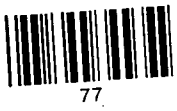

BARD

FINAL REPORT

PROJECT NO. IS-3048-98

A Novel Approach to Parasitic Weed Control Based on Inducible Expression of Cecropin Transgenic Plants

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Project Title: A Novel Approach to Parasitic Weed Control Based on Inducible Expression of Cecropin in Transgenic Plants.

Investigators	Institutions
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Keywords: Sarcotoxin IA, *Orobanche* spp., Broomrape, tobacco, tomato

Abbreviations commonly: GFP, green fluorescent protein; HIS, hexa-histidine residue tag HMG2, 3-hydroxy-3-methylglutaryl CoA reductase isogene 2; ps, patatin transit signal sequence; SLP, sarcotoxin-like protein; ss, Sarcotoxin IA signal sequence;.

Budget: IS: $ 109,000 US: $ 156,000 Total: $ 265,000

Signature
Principal Investigator

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Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)				
Submitted, in review, in preparation	3			3
Invited review papers				
Book chapters		3		3
Books				
Master theses			1	1
Ph.D. theses		1		1
Abstracts	7		2	9
Not refereed (proceedings, reports, etc.)	3			3

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1		1
Longer Visits (Sabbaticals)		1		1

Description of Cooperation:

See Final Report

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted			1	1
Issued (allowed)				
Licensed				

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Final Report

A Novel Approach to Parasitic Weed Control Based on Inducible Expression of Cecropin in Transgenic Plants.

Radi Ali, Carole L. Cramer and James H. Westwood

Abstract

Our overall goal was to engineer crop plants with enhanced resistance to *Orobanche* (broomrape) based on the inducible expression of sarcotoxin-like peptide (SLP). A secondary objective was to localize small proteins such as SLP in the host-parasite union in order to begin characterizing the mechanism of SLP toxicity to *Orobanche*. We have successfully accomplished both of these objectives and have demonstrated that transgenic tobacco plants expressing SLP under control of the HMG2 promoter show enhanced resistance to *O. aegyptiaca* and *O. ramosa*. Furthermore, we have shown that proteins much larger than the SLP move into *Orobanche* tubercles from the host root via either symplastic or apoplastic routes.

This project was initiated with the finding that enhanced resistance to *Orobanche* could be conferred on tobacco, potato, and tomato by expression of SLP (Sarcotoxin IA is a 40-residue peptide produced as an antibiotic by the flesh fly, *Sarcophaga peregrina*) under the control of a low-level, root-specific promoter. To improve the level of resistance, we linked the SLP gene to the promoter from HMG2, which is strongly inducible by *Orobanche* as it parasitizes the host. The resulting transgenic plants express SLP and show increased resistance to *Orobanche*. Resistance in this case is manifested by increased growth and yield of the host in the presence of the parasite as compared to non-transgenic plants, and decreased parasite growth. The mechanism of resistance appears to operate post-attachment as the parasite tubercles attached to the transgenic root plants turned necrotic and failed to develop normally. Studies examining the movement of GFP (approximately 6X the size of SLP) produced in tobacco roots showed accumulation of green fluorescence in tubercles growing on transformed plants but not in those growing on wild-type plants. This accumulation occurs regardless of whether the GFP is targeted to the cytoplasm (translocated symplastically) or the apoplastic space (translocated in xylem). Plants expressing SLP appear normal as compared to non-transgenic plants in the absence of *Orobanche*, so there is no obvious unintended impact on the host plant from SLP expression.

This project required the creation of several gene constructs and generation of many transformed plant lines in order to address the research questions. The specific objectives of the project were to:

1. Make gene constructs fusing *Orobanche*-inducible promoter sequences to either the sarcotoxin-like peptide (SLP) gene or the GFP reporter gene.
2. Create transgenic plants containing gene constructs.
3. Characterize patterns of transgene expression and host-to-parasite movement of gene products in tobacco (*Nicotiana tabacum* L.) and *Arabidopsis thaliana* (L.).

4. Characterize response of transgenic potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) to *Orobanche* in lab, greenhouse, and field.

Objectives 1 and 2 were largely accomplished during the first year during Dr. Aly's sabbatical visit to Virginia Tech. Transforming and analyzing plants with all the constructs has taken longer than expected, so efforts have concentrated on the most important constructs. Work on objective 4 has been delayed pending the final results of analysis on tobacco and *Arabidopsis* transgenic plants.

The implications of this work are profound, because the *Orobanche* spp. is an extremely destructive weed that is not controlled effectively by traditional cultural or herbicidal weed control strategies. This is the first example of engineering resistance to parasitic weeds and represents a unique mode of action for selective control of these weeds. This research highlights the possibility of using this technique for resistance to other parasitic species and demonstrates the feasibility of developing other novel strategies for engineering resistance to parasitic weeds.

