
CONTENTS

Abstract.....	V
Resumen.....	VII
Resum.....	XI
1 Introduction.....	1
Plant viruses.....	1
Virus evolution.....	1
Virus biology.....	2
Control of plant viral diseases.....	5
Plant viruses used in this study.....	5
Genus <i>Fabavirus</i>	5
Genus <i>Tobamovirus</i>	7
<i>Tomato spotted wilt virus</i>	8
2 Interest and objectives.....	11
3 Development of detection and quantification methods.....	13
Introduction.....	13
Results and discussion.....	15
1. Development of flow-through hybridization of BBWV-1 tissue prints.....	15
2. Development of a RT-qPCR procedure for BBWV-1 and BBWV-2.....	18
3. Improvement of the RT-qPCR procedure for TMV and ORMV.....	21
4 Generation of BBWV-1 infectious transcripts from cDNA clones.....	23
Introduction.....	23
Results and discussion.....	24
1. Cloning and <i>in vitro</i> generation of infectious clones.....	24
2. Evaluation and comparison of transcripts with different 5' terminal sequences.....	25
3. Evaluation and comparison of a BBWV-1 isolate and <i>in vitro</i> transcripts	27
5 Effect of host, vector and another virus on BBWV-1 fitness.....	29
Introduction.....	29
Results.....	31
1. Temporal accumulation of BBWV-1 in plants: effect of inoculum dose, plant development and host type.....	31
2. Effect of a systemic acquired resistance activator on BBWV-1 infection	33
3. Effect of co-inoculating TSWV, which triggers a hypersensitive response	34

4. Transmissibility of BBWV-1 by aphids: effect of inoculum dose, virus genotype and aphid species.....	35
Discussion.....	38
6 Effect of host interactions on fitness and evolution of TMV	43
Introduction.....	43
Results.....	47
1. Effect of mutations in <i>Arabidopsis thaliana</i> genes related to microtubule dynamics and defense on symptoms induction and fitness of TMV and ORMV	47
2. Experimental evolution and adaptation analysis of TMV in <i>A. thaliana</i> mutants.....	49
Genetic analysis of TMV lineages evolved in <i>A. thaliana</i> genotypes.....	51
Infectivity and accumulation of TMV lineages evolved in <i>A. thaliana</i> mutant <i>tor1</i>	52
Infectivity and accumulation of TMV lineages evolved in <i>A. thaliana</i> mutant <i>tor2</i>	53
Infectivity and accumulation of TMV lineages evolved in <i>A. thaliana</i> mutant <i>npr1</i>	54
Infectivity and accumulation of TMV lineages evolved in <i>A. thaliana</i> mutant <i>cpr5</i>	55
Infectivity and accumulation of TMV lineages evolved in <i>A. thaliana</i> ecotypes Ler and Col-0.....	56
Discussion.....	57
7 General conclusions.....	61
8 Materials and methods.....	63
Biological material	63
Viruses	63
Plants	64
Aphids	64
Purification.....	64
Total RNA purification	64
Purification of virions	65
Detection of viruses.....	65
DAS-ELISA	65
RT-PCR	
Real time quantitative RT-PCR	
Molecular hybridization	
Cloning and generation of capped transcripts.....	69
Cloning	

Transcription of capped RNAs	
Biological assays.....	69
Mechanical inoculation of virus.....	69
Application of BTH	70
Transmission assays	70
Experimental evolution protocol.....	70
Nucleotide and statistical analyses.....	71
Nucleotide sequence determination and analyses.....	71
Statistical analyses.....	71
9 References.....	73
Acknowledgements / Agradecimientos / Agreements	83