

The role of JAZ proteins in plant defence and hormone crosstalk.

Garoufalia Eleftheriadou to the University of Exeter as a thesis for the degree of Masters by Research in Biosciences (March, 2012).

This thesis is available for library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved For the award degree for this or any other University.

ABSTRACT

Phytohormones have a crucial role in plant defence responses. The role of each specific phytohormone is contingent on whether the pathogen is a bio- or a necrotroph. In the case of the hemibiotrophic *Pseudomonas syringae* pv *tomato* (DC3000) and the model plant *Arabidopsis thaliana*, the interaction of the three key plant hormones, salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA), ultimately dictates the outcome of the infection. Post-invasion defence responses, chiefly regulated by the SA dependent pathway are compromised by the antagonistic action of JA and ABA. It is this complex crosstalk between the hormone pathways that propels susceptibility or resistance. The Jasmonate ZIM domain (JAZ) transcription repressors are crucial part of the JA signaling cascade. JAZs form a co-receptor with F-BOX Coronatine Insensitive (COI) 1 that binds the bioactive form of JA, Jasmonic Isoleucine (JA-Ile). Subsequent to this JA perception is the specific degradation of JAZ proteins that allows transcription of early JA-related genes. Interestingly the bacterial phytotoxin coronatine, which is a JA-Ile mimic, increases bacterial virulence by exploiting the JA signaling pathway. In this project we establish the DC3000 induced susceptibility of *jaz10* and *jaz5/10* loss of function mutants that substantiates JAZ specificity and confirms coronatine-induced virulence. In addition, we demonstrate the different manifestation of symptom development caused by absence of key positive regulators of plant defence, SA and JAZ5/JAZ10 and uncover the severely compromised phenotype of *jaz5/jaz10/aao3/sid2-1*. Finally we produce the tools that will allow further research of JAZ protein interactions.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES	5
LIST OF TABLES AND FIGURES	6
LIST OF ABBREVIATIONS	7
1.INTRODUCTION	8
1.1 Plant defence mechanisms.	8
1.2 Hormones in defence.	9
1.2.1 Salicylic acid.	9
1.2.2 Abscisic acid.	10
1.2.3 Jasmonates.	11
1.2.3.a JA biosynthesis and signalling.	11
1.2.3.b Jasmonate ZIM domain (JAZ) proteins.	14
i. ZIM domain dimerization.	14
ii. The jas domain and the JAZ-CO11 co-receptor.	15
iii. Interaction with transcription factors and other proteins.	15
1.2.3.c JA signalling curtailment.	16
1.2.3.d JA/SA and JA/ABA crosstalk.	17
1.3 <i>Pseudomonas syringae</i> .	18
1.3.1 <i>P. syringae</i> pv tomato DC3000.	18
1.3.2 Coronatine	19
1.4 <i>B. cinerea</i>	19
2. MATERIALS AND METHODS	20
2.1 Plant material and growth conditions.	20
2.2 Pathogen material.	20
2.3 Genotyping of <i>jaz</i> knock-out mutants.	20
2.3.1 DNA extraction protocol.	20
2.3.2 PCR	21
2.3.3 Gel electrophoresis.	21
2.4 Generation of new <i>jaz</i> mutant combinations.	23
2.5 <i>Pst</i> challenges.	23
2.5.1 Leaf infiltration and spraying treatment.	23
2.5.2 Population counts and data analysis.	23
2.6 <i>B. cinerea</i> inoculations.	23
2.7 Construct generation.	23

3. RESULTS	26
3.1 Genotyping and generation of new <i>jaz</i> mutant genotypes.	26
3.2 Pathogenicity tests on <i>jaz</i> knock-out mutants.	29
3.2.1 The impact of the <i>jaz</i> mutations on the outcome of infection.	29
3.2.1a The stark chlorotic phenotypes of <i>jaz10</i> and <i>jaz5/10</i> .	29
3.2.1b <i>jaz5/10</i> is a unique loss of function combination that causes a dominant phenotype amongst the JAZs.	30
3.2.1 c Chlorotic symptoms are 100% linked to the presence of coronatine.	32
3.2.1 d The susceptible genotype <i>jaz5/10</i> is subject to a threshold level of bacteria.	33
3.2.2 The effect of <i>jaz</i> mutation in hormone deficient background.	34
3.2.2.a Rapid collapse of leaf tissue in the quadruple mutant <i>jaz5/jaz10/aao3/sid2-1</i> following DC3000 infiltration .	34
3.2.2b Different levels of bacterial growth of <i>aao3</i> and <i>sid2-1</i> genotypes in <i>jaz</i> KO background 72 hpi.	36
3.3 Putative resistance of <i>jaz5/10</i> and <i>jaz6</i> mutants to <i>B. cinerea</i> pv pepper.	37
3.4 Generation of fusion PCR amplicon insert.	38
4. DISCUSSION	39
5. APPENDIX	42
6. BIBLIOGRAPHY	51