Achievements

Due to the persistent difficulty in controlling parasitic weeds throughout much of the world there is a pressing need for efficient, environmentally safe, and inexpensive strategies for limiting damage caused by these parasites. Parasitic weeds such as *Orobanche* are difficult to control because they are closely associated to the host root and are concealed underground for most of their life cycle. Currently the best control method is to kill seeds in the soil by fumigation with methyl bromide. This method is expensive, laborious, and extremely hazardous to the environment, (methyl bromide use is being phased out by international agreement to protect the global environment). The best long-term strategy for limiting damage caused by parasitic weeds is the development of parasite-resistant crops. While conventional plant breeding has produced few crops with stable resistance, we believe that genetic engineering offers the possibility of creating novel resistance mechanisms with the potential to be introduced into many commercial crops. Our research consistently demonstrates that SLP is a selective inhibitor of *Orobanche* growth. Initial studies indicated that SLP synthesized by yeast (*Saccharomyces cerevisiae*) (Aly et al. 1999) inhibited *O. aegyptiaca* seed germination and radicle elongation (Aly and Plakhine, unpublished results). The SLP gene was then linked to the constitutive, root specific *Tob* promoter (Mahler-Slasky et al. 1996), and this construct was transformed into potato, tomato, and tobacco. Each of these transformations yielded lines with enhanced resistance to *O. aegyptiaca* as compared to non-transformed control plants (Fig. 1A). Observations of parasite growth in association with these plants indicated that parasites were able to attach to the host root, but grew abnormally and had an increased

mortality rate as compared to parasites growing on non-transformed plants (Fig. 1B). In the absence of *Orobanche*, growth and development of crops expressing *Tob:SLP* was equal to that of non-transformed plants, suggesting that low levels of SLP are not detrimental to the plants. However, *Orobanche* resistance in these plants was incomplete, and we hypothesized that this is due to the low level of SLP expression. We therefore proposed to increase the efficacy of SLP by regulating its expression with the *HMG2* promoter, which we had previously shown to have strong and sustained expression in host roots at the site of *Orobanche* entry (Westwood et al. 1998).

The expression pattern of the *HMG2* promoter in response to *O. aegyptiaca* represents many desirable traits of an optimal promoter for engineering resistance: expression is induced immediately following parasite penetration of the host root, occurs specifically in the area immediately surrounding the point of attachment, and continues throughout development of the parasite. *HMG2* is one of four differentially-regulated genes in tomato that encode 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), considered the rate limiting enzyme in the isoprenoid biosynthetic pathway (Chappell 1995; Cramer et al. 1993).

We have generated transgenic plants expressing several variations of the *HMG2:SLP* construct (Table 1, Fig. 4 A, B) and compared *Orobanche* interactions of non-transgenic tobacco with lines expressing *HMG2:ss:SLP*.

Our results confirm that SLP reduces parasite growth and increases host biomass while having no obvious effect on the host plant. Seven tobacco lines containing this construct (confirmed by PCR using primers specific to SLP) were challenged in two experiments in pots containing soil inoculated with either *O. aegyptiaca* or *O. ramosa*. In both experiments the lines varied in response to parasitism, as expected for the initial generation of transformants where transgene copy number and position effects can greatly influence expression. However, six lines showed some reduction in parasitism, and lines L3 and L7 exhibited the best response, as confirmed in experiments conducted independently in Israel and the US. When grown in soil inoculated with *O. ramosa*, L3 and L7 had greater height and biomass than control plants (Fig 2 A, B). An experiment conducted in polyethylene bags to facilitate observation of host-parasite interactions indicated that *O. aegyptiaca* tubercle mortality was high on L3 and L7 (Fig. 2C). Results from the experiment challenging plants in soil inoculated with *O. ramosa* showed dramatically reduced numbers and fresh weights of tubercles, particularly L3, which had parasite numbers approximately 10% of the control (Fig. 3). Observations of the roots of transgenic

plants expressing sarcotoxin IA revealed significantly deader parasite tubercles compared to normal growth of the parasite tubercles on non-transgenic plants (Figure 3). In all experiments the transgenic plants were indistinguishable from non-transformed when grown in the absence of *Orobanche* seeds suggesting no obvious effect of the transgene on plant growth and development. We confirmed that the plants contained the transgene by DNA hybridization analysis. The best expressing line, designated L3 was found to have two copies of the gene (data not shown).

Western blot analysis then confirmed the presence of SLP transgene expression. Total soluble proteins were extracted from root tissue adjacent to the parasite penetration site (1.5 cm from each side of the parasite tubercles) separated by polyacrylamide gel electrophoresis, and probed using a specific anti-sarcotoxin antibody. The immunoreactive band detected around 8.2 kDa. in transgenic plants (Fig. 4 C) is likely dimmers of sarcotoxin IA, which has an expected size as a monomer of around 4.2 kDa. (Mitsuhara et al., 2000).

