

**THE UTILIZATION OF THE *HMG2* INDUCIBLE PROMOTER TO
GENETICALLY ENGINEER PARASITE RESISTANCE
IN TOBACCO**

by
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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 intracellularly retained or secreted form of tomato PI-I under the control of the nematode-inducible promoter, derived from tomato (*Lycopersicon esculentum* L.) *Hmg2* 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 *Hmg2* 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 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 (*Phaseolus 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.

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Dedication

This dissertation is dedicated to the memory of grandparents,
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List of Abbreviations

TCN	tobacco cyst nematode
UPGMA	unweighted pair group method
SCN	soybean cyst nematode
HR	hypersensitive response
<i>Avr</i>	avirulence gene
LRR	leucine rich repeat
NBS	nucleotide binding site
TIR	<i>Drosophila</i> 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 -glucuronidase reporter gene
PIs	Proteinase inhibitors
PIIF	protease inhibitor-inducing factor
CpTI	cowpea trypsin inhibitor
CaMV	cauliflower mosaic virus
Oc-I	oryzacystatin
Oc-I Δ 86	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-transcriptase polymerase chain reaction
AE	alternative exon
MS	Mushige and Skoog
MVA	Mevalonic Acid
PP	Pyrophosphate
IPP	Isopentyl diphosphate, isopentyl pyrophosphate
NAPDH	β -nicotinamide adenine dinucleotide phosphate, reduced form