

Contents

List of Abbreviations	7
List of Figures	9
List of Tables	11
1. Introduction	13
1.1. Description and history of the plant disease fire blight	13
1.2. Conventional control methods of fire blight	14
1.3. Genetic engineering to improve host resistance against fire blight	16
1.4. General aspects of plant-pathogen interactions	18
1.5. Genetics of fire blight	19
1.6. The <i>E. amylovora</i> effector AvrRpt2 _{EA}	20
1.7. Aim of the study	21
2. Materials and methods	23
2.1. Materials	23
2.1.1. Plant material	23
2.1.2. Bacteria and yeast strains	23
2.1.3. Vectors and plasmids	24
2.2. Methods	25
2.2.1. Inoculation of apple shoots	25
2.2.2. Cultivation of bacteria	25
2.2.3. Cultivation of yeast	25
2.2.4. DNA extraction	26
2.2.5. RNA extraction	26
2.2.6. cDNA synthesis	27
2.2.7. PCR based methods	27

2.2.8. Cloning of <i>E. coli</i>	30
2.3. Sequencing of the <i>avrRpt2_{EA}</i> gene of different <i>E. amylovora</i> strains	31
2.4. Establishment of complemented <i>E. amylovora</i> strains	31
2.5. Yeast two-hybrid assay	32
2.5.1. Cloning	32
2.5.2. Mating	34
2.6. Western blot	35
2.6.1. Protein extraction	35
2.6.2. Electrophoresis of proteins	35
2.6.3. Membrane transfer and detection	36
2.7. RNA-seq	37
2.7.1. Sample preparation and RNA extraction	37
2.7.2. cDNA library construction, sequencing and mapping	37
2.7.3. Data analysis and bioinformatics	37
2.8. Gene expression analysis with the BioMark™ HD system	38
2.8.1. Primer development and optimisation	38
2.8.2. Differential gene expression analysis	39
2.8.3. Statistical analysis	40
3. Results	41
3.1. Analysis of the AvrRpt2 _{EA} effector	41
3.1.1. Sequencing of the <i>avrRpt2_{EA}</i> gene	41
3.1.2. Development and virulence analysis of the complemented <i>E. amylovora</i> strains	42
3.1.3. Real-Time qRT-PCR with the complemented <i>E. amylovora</i> strains	44
3.2. RIN4 of <i>Malus × robusta</i> 5	47
3.2.1. Sequence analysis of <i>RIN4</i> from Mr5	47
3.2.2. Western blot with the RIN4 antibody	50
3.3. Yeast two-hybrid assay to prove protein-protein interactions	51
3.4. RNA-seq and gene expression analysis with the BioMark™ HD system	54
3.4.1. Differential transcriptome analysis by RNA-seq	54
3.4.2. Gene expression analysis with the BioMark™ HD system	60
3.4.3. Comparison of the results obtained by RNA-seq and the BioMark™ HD system	67

3.4.4. Comparison between prediction, sequencing and RNA-seq data in case of <i>RIN4</i> from Mr5	68
3.5. Fire blight resistance gene <i>FB_MR5</i> of Mr5	70
4. Discussion	73
4.1. Role of the <i>E. amylovora</i> effector AvrRpt2 _{EA}	73
4.2. Identification and characterisation of <i>RIN4</i> of Mr5	76
4.3. Protein-protein interaction in the system Mr5 and <i>E. amylovora</i>	79
4.4. Differential transcriptome analysis of Mr5 by RNA-seq	81
4.5. Gene expression analysis of Mr5 with the BioMark™ HD system	87
4.6. Fire blight resistance gene <i>FB_MR5</i> of Mr5	90
5. Conclusion	92
Bibliography	95
Appendix	108
A. Materials	108
A.1. Used chemicals	108
A.2. Composition of buffers and media	109
A.3. Laboratory equipment	110
A.4. Consumables	113
B. <i>Erwinia amylovora</i> strains	114
C. Tables of primers	116
C.1. General primers	116
C.2. Primers for the synthesis of the complemented strains	117
C.3. Primers used in Y2H assay	118
C.4. Primers for RACE-PCR of <i>FB_MR5</i> and <i>RIN4</i>	119
C.5. Primers used in gene expression analysis with the BioMark™ HD system	120
D. RT-PCR of the complemented <i>E. amylovora</i> strains	124
E. Analysis of Real-Time q-RT data of the complemented <i>E. amylovora</i> strains	125
F. Sequence of the <i>avrRpt2_{EA}</i> -eu gene	126
G. Alignment of the sequences received by 5' RACE-PCR of <i>RIN4</i> from Mr5	127
H. Protein sequence alignment of RIN4	131

I.	Protein sequence identity matrix of RIN4	134
J.	RNA Integrity Number of the samples used for RNA-seq	135
J.1.	RIN value of the sample Ea1189 2 hpi	135
J.2.	RIN value of the sample Ea1189 48 hpi	136
J.3.	RIN value of the sample ZYRKD3-1 2 hpi	137
J.4.	RIN value of the sample ZYRKD3-1 48 hpi	138
K.	FastQC results of the transcriptome analysis	139
L.	Significant DEGs obtained from RNA-seq	140
L.1.	DEGs at 2 or 48 hpi	140
L.2.	DEGs at 2 and 48 hpi	144
M.	Assignment of RNA-seq data via MapMan	145
N.	CNRQ values of the genes analysed by BioMark™ HD system	147
O.	Genes analysed by BioMark™ HD system	155
P.	Statistical analysis by SAS of the CNRQ values of the genes analysed by BioMark™ HD system	156
P.1.	Shapiro-Wilk, Kruskal-Wallis and Levene's test	156
P.2.	ANOVA and Tukey's HSD test	157
P.3.	Mann-Whitney U test	159
Q.	Sequences of the <i>FB_MR5</i> gene obtained by 3' and 5' RACE-PCR	160
Q.1.	5' RACE-PCR of the <i>FB_MR5</i> gene	160
Q.2.	3' RACE-PCR of the <i>FB_MR5</i> gene	162
	Acknowledgements	164
	Declaration of Authorship	165
	List of publications	166
	Curriculum vitae	168