It is important to note that we have only begun to analyze some of the many transgenic lines that we have created (Table 1). To facilitate identification of the optimal SLP expressing lines we have generated an antibody to the hydrophilic region of the sarcotoxin IA protein. This antibody is currently being optimized for use in screening transformants. We expect that we will identify a wide range of SLP-expressing plants that will provide a better estimate of the maximum potential of this strategy for *Orobanche* resistance.

The finding that SLP has selective toxicity to *Orobanche*, an angiosperm plant, is unexpected given our understanding of its mechanism of action. Selectivity of these toxins for bacteria is attributed to the high level of positively-charged residues on the hydrophilic end of the peptide, causing it to associate preferentially with bacterial membranes, which carry a more negative charge than eukaryotic cells (Shai 1999). Therefore, the toxins disrupt bacterial cells at concentrations one to two orders of magnitude lower than those that disrupt mammalian or plant cells (Jaynes et al. 1989; Nordeen et al. 1992). Despite this difference in sensitivity, it is important to understand how SLP acts and where accumulates in the parasite.

A fundamental question relating to this research is whether it is even possible for a macromolecule such as SLP to move from the host to the parasite. To address this question and to optimize toxin delivery by characterizing pathways of protein movement between host and

parasite, we replaced the gene coding for sarcotoxin IA by a gene coding for green fluorescent protein (GFP) (Fig. 4 B). GFP is a fluorescent protein with a unique combination of small size (27 kDa), stability, and ability to be easily detected in living tissues without the need for additional staining or tissue fixation (Yang et al. 1996). We have generated transgenic *Arabidopsis* and tobacco with GFP targeted to the cytosol (lacking signal peptide) or targeted for secretion to the extracellular space (linked to the patatin signal peptide). We are also using other mobile fluorescent dyes that enable us to track the movement of various sized molecules across the host-parasite interface.

A result of research with fluorescent markers suggests that small proteins such as sarcotoxin are translocated directly into the *Orobanche* tubercle from the host rather than accumulating at the haustorial surface. Green fluorescence was observed at the point of attachment and inside tubercles growing on *Arabidopsis* plants engineered to produce GFP targeted to the cytosol, while any autofluoresence observed in tubercles growing on non-transformed *Arabidopsis* appeared to be restricted to the host root at the junction with the tubercle (Fig. 5A and B). Although autofluorescence in tobacco roots presents a major challenge to this research because it interferes with visual detection of GFP, use of anti-GFP antibodies has enabled us to confirm appropriate expression of HMG2-regulated GFP in these tissues. In addition, *Orobanche* tubercles do not exhibit autofluorescence, so it is possible to examine the tubercles directly for the presence of GFP, which must be host-derived. This is done by squashing the normally opaque tubercles to reveal the presence of fluorescent compounds inside (Fig. 5C-E). Thus, we have observed fluorescence in tubercles growing on transgenic tobacco plants that have GFP targeted either for symplastic or apoplastic localization. The results (Fig. 5C-E) show that proteins the size of GFP can be translocated into *Orobanche* tubercles by symplasitic or apoplastic routes.

A complimentary strategy is using fluorescent dyes such as carboxyfluorescein to monitor movement from host to parasite. This relatively small molecule (0.5 kDa) moves rapidly through both the xylem and phloem tissues into *O. aegyptiaca* tubercles parasitizing *Arabidopsis* (Fig. 5). This research demonstrates that the parasite is a very strong sink on the host root system and readily absorbs molecules spanning the size range above and below that of sarcotoxin. Ongoing work will provide a more detailed picture of the final point of accumulation of these molecules inside the parasite.

In summary, this project has led to the development of the first genetically engineered *Orobanche*-resistant plants. Furthermore, the resistance is based on a novel mechanism that

involves only the expression of a single protein. Although the SLP-mediated resistance mechanism described to date does not provide complete protection, we have only begun to evaluate all of our transgenic lines and expect to find even more interesting lines. Although the mechanism of SLP action remains unclear, we are also making progress in this area. The SLP is toxic to germinating *O. aegyptiaca* seeds in Petri dish studies, and inhibits growth of *Orobanche* tubercles attached to SLP-expressing plants. The ability of the parasites to absorb and accumulate SLP may play a role in the mechanism and selectivity of the toxin between host and parasite.

Implications of this work will extend beyond *Orobanche*, to include potential strategies for reducing crop losses caused by other parasitic weeds such as *Striga* spp. and *Cuscuta* spp., and even other pathogens such as bacteria and fungi. Development of an effective resistance mechanism to *Orobanche* would be a clear benefit to Israel and other nations, including the US that are plagued or threatened by parasitic weeds. Furthermore, the strategy we are developing offers an alternative to the use of pesticides such as methyl bromide with its associated risks to human health and the environment.

