THE UTILIZATION OF THE *HMG*2 INDUCIBLE PROMOTER TO GENETICALLY ENGINEER PARASITE RESISTANCE IN TOBACCO

by
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Dissertation Submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in Plant Pathology, Physiology, and Weed Science

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April, 2003

Blacksburg, Virginia

Key Words: *Hmg*2 promoter, proteinase inhibitors, cyst nematode, tobacco N gene, *Orobanche aegyptiaca*

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ABSTRACT

The cyst nematode, *Globodera tabacum tabacum* Behrens, and the parasitic angiosperm, Egyptian broomrape, *Orobanche aegyptiaca* Pers., are obligate root parasites that cause severe yield and quality loss of many important crop hosts. Although these represent two diverse classes of parasites, they have significant similarities in the modes of parasitism and complex interactions with their hosts. Conventional control methods have had limited success in controlling these parasites. The overall objective of this research was to engineer resistance to the cyst nematode and Egyptian broomrape by expressing genes encoding parasite specific toxins under the control of parasite-responsive promoters using tobacco (*Nicotiana tabacum* L. cv. Xanthi). For nematode resistance, an anti-feeding strategy was employed utilizing the tomato proteinase inhibitor I (PI-I) gene as a nematode specific toxin. Transgenic tobacco plants were generated that expressed genes encoding an intracellarly retained or secreted form of tomato PI-I under the control of the nematode-inducible promoter, derived from tomato (*Lycopersicon esculentum* L.) *Hmg*2 gene. Our goals were to determine the effectiveness of local PI-I expression on nematode resistance and to determine if intracellular or extracellular PI-I deposition enhances resistance. Two constructs were generated that contained either the coding region of the tomato PI-I gene, lacking the signal sequence (EM1), or the coding region of PI-I including the signal sequence (EM2), fused to the nematode-responsive *Hmg*2 promoter. Transgenic PI-I plants were inoculated with *G. t. tabacum* cysts and evaluated for nematode interactions. Our results suggest that local expression of intercellular of PI-I significantly reduced cyst production when compared to the nontransformed controls. For broomrape resistance, a well characterized *R*/*avr* gene pair, the tobacco N resistance gene and the tobacco mosaic virus replicase (TMV) gene, was utilized to create novel gene-for-gene resistance via a N gene-mediated hypersensitive response (HR) to limit broomrape parasitism. The bean (*Phaselous vulgaris* L.) chalcone synthase 8 (CHS8) promoter has been characterized as a broomrape–responsive promoter. We introduced the CHS8:TMV replicase gene

construct into tobacco plants that contains an endogenous N gene. Transgenic tobacco plants were inoculated with *O. aegyptiaca* seeds and monitored for parasite attachment and development. The expression of the TMV replicase leads to a significant reduction in broomrape parasitism. These genetic engineering strategies show promise in enhancing resistance to these destructive parasites.

Acknowledgements

First and foremost I would like to thank my major advisor, Dr. Carole Cramer, for providing so much more than just a the normal professor-graduate student relationship. Thank you for giving me the opportunity to work in your lab and teaching me what it truly means to be a great scientist who has passion for their work. Thank you for always being there and encouraging me to persevere - you are truly a "superwoman".

I would like thank the other members of my committee, Dr. James Westwood, Dr.Elizabeth Grabau, Dr.George Lacy, and Dr. Carole Wilkinson for their support and guidance.

I would also wish to thank the MAOP program for providing financial support.

The Cramer lab for always supporting my every effort and helping me throughout my graduate career. It was wonderful to be a part of such a fun-loving lab. Thank you for always keeping the atmosphere light and full of laughter. I would especially like to thank Dr. Cynthia Denbow and Dr. Maureen Dolan for always giving great advice and being such remarkable examples of strong women in science. I could not have put all the pieces together without the help everyone including a phenomenal undergraduate student, Ms. Kristin Reedy. Thank you for being so dedicated and hard-working.

I want to thank the Westwood and Eisenback labs for their expertise and help with my research projects.

I want to thank all of my close friends Kimberly Smith, Amer Fayad, Amanda Griffitts, Bakhitah Abdul-Rauf, and Ann Michaels for lending you ears and words of encouragement.

I would like to thank my brother, Anthony Williamson, for being here with me through thick and thin. I love you dearly.

Finally, I wish to offer a sincere thanks to Dr. Selester Bennett, who has surpassed being just a friend, you are like a brother to me. Thank you for taking me under your wing and always having my back no matter what. I will always look up to you and hope that I can positively impact other lives the way that you have mine.

Dedication

This dissertation is dedicated to the memory of grandparents,
Willie and Dorothy Winston.

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List of Abbreviations

TCN	tobacco cyst nematode
UPGMA	unweighted pair group method
SCN	soybean cyst nematode
HR	hypersensitive response
Avr	avirulence gene
LRR	leucine rich repeat
NBS	nucleotide binding site
TIR	*Drosophila* Toll and mammalian interleukin-1 receptor
ARF 18	Arkansas Fungus 18
PR	pathogenesis-related proteins
HRGP	hydroxyproline-rich glycoproteins
SAR	systemic acquired resistance
SA	salicylic acid
INA	2,6-dichloroisonicotinic acid
BTH	benzothiadiazole
TMV	tobacco mosaic virus
DAI	days after inoculation
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
HMG CoA	3-hydroxy-3-methylglutaryl-CoA
HMGR	HMG CoA reductase
IPP	isopentyl pyrophosphate
GUS	β-glucoronidase reporter gene
PIs	Proteinase inhibitors
PIIF	protease inhibitor-inducing factor
CpTI	cowpea trypsin inhibitor
CaMV	cauliflower mosaic virus
Oc-I	oryzacystatin
Oc-IΔ86	engineered oryzacystatin
GO	galactose oxidase
PsMTa	plant metallothionin-like protein
Ha	hectares
PME	pectin methylesterase
PGA	polygacturonase
a.i.	active ingredient
R	resistance genes
CHS	Chalcone synthase
RT-PCR	reverse-transciptase polymerase chain reaction
AE	alternative exon
MS	Mushige and Skoog
MVA	Mevalonic Acid
PP	Pyrophosphate
IPP	Isopentyl diphosphate, isopentyl pyrophosphate
NAPDH	ß-nicotinamide adenine dinucleotide phosphate, reduced form