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Advanced Technologies for Parasitic Weed Control

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Parasitic weeds such as *Phelipanche* and *Orobanche* are obligate holoparasites that attack roots of almost all economically important crops in semiarid regions of the world. A wide variety of parasitic weed control strategies (chemical, biological, cultural, and resistant crops) has been tried. Unfortunately, most are partially effective and have significant limitations. The current mini review will discuss the needs for alternative methods and will summarize current and new biotechnology-based approaches for broomrape control. At present, we have generated transgenic tobacco plants expressing a cecropin peptide (*sarcotoxin IA*), under the control of the inducible *HMG2* promoter. Transgenic lines enhanced host resistance to the parasitic weed; transgenes showed higher numbers of aborted parasitization events, reduced *Phelipanche* biomass, and increased host biomass. *Sarcotoxin IA* had no obvious effect on growth and development of transgenic host plants. Mannitol content in the parasite is regulated by Mannose 6-Phosphate Reductase (*M6PR*) gene, an essential process to broomrape species for water and nutrient uptake from the host. In our study, we used the inverted repeat technique to silence the parasite target gene, *M6PR*. In this study it was shown that the endogenous *M6PR* mRNA from *P. aegyptiaca* tubercles or shoots grown on transgenic tomato plants harboring the *M6PR* silencing construct was reduced by 60 to 80%. The number of dead tubercles was also increased significantly on transgenic plants as compared with the control plants. The strategies presented here are potentially superior to other methods in that they are effective, have a low cost of implementation for producers, and are safe for the environment.

Nomenclature: *Phelipanche aegyptiaca* Pers. (syn. *O. aegyptiaca*) ORAAE; tomato, *Solanum lycopersicum* Mill.; tobacco, *Nicotiana tabacum* cv. Xanthi.

Key words: Transgenic tobacco, transgenic tomato, parasitic weeds, *Sarcotoxin IA*, Mannose 6-Phosphate Reductase.

About 20 families (3,000 to 5,000 species) of higher plants contain species that are parasitic on other members of the plant kingdom, and may cause production losses of 30% to 80% in staple food and industrial crops. Broomrapes (*Phelipanche* and *Orobanche* spp.) are obligate holoparasites that attack roots of almost all economically important crops in semiarid regions of the world (Joel et al. 2006). Annual food crop losses from these weeds in the Middle East can be conservatively estimated at $1.3 billion to $2.6 billion (Aly 2007). Unlike other weeds, parasitic weeds are directly connected, through haustoria, to the vascular system of the crop plants (Joel and Portnoy 1998) that serve as their hosts, and successfully compete for host nutritional resources. The parasite develops underground and exerts the greatest damage prior to its emergence; therefore, the majority of field loss may occur before diagnosis of infection.

The broomrapes include more than 100 species, with five (*P. aegyptiaca* (Wallr.), *P. ramose* (L.), *O. minor* (Sm.), *O. cumana* (Pers.), *and O. crenata* (Forsk.)) being considered significant parasites of crops including members of the Solanaceae, Fabaceae, Compositae, Cruciferae, and Umbelliferae families (Parker and Riches 1993).

Broomrape seeds persist in soil for many years, often for decades, and its management is in most cases impossible, due to the lack of sources for resistances and suitable selective herbicides for most of the susceptible crops (Joel et al. 2006; Pérez de Luque et al., 2009). Having no effective means to control the parasite in the majority of the crops, and anticipating the development of herbicide-resistant broomrapes and new broomrape races that may successfully attack resistant crops, there is an immediate need to explore innovative approaches and technologies to control the parasite. In this mini review, we will briefly discuss conventional control methods and progress made recently to develop innovative technologies to parasitic weed control.

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Conventional Broomrape Control