Details of cooperation

This collaboration is based on the interdependent distribution of work. The initial phase of the project was conducted as a single team while Dr. Aly was on sabbatical (July 2000 – July 2001) at Virginia Tech, and during this time the constructs were made and plant transformations initiated. The resulting transgenic plants have been shared among the two teams to analyze cooperatively. Since that time both teams have worked on characterizing the *HMG2:SLP* plants, even conducting replicate experiments in Israel and the US. A specific example of the interaction is that Southern blots of the transgenic plants were conducted in Israel while SLP antibody development has been done in the US. This requires that results are shared frequently, which has been facilitated by frequent email contact. Other aspects of the project are more clearly separated as required by availability of facilities, such as the characterization of protein movement from host to parasite, being conducted at Virginia Tech where extensive fluorescence and confocal microscopy facilities are available. On the other hand, extensive greenhouse screening has been performed at Newe Ya'ar that would not be possible in the US due to quarantine restrictions.

This project has resulted in several co-authored research presentations, papers (in preparation) and one patent application.

These interactions have greatly contributed specifically to the progress made in this project and promoted the successful completion of the project, as planned. Two students, M.Sc. student (at Newe Ya'ar) and a Ph.D. student (at Virginia Tech) were involved in the research, greatly contributing to the project's success.

Table 1. Constructs and transgenic plants generated as of May, 2003.

Constructs made	Rationale	Transformation progress	
		Tobacco	*Arabidopsis*
HMG2:GFP	Cytosolic GFP (without signal sequence) will reveal protein movement via symplastic connections	21 lines	77 lines
HMG2:ps:GFP	ps signal sequence targets GFP to extracellular space to reveal apoplastic movement	14 lines	105 lines
HMG2:ss:SLP	Test efficacy of SLP for *Orobanche* resistance using endogenous signal peptide	11 lines	30 lines
HMG2:ss:SLP-HIS	As above but allows localization of SLP by the HIS tag; may also increase SLP stability	15 lines	Transformed
HMG2:ps:SLP	Replaces the fly signal peptide with the plant signal peptide	23 lines	Transformed
HMG2:ps:ss:SLP	Adds the plant signal peptide to that of the fly for targeting SLP to apoplast	26 lines	Transformed
HMG2:ps:SLP-HIS	As above but allows localization of SLP by the HIS tag; may also increase SLP stability	Lower priority; Ready for transformation if needed	
HMG2:ps:ss:SLP-HIS	As above but allows localization of SLP by the HIS tag; may also increase SLP stability	Lower priority; Ready for transformation if needed	

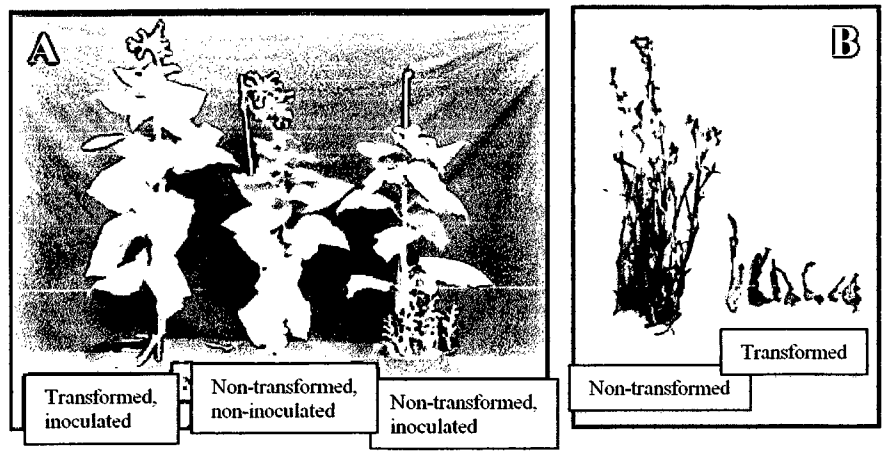

Figure 1. Effect of *tob:ss:SLP* (constitutive, root expressing promoter) construct on tobacco resistance to *O. aegyptiaca*.
A) Transformed plants grown in either non-inoculated or inoculated soil are compared to non-transformed control growing in inoculated soil. **B)** Shoots of *O. aegyptiaca* harvested from the non-transformed and transformed host plants.

