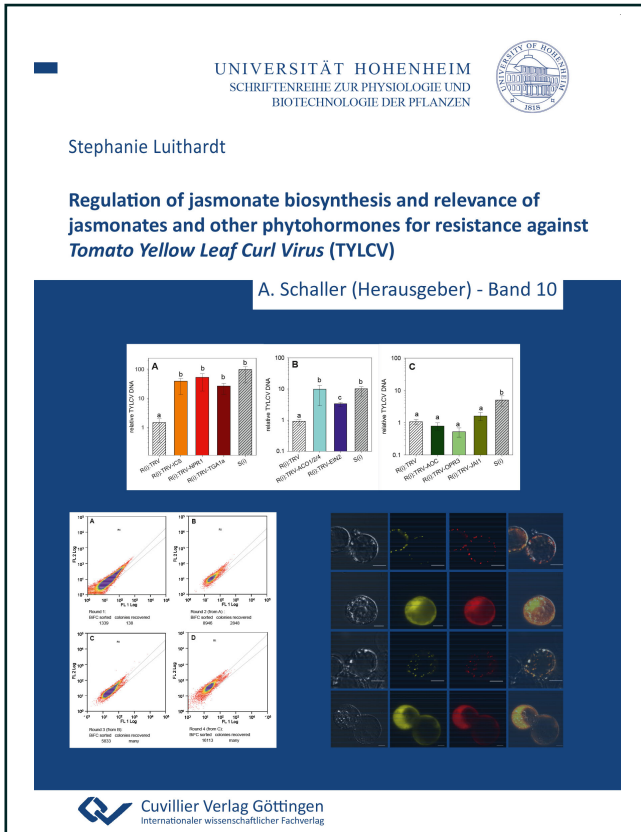




Stephanie Luithardt (Autor)
Regulation of jasmonate biosynthesis and relevance of jasmonates and other phytohormones for resistance against Tomato Yellow Leaf Curl Virus (TYLCV)



<https://cuvillier.de/de/shop/publications/7701>

Copyright:
Cuvillier Verlag, Inhaberin Annette Jentsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,
Germany
Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>



CONTENTS

Contents	I
Tables	V
Figures	V
Abbreviations	VII
Summary	XI
Zusammenfassung.....	XIII
1 Introduction	1
1.1 Defense mechanisms of plants against pathogens and herbivores	1
1.2 Phytohormones regulating biotic defenses in plants	3
1.3 Defense mechanisms against plant viruses and vectors	10
1.4 Regulation mechanisms of JA biosynthesis.....	14
1.5 Post-translational modifications of OPR3	15
1.6 Objectives of this work.....	18
2 Material and Methods	21
2.1 Material.....	21
2.1.1 Chemicals and antibiotics	21
2.1.2 Enzymes	21
2.1.3 Antibodies	21
2.1.4 Primers	21
2.1.5 Vectors	24
2.1.6 Constructs	25
2.1.7 Media	27
2.2 Organisms.....	28
2.2.1 Bacteria	28
2.2.2 <i>Solanum lycopersicum</i>	28
2.2.3 <i>A. thaliana</i> cell culture	29
2.3 Methods for determination of TYLCV resistance.....	29
2.3.1 Cultivation of tomato plants on soil.....	29



Contents

2.3.2	Infiltration of VIGS constructs into tomato radicles	29
2.3.3	Determination of silencing vector systemic spread	30
2.3.4	TYLCV inoculation.....	30
2.4	Harvest of plant material	30
2.5	Sequencing <i>Ty-1/Ty-3</i> resistance loci.....	31
2.6	Methods for the analysis of PPI	31
2.6.1	Preparation of <i>A. thaliana</i> cell culture protoplasts	31
2.6.2	Transfection of cell culture protoplasts.....	31
2.6.3	Treatment and harvest of cell culture protoplasts.....	32
2.6.4	Microscopy.....	32
2.6.5	Protoplast co-immunoprecipitation	32
2.6.6	cDNA library screen by fluorescence-activated cell sorting	33
2.6.7	Fluorescence activated cell sorting.....	35
2.6.8	Flow cytometric analysis.....	35
2.6.9	Next generation sequencing (NGS) of sorted cDNA libraries	35
2.7	Molecular methods.....	38
2.7.1	Extraction of nucleic acids	38
2.7.1.1	RNA isolation.....	38
2.7.1.2	Isolation of genomic DNA from plant material.....	38
2.7.1.3	Isolation of plasmid DNA for cloning procedures.....	38
2.7.1.4	Plasmid DNA isolation for small scale and large scale transfections.....	39
2.7.1.5	Plasmid DNA isolation for single clone screens	39
2.7.2	Reverse transcription (RT) PCR	40
2.7.3	Estimation of nucleic acid concentration and purity.....	40
2.7.4	Polymerase chain reaction (PCR)	40
2.7.5	Colony PCR	41
2.7.6	Quantitative (q)PCR	41
2.7.7	Bacterial cultivation	42
2.7.8	Expression and purification of <i>Taq</i> polymerase from <i>E. coli</i>	42
2.7.9	Bacterial transformation.....	43
2.7.10	TOPO® cloning.....	44



Contents

2.7.11	DNA sequencing.....	44
2.7.12	Restriction digestion	44
2.7.13	Separation of DNA on agarose gels	44
2.7.14	Elution of DNA fragments from agarose gels	45
2.7.15	Dephosphorylation of DNA fragments	45
2.7.16	Ligation.....	45
2.7.17	Denaturing gel electrophoresis (SDS-PAGE)	45
2.7.18	Western blot analysis.....	46
2.8	Statistics	46
3	Results.....	47
3.1	Silencing genes in three plant defense hormone pathways.....	47
3.1.1	Resistance loci in the R and S line	47
3.1.2	Defense gene expression after TYLCV infection	51
3.1.3	Impact of defense signaling pathways on TYLCV resistance	52
3.2	Identification of potential regulators of OPR3.....	60
3.2.1	Subcellular site of OPR3 dimerization.....	61
3.2.2	Identification of OPR3 interaction partners by cDNA library screening in <i>Arabidopsis</i> protoplasts using fluorescence activated cell sorting (FACS)	64
3.2.3	FACS screen for identification of OPR3 interaction partners - Screen I	66
3.2.4	Co-immunoprecipitation (Co-IP) of OPR3 with the putative interacting proteins At1g06650 and LTI78	75
3.2.5	cDNA library screen in <i>Arabidopsis</i> protoplasts using FACS to identify OPR3 interaction partners - Screen II	77
3.2.6	Analysis of pooled plasmids of Screen II-1 and Screen II-3 by Next generation sequencing (NGS).....	82
4	Discussion	93
4.1	<i>Ty-1/Ty-3</i> loci are not involved in TYLCV resistance of R plants	93
4.2	TYLCV resistance in the R line depends on SA- and ET-signaling.....	94
4.2.1	The ET pathway contributes to TYLCV resistance in the R line	97
4.3	Identification of potential regulators of OPR3.....	100
4.3.1	Subcellular site of OPR3 dimerization.....	101



Contents

4.3.2	Evaluation of the cDNA library screen	101
4.3.3	Identification of interacting proteins of OPR3 by a combination of BiFC/FACS and NGS.....	103
4.3.4	Putative interaction partners of OPR3.....	105
4.4	Conclusion and future perspectives.....	108
5	Bibliography.....	111
6	Appendix.....	153
A.1.	TYLCV quantification	153
A.2.	Co-expression analysis and Pearson correlation of OPR3 together with potential interactors of Screen II-3.....	154
	Danksagung	157
	Eidesstattliche Erklärung.....	158
	Lebenslauf	159