Although the simplest and most effective approach to parasitic weed control—host resistance—remains an unrealized goal for agriculture, a wide variety of conventional methods (chemical, biological, cultural, and resistant crops) have been tried. Unfortunately, most are only partially effective and have significant limitations (labor intensive and time consuming) (Aly 2007). The best long-term strategy for controlling parasitic weeds may be through the identification and breeding of resistant genotypes (Cubero and Hernández 1991; Ejeta et al. 1991). Despite many years of work by plant breeders, resistant cultivars of only a small handful of crops are available. A limited number of cases of genetic resistance against broomrape have been found and have been bred into sunflower and a few legumes (Labrousse et al. 2001; Pérez -Vich et al. 2004; Rubiales 2003). However, most genetic resistance mechanisms were successively overcome by new races of *O. cumana* (Antonova 1998; Fernández-Martínez et al. 2000; Rubiales 2003). Parasitic plants could not be effectively controlled by application of herbicides (Foy et al. 1989). Very few herbicides are selective enough to be applied in the field against *Striga* (Kanampiu et al. 2003) or broomrape (Aly et al. 2001; Goldwasser and Kleifeld 2004). The herbicides that are currently in use for broomrape control are glyphosate (inhibitor of EPSP synthase), imidazolinones, and sulfonylureas (inhibitors of acetolactate synthase, ALS) (Aly et al. 2001; Eizenberg et al. 2004; Garcia-Torres et al. 1995; Goldwasser and Kleifeld 2004). Recently a high number of weeds have developed resistance against herbicides especially against ALS inhibitors, triazines, and glyphosate (Ian Heap, http://www.weedscience.com). One ALS inhibitor, sulfosulfuron, has recently been licensed in Israel for use against broomrape in tomato fields, but it is only partially effective, and its residues may damage subsequent crops. The lack of new sources for resistance limits

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Table 1. Selected engineered crops allowed in the market.[a]

Engineered crop	Producer (company)	Trait and function
Canola	Bayer	Glufosinate herbicide resistant for weed control
Canola	Monsanto	Altered oil for soap and food products
Corn	Bayer	Glufosinate herbicide resistant for weed control
Corn	Monsanto	*Bacillus thuringiensis* (Bt) toxin to control insect pests (European corn borer)
Corn	Monsanto	Glyphosate herbicide resistant for weed control and Bt toxin to control insect pests
Cotton	Monsanto/Bayer	Bromoxynil herbicide Resistant to control weeds
Cotton	Mycogen/Dow	Bt toxin to control insect pests/resist glufosinate herbicide to control weeds
Papaya	Cornell University/Hawaii University	Resistant to papaya ringspot virus
Potato	Monsanto	Bt toxin to control insect pests (Colorado potato beetle)
Rice	Bayer	Glufosinate herbicide resistant for weed control
Soybean	Monsanto	Glyphosate herbicide resistant for weed control
Soybean	DuPont	Altered oil to increase stability, reduce polyunsaturated fatty acids
Sugarbeet	Bayer	Glufosinate herbicide resistant for weed control
Tomato	Monsanto	Altered ripening to enhance fresh market value

[a] Sources: U.S. Department of Agriculture, U.S. Environmental Protection Agency, and U.S. Food and Drug Administration.

our ability to cope with the newly developing, more virulent, *O. cumana* races. Furthermore, the transfer of available resistances from a resistant crop (like sunflower) to other crops is currently impossible. In fact, the best control method used in Israel is fumigation with methyl bromide, which kills seeds in the soil (Goldwasser and Kleifeld 2004; Jacobsohn 1994). This method, however, is expensive, laborious, and it was being phased out because it is extremely hazardous to the environment.

The Need for Innovative Technologies

The nature of parasitic weeds makes control extremely difficult by conventional means due to their life style: parasites are concealed underground, intimately involved with the host roots and undiagnosed until they irreversibly damage the crop. Broomrape has so much metabolic overlap with the host that selective treatments are very difficult to develop.

Biotechnology in agriculture encompasses a wide range of technologies and they can be applied for a range of different purposes, such as genetic improvement and modification of plant resistance varieties in order to increase their yields.

Since 1980 the world is facing food crisis and challenges particularly for the rural poor populations (http://www.fao.org/biotech/abdc/). Nearly a billion people worldwide don't have reliable access to food, according to United Nations estimates, and the situation is even worse in East Africa because over 11 million people there are at risk of starvation. This is further threatened by climate change because climate models predict that extreme weather events, like droughts and floods, will become more common in the future, threatening farmers' ability to produce reliable harvests. Recently, the area under agriculture has declined as well as the rate of growth in agricultural productivity, while the demand for food continues to escalate (http://www.fao.org/biotech/abdc/). Therefore, innovative technologies are needed to produce more food and energy.