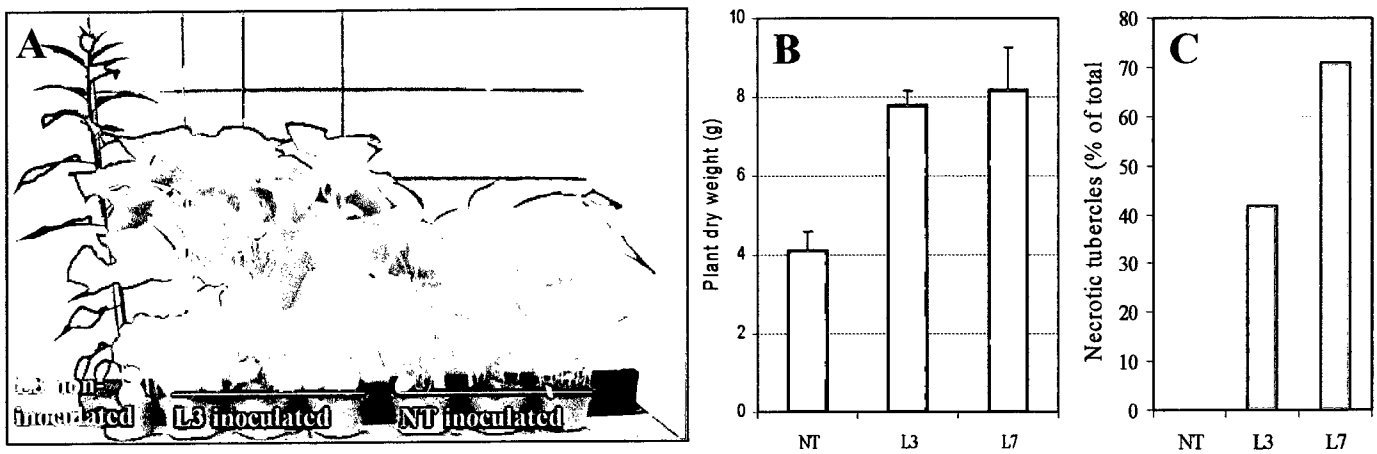

Figure 2. Growth and biomass accumulation of non-transformed (NT) and transgenic tobacco lines expressing the *HMG2:ss:SLP* transgene. **A:** Photo of L3 and NT plants. **B:** Quantification of results shown in (A). Mean dry wt. of tobacco shoots grown in soil inoculated with *O. aegyptiaca*. Bars are means of three observations with vertical lines indicating SE. **C:** Percentage of necrotic *O. aegyptiaca* tubercles growing on non-transgenic and transgenic tobacco in polyethylene bags. A and B are results from the US, while experiment in C was conducted in Israel.

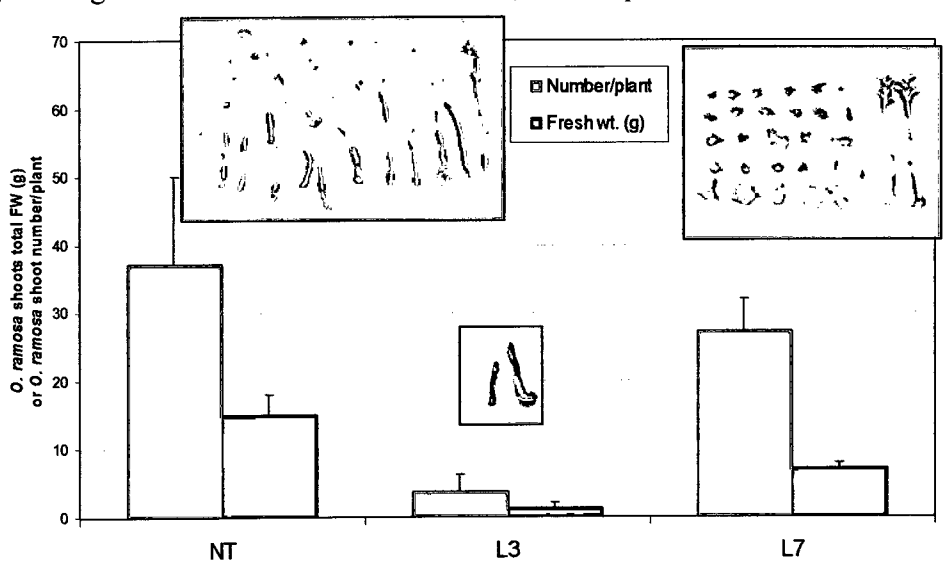

Figure 3. Number and fresh weights of *O. ramosa* plants growing on non-transformed and *HMG2:ss:SLP* transgenic tobacco. Plants were grown in inoculated soil. Data are means of 5 non-transformed (NT) and 4 (L3 and L7) replicates, with vertical bars representing SE. Insets illustrate the corresponding quantitative data. Note

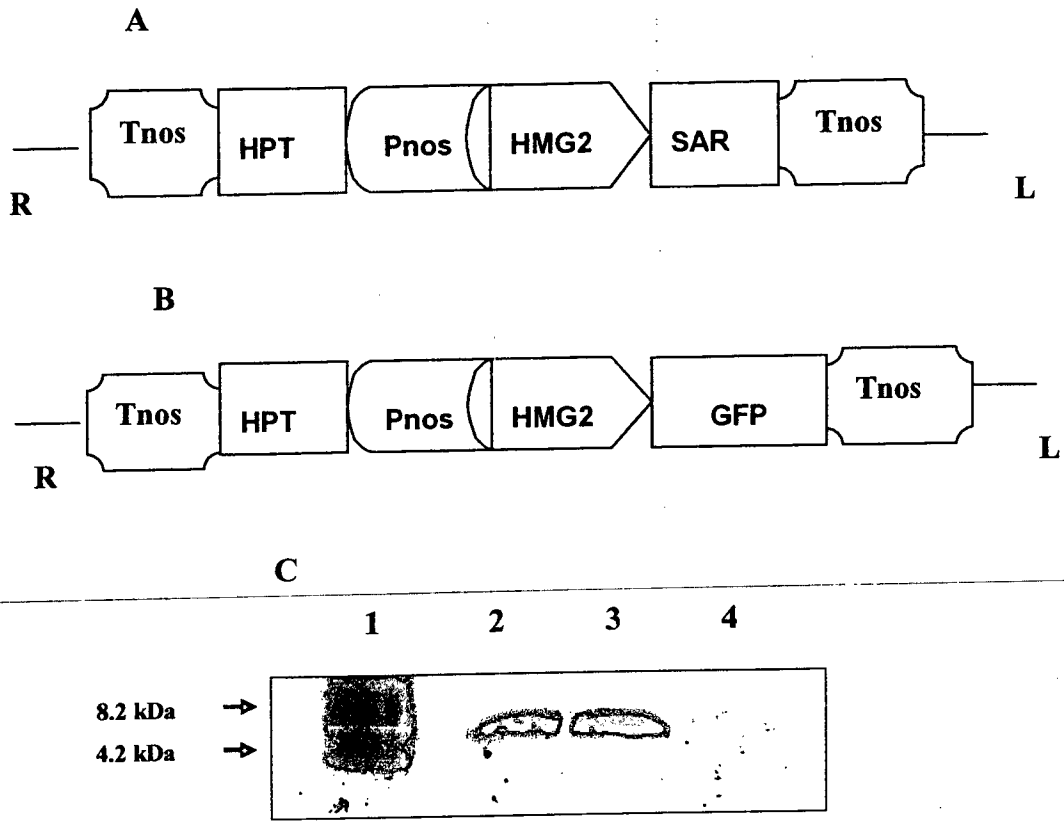

Figure 4. Structure of the expression vectors and Western blot analysis.
A, B: Schematic representation of sarcotoxin IA (SAR) and the green fluorescent protein (GFP) expression vectors respectively. Both genes, GFP – 0.7 kb. and SAR – 0.284 kb were fused under the control of a 3-hydroxy-3-methylglutaryl CoA reductase (HMG2) promoter and constructed into the binary vector pBIB. R and L, right and left border of T-DNA of the *Agrobacterium tumefaciens* Ti plasmid; HPT, coding sequence of hygromycin selectable marker; Pnos, the promoter sequence of *A. tumefaciens* nopalin synthase gene; Tnos, the terminator sequence of *A. tumefaciens* nopalin synthase gene. **C:** Western blot of putative tobacco transgene expressing sarcotoxin IA. Transgenic and non-transgenic tobacco plants were inoculated with *O. aegyptiaca* seeds in PEB. Ten days after inoculation, extracts were subjected to protein gel blotting with anti-sarcotoxin IA antibody.
Total proteins were extracted from roots of two independent transgenic tobacco lines (3 and 6) adjacent to *O. aegyptiaca* penetration site (lanes: 2,3) respectively and from non-transgenic control plants (lane: 4). Lane 1 (positive control), 40 ng of synthetic sarcotoxin IA peptide.