Current Uses of Biotechnology in Agriculture

The global area of biotech crops has increased since 1996. The most widespread application of genetic engineering in agriculture is in engineered crops such as soybean, maize, cotton, rice canola, tomato, and potato. The traits most commonly introduced into crops are herbicide tolerance, insect tolerance, and virus tolerance (Table 1). Since 2008, more than 90 and 45% of the consumed soybean or maize, respectively, were commercialized as engineered crops. Genetically modified organisms have a great potential to address challenges in our constraint global food and agricultural. However, we need to move carefully, with a full understanding of all factors involved including biosafety, food quality, security, and sustainability.

Because a high number of genes have been isolated and are available for transfer to other crops, the global area of biotech crops is growing daily. Currently, the technology is used primarily to modify crops, although a number of other applications are in the pipeline such as vaccine development, genetic improvement of plant varieties, and animal populations to increase their yields or efficiency.

New Approaches to Control Parasitic Weeds Based on Biotechnology

Genomic research on root parasites is likely to help in an overall understanding of some key aspects of parasitism. Along with the model parasite *Triphysaria versicolor*, which serves as valuable sources of genomic understanding of host–parasite interaction (Yoder 1997), other model plants such as *Arabidopsis thaliana* (L.) Heynh. and *Medicago truncatula* (Gaertn.) have been used for studies on host reactions to parasitic plant infection (Rodríguez-Conde et al. 2004; Westwood 2000). Development of effective genetic engineering strategies for resistance to parasitic weeds require: (1) Identification of genes whose products selectively inhibit the parasite growth or silence a key target gene of the parasite; (2) Capability of the transgene products or signals to move from host to parasite.

The development of herbicide-resistant crops has recently offered another broomrape control approach, based on herbicide translocation through the host to the parasite (Joel et al. 1995; Surov et al. 1998). However, this approach depends on commercial availability of herbicide-resistant crops, requires correct chemical application, and may be countered by the development of herbicide-resistant populations of the parasite (Gressel et al. 1996). In our laboratory we used two biotechnological approaches to control *P. aegyptiaca*.

***Phelipanche* Control Based on Inducible Expression of Cecropin in Transgenic Plants (Aly et al., 2006; Hamamouch et al. 2005).** A novel strategy was designed to enhance

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host resistance to *Phelipanche* based on parasite-induced expression of a selective sarcotoxin IA polypeptide. Sarcotoxin is an antimicrobial polypeptide of the cecropin family, produced by the flesh fly *Sarcophaga peregrina* (Kanai and Natori 1989). Initial studies indicated that sarcotoxin IA peptide overproduced by yeast (*Saccharomyces cerevisiae*) inhibited *P. aegyptiaca* seed germination and radicle elongation (Aly et al. 1999). Based on this study it was proposed to increase the efficacy of sarcotoxin-producing plants by regulating its expression with the *HMG2* promoter. The *HMG2* gene is involved in isoprenoid biosynthesis pathway and is activated specifically during defense responses (Cramer et al. 1993). The *HMG2* promoter is specifically induced in host roots around the site of *Phelipanche* penetration (Westwood et al. 1998) and encodes a protein associated with phytoalexin and sesquiterpene production.

Transgenic tobacco plants harboring the *sarcotoxin IA* gene, under the regulation of the *HMG2* promoter showed enhanced resistance to *Phelipanche* resulting in higher numbers of aborted parasitization events, reduced *Phelipanche* biomass, and yielded greater host biomass following parasite inoculation, compared to nontransgenic controls (Hamamouch et al. 2005). Aly et al. (2006) showed enhanced resistance to *P. aegyptiaca* when the *sarcotoxin IA* gene was linked to the constitutive root-specific *Tob* promoter to generate sarcotoxin-expressing tomato plants. The parasites were able to attach to the host root but grew abnormally with increased mortality. In the absence of *Phelipanche*, growth and development of transgenic tomato expressing *Tob:sarcotoxin* was equal to that of nontransgenic plants, suggesting that sarcotoxin peptide was not detrimental to the plants. However, this transgenic resistance to *Phelipanche* was incomplete and did not provide adequate protection, possibly due to instability of sarcotoxin peptide produced in transgenic plants. Therefore, protein stability may be the most critical limiting factor, because sarcotoxin IA peptide is subject to rapid degradation in plants from extracellular proteases (Okamoto et al. 1998). More research is needed to understand the mechanism of sarcotoxin IA selectivity toward *Phelipanche* and optimize this mechanism for engineering parasite-resistant crop species.