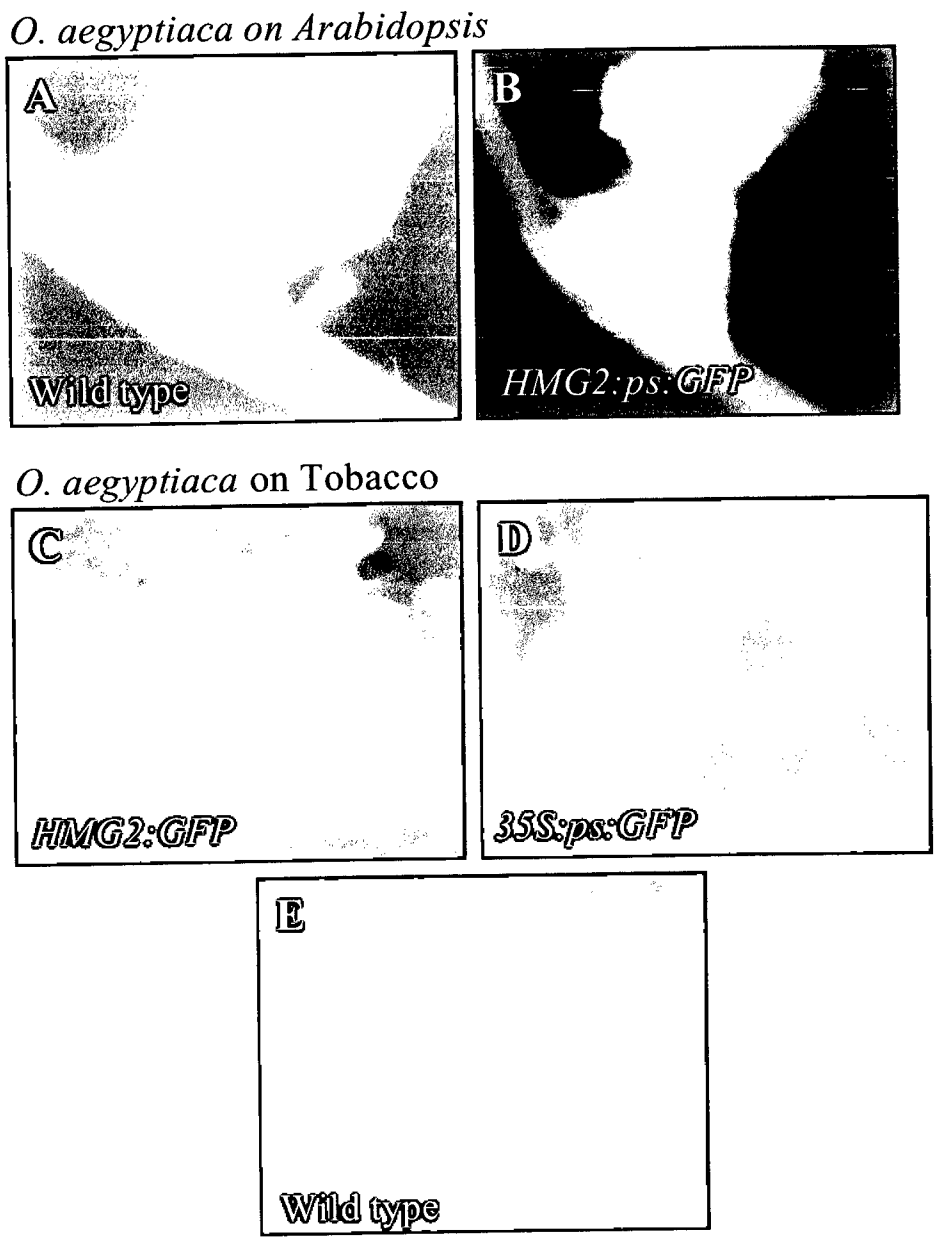

Figure 4? Translocation of GFP from host roots to *Orobanche*. Tubercles of *O. aegyptiaca* parasitizing transgenic *Arabidopsis* or tobacco were photographed using fluorescence microscopy. Tubercles parasitizing *Arabidopsis* show some autofluorescence on wild type plants (A), but it is distinct from those growing on plants expressing *HMG2:ps:GFP* (B). Tubercles are optically dense after they begin to grow, but do not show autofluorescence. Tubercles growing on transgenic tobacco lines with GFP targeted to cytoplasm (D) or apoplast (D) were squashed to reveal fluorescent contents. This fluorescence is not found in similarly treated wild type tubercles.

APPENDIX

Abstracts of presentations at professional meetings:

Aly, R., D. Plakhin, N. Hamamouch, C. L. Cramer, and J. H. Westwood. 2001. Expression of sarcotoxin IA like peptide enhances host resistance to *O. aegyptiaca* in transgenic plants. P. 101 in: A. Fer, P., Thalouarn, D. M. Joel, L. J. Musselman, C. Parker, and J.A.C. Verkleij, eds., Proc. 7th Intl. Parasitic Weed Symp., Faculté des Sciences, University of Nantes, France.

Aly, R., and D. Plakhine and J. H. Westwood . 2001. Broomrape (*Orobanche aegyptiaca* Pers.) control based on expression of cecropin in transgenic plants. WSSA Abstracts 41:129-130.

Aly, R. and D. Plakhin. 2001. Sarcotoxin IA enhances host resistance *Orobanche aegyptiaca* in transgenic Plants. European Cooperation (COST) 849 Meeting. Parasitic plant management in sustainable agriculture. Bari, Italy, Oct. 18-20.

Aly, R. and D. Plakhin. 2001. Sarcotoxin IA enhances host resistance *Orobanche aegyptiaca* in transgenic Plants. European Cooperation (COST) 849 Meeting. Parasitic plant management in sustainable agriculture. Sofia, Bulgaria, March 14-17.

Hamamouch, N, R. Aly, C. L. Cramer, and J. H. Westwood. 2002. Inducible expression of GFP as a tool to study protein movement across the host-broomrape (*Orobanche*) interface. WSSA Abstracts 42:20.

Hamamouch, N, R. Aly, C. L. Cramer, and J. H. Westwood. 2002. Inducible expression of GFP as a tool to study protein movement across the host-broomrape (*Orobanche*) interface. Moroccan Biologists Association, Third Annual Meeting, May 25-26, 2002, Johns Hopkins University School of Medicine.

Hamamouch, N, R. Aly, C. L. Cramer, and J. H. Westwood. 2002. Inducible expression of GFP as a tool to study protein movement across the host-broomrape (*Orobanche* aegyptiaca Pers.) interface. Plant Biotechnology 2002 and Beyond: 10th International Association for Plant Tissue Culture & Biotechnology Congress. P. 35-A.

Hamamouch, N., R. Aly, C. Cramer, and J. Westwood. 2003. Induced expression of sarcotoxin IA enhances host resistance against Egyptian broomrape (*Orobanche aegyptiaca* Pers.) WSSA Abstracts 43:52.

Hamamouch, N., R. Aly, C. Cramer, and J. Westwood. 2003. Visualizing the movement of host proteins to Egyptian broomrape using green fluorescent protein. Proceedings of the Northeastern Weed Science Society 57:3

Patents:

Inventor(s): Radi Ali (Volcani Center, Israel), James Westwood, and Carole Cramer (Virginia Tech).
Transgenic Plants Protected Against Parasitic Plants.
Publication date: 2002-11-28
Application No.: WO2002US22520 20020125

Manuscripts submitted, in preparation:

Aly R., Hamamouch N., Lerer, I., Plakhin D., Cramer C. & Westwood J. 2003. A gene from insect protect crop plants from parasitic weeds. (to be submitted to Nature biotechnology).

Aly, R., N. Hamamouch, C. Cramer, and J. Westwood. Growth of the parasitic plant *Orobanche aegyptiaca* is selectively inhibited by sarcotoxin, an antibitotic compound. (in preparation).

N. Hamamouch, R. Aly, C. Cramer, and J. Westwood. Macromolecule translocation from tobacco and Arabidopsis to the parasitic angiosperm broomrape.

Book chapters:

Goldwasser, Y., J. H. Westwood, and J. I. Yoder. April 4, 2002. The use of *Arabidopsis* to study interactions between parasitic angiosperms and their plant hosts. In C.R. Somerville and E.M. Meyerowitz, eds., The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, doi/10.1199/tab.0035, http://www.aspb.org/publications/arabidopsis/.

Westwood, J. H. Plant defense responses to parasitic weeds. Pp. 27-43 In: Biochemical & Molecular Responses of Plants to the Environment. 2002. Wood, AJ (Ed.) Research Signpost, Trivandrum India. pp127. ISBN: 81-7736-167-8

Westwood, J. H. Molecular aspects of host-parasite interactions: opportunities for engineering resistance to parasitic weeds. In Inderjit, ed., Principles and Practices in Weed Management: Weed Biology & Weed Management. Kluwer Academic Publishers, The Netherlands. In press.

REFERENCES

Aly, R., D. Granot, Y. Mahler-Slasky, N. Halpern, and E. Galun. 1999. *Saccharomyces cerevisiae* cells, harboring the gene encoding Sarcotoxin IA secrete a peptide that is toxic to plant pathogenic bacteria. Prot. Expr. Purif. 16:120-124.

Chappell, J. 1995. Biochemistry and molecular biology of the isoprenoid biosynthetic pathway in plants. Annu Rev. Plant Physiol. Plant Mol. Biol. 46.

Cramer, C. L., D. L. Weissenborn, C. K. Cottingham, C. J. Denbow, J. D. Eisenback, D. N. Radin, and X. Yu. 1993. Regulation of defense-related gene expression during plant-pathogen interactions. J. Nematol. 25:507-518.

Jaynes, J. M., G. R. Julian, G. W. Jeffers, K. L. White, and F. M. Enright. 1989. *In vitro* cytocidal effect of lytic peptides on several transformed mammalian cell lines. Pept. Res.:157-160.

Mahler-Slasky, Y., S. Galili, A. Perl, R. Aly, S. Wolf, D. Aviv, and E. Galun. 1996. Root directed expression of alien genes in transgenic potato: sarcotoxin and GUS. Pp. 187-192 *in* A. Altman and Y. Waisel, eds., Biology of Root Formation, N.Y.: Plenum Press.

Mitsuhara, I., H. Matsufuru, M. Ohshima, H. Kaku, Y. Nakajima, N. Murai, S. Natori, and Y. Ohashi. 2000. Induced expression of sarcotoxin IA enhanced host resistance against both bacterial and fungal pathogens in transgenic tobacco. Mol. Plant Microbe Interact. 13:860-868.

Nordeen, R. D., S. L. Sinden, J. M. Jaynes, and L. D. Owens. 1992. Activity of cecropin SB37 against protoplasts from several plant species and their bacterial pathogens. Plant Sci. 82:101-107.

Shai, Y. 1999. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochim. Biophys. Acta 1462:55-70.

Westwood, J. H., X. Yu, C. L. Foy, and C. L. Cramer. 1998. Expression of a defense-related 3-hydroxy-3-methylglutaryl CoA reductase gene in response to parasitization by *Orobanche* spp. Mol. Plant-Microbe Interact. 11:530-536.

Yang, F., L. G. Moss, and G. N. Phillips Jr. 1996. The molecular structure of green fluorescent protein. Nature Biotechnol 14:1246-1251.

Mol. Plant Microbe Interact. 13:860-868.