***Phelipanche* Control Based on Silencing of a Key-Target Gene in the Parasite (Aly et al. 2009).** Gene-silencing has already been shown to effectively control nematodes (Atkinson et al. 2003; Huang et al. 2006) and viruses (Prins et al. 2008). The introduction of double-stranded RNA (dsRNA) is a powerful tool for suppressing gene expression through a process known as post-transcriptional gene silencing in plants (Voinnet 2008). dsRNA can be produced in vivo by host or virus-encoded RNA-dependent RNA polymerases and by transcription either through promoters or through inverted repeats and RNA synthesis of sense or antisense orientation. In all these cases, the main element in the silencing process is a small RNA molecule, a short interfering RNA that facilitates sequence-specific gene suppression by degradation of the target mRNA.

Parasitic weeds such as *Phelipanche* accumulate high amounts of mannitol during their development. Mannitol content in the parasite is regulated by M6PR, an essential process to *Phelipanche* for water and nutrient uptake from the host (Delavault et al. 2002). In our study, we suggested to control *Phelipanche* by engineering a potential host (tomato) to produce a systemic signal to effect silencing of critical metabolic activity in the parasite. This was accomplished by expression of the M6PR-silencing inverted-repeat construct in the host plant (Aly et al. 2009). Our results showed that the endogenous M6PR mRNA from *P. aegyptiaca* tubercles or shoots grown on transgenic tomato plants harboring the M6PR silencing construct was reduced by 60 to 80%. Concomitant with M6PR mRNA suppression, the number of *Phelipanche* dead tubercles was significantly increased on transgenic lines and a significant decrease in mannitol level was measured in the parasite tissue grown on transgenic plants as compared with the control plants (Aly et al. 2009). The results of these experiments indicate that the silencing of the target gene M6PR was incomplete. We hypothesize that a small amount of the silencing signal was produced and transferred from the host roots to the parasite organs. Therefore, amplification and accumulation of the RNAi signal in the transgenic host roots seems to be a threshold for the success of the silencing strategy.

Conclusions

Considering the importance of parasitic weeds to world agriculture and the difficulty in obtaining resistance by conventional methods, the developing of strategies-based biotechnology is urgently needed. Optimal parasitic weed control could be achieved by either the use of parasite-resistant crops (from conventional breeding), or by crops genetically engineered for resistance. So far only a few crop varieties with stable resistance have been developed after decades of conventional plant breeding and unfortunately, genetic resources for resistance genes are rare. Over 1,000 lines of tobacco (and related species and mutants) have been screened for resistance to *P. aegyptiaca*, *P. ramosa*, and *O. cernua* over the past 30 yr with little success (Alonso 1998). In this context, the resistance to *Phelipanche* of the sarcotoxin IA-expressing plants is remarkable because it was conferred by the addition of just a single gene (Hamamouch et al. 2005).

Until recently, genomic resources associated with resistance to parasitic weeds due to RNA interference were sparse. Beside the great progress made in the Parasitic Plant Genome Project (http://ppgp.huck.psu.edu/) (Westwood et al. 2011), genetic resistance based on silencing of a target gene in the parasite is now feasible. This should facilitate identifying key- parasite genes to target via the silencing technology. However, genetic resistance based on the silencing of a key gene in the parasite has already been demonstrated (Aly et al. 2009; Tomilov et al. 2008; Yoder et al. 2009).

Unlike other resistances, the gene silencing approach could be applied to various other susceptible crops, not necessarily of the same plant family. Also, this method could be effective against a broad spectrum of broomrape species and will have implications for the control of other plant parasites and pathogens. In addition, multiple silencing constructs can be pyramided in susceptible hosts, and this new biotechnology could be combined with genetic or tolerant resistances, thus significantly reducing the risk of the development of new virulent races of the parasite. Silencing of multiple loci is technically feasible by making chimeric constructions, and currently there are many potential parasite genes to target (http://ppgp.huck.psu.edu/).

Although, there is still a need for further research into the mechanisms involved in the translocation and regulation of

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macromolecules within host–parasite interactions, advantages of biotechnological approaches will include greater efficiency, reducing labor and lowering expenses, increasing cropping choices, and eliminating the need for chemicals that may be harmful to the environment. By this we wish to ensure reduction of broomrape damage and a significant gain in yields to the benefit of the farmers and the industries involved.

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