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# Agricultural Genomics and Subterranean Plant-Plant Communications

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**Abstract** Agricultural genomics has the potential to dramatically enrich the availability and quality of food supplies worldwide. However, because thousands of different plant species are grown for food, the application of genomics to crop improvement faces issues distinct from those in medical research. The challenge to agricultural plant scientists is to exploit the databases being generated for rice, maize, and *Arabidopsis* toward the genetic improvement of non-model crop species. The work in our lab illustrates one example of how genomic approaches can be applied to a non-model plant. Our overall goal is to understand how roots of different plants interact and use this information to improve the subterranean performance of crops in relation to weeds. The most obvious manifestation of root-root interactions is haustoria development. Haustoria are parasitic plant-specific organs that invade host plants and rob them of water and nutrients. Parasitic members of the Scrophulariaceae develop haustoria *in vitro* when exposed to molecules released by host roots. This is a useful phenotype for investigating plant-plant interactions because it is rapid, highly synchronous, and strictly dependent on exogenous haustoria-inducing factors (HIFs). Using a PCR-based subtractive hybridization, we cloned several hundred cDNAs representing transcripts one to two orders of magnitude more abundant in the roots of a parasitic plant after HIF exposure. Putative functions for about 90% of these transcripts could be assigned by searching the public databases. These have been arrayed on nylon filters and interrogated with a variety of probes from different parasitic and nonparasitic plants. Results from these experiments allowed us to identify likely candidate genes for the perception and processing of root signals by neighboring plants. J. Cell. Biochem. 80: 203–207, 2000. 

Almost exactly 150 years ago, following the discovery of gold at the site of Sutter's Mill in California, the Sierra Mountains became ground zero for a worldwide gold rush. Fortunes in gold were to be made and a hearty band of entrepreneurs from various walks of life answered the call and transformed themselves into gold miners. The symbolism of tough risk-taking miners searching for gold is all too appropriate to the task of mining sequence data being generated by the Human Genome Project and other sequencing efforts. Large public and private sequencing projects will continue to release genomic data for several major model organisms. Databases archived into public and private data warehouses are the streambeds from which nuggets can be harvested by those able to mine the resources.

How can genomic databases be exploited toward the improvement of agricultural crop species? One of the key attributes of agricultural plant biology is the huge number and diversity of plant species of interest. Thousands of species are grown worldwide for use as food, feed, or fiber. There are approximately 350 different plant crops (with a combined yearly value of $26.8 billion for 1997) produced in California alone [CDAF, 1999]. Of these, only rice is being studied at a full genomics scale. In addition, numerous plants that are not yet cultivated are known to produce secondary metabolites of medicinal, nutritive, or commercial interest. The ability of many plants to be readily transformed with foreign genes further expands the pool of germplasm available for crop improvement. Large-scale sequencing or expression profiling of the enormous number of potential crops is clearly not economically feasible or practical at this time. Therefore cross species comparative studies are critical in order to mine the available genomics data for the improvement of most crop plants.

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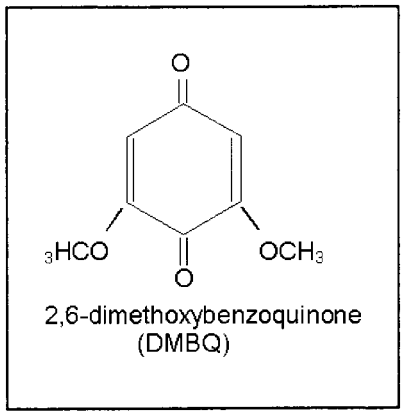
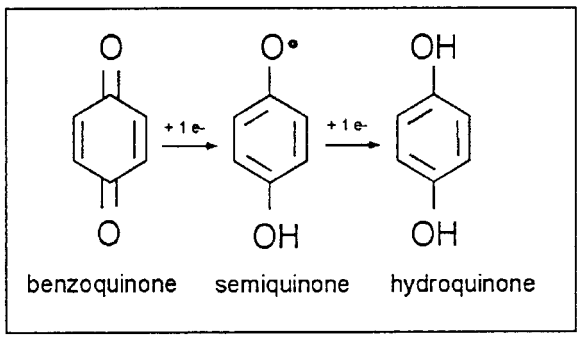
## Parasitic Plants as a Model for Subterranean Plant-Plant Interactions

Vegetation management is the leading constraint in agriculture worldwide. Weeds are the most universal agricultural pests and their control is a critical component of virtually all agricultural systems. Weeds are typically controlled by physically removing the offending plants or by the application of chemical herbicides. Both of these alternatives are expensive with regard to human labor, energy input, and chemical dependency. More time and money is spent controlling weeds than on any other agricultural pest and herbicides are by far the most commonly used agricultural chemical. Biological approaches are needed for controlling unwanted vegetation in managed ecosystems. We predict that by better understanding how plants interact and compete with one another underground we will be able to develop novel control strategies for biologically controlling weeds.




Haustorium development is probably the most obvious phenotype resulting from a subterranean plant-plant interaction. Parasitic plants use haustoria to attach to and invade host roots, forming a vascular connection through which they rob the host of fluid and nutrients [Kuijt, 1969]. In this rudimentary form of communication between plants, molecular recognition signals are exchanged and processed [Estabrook and Yoder, 1998]. Recognition signals belong to a broad class of small organic molecules, known as haustorium-inducing factors (HIFs). The first HIFs identified were the phenylpropanoids xenognosin A and xenognosin B [Lynn et al., 1981]. A simple quinone, 2,6-dimethoxybenzoquinone (DMBQ), was later identified as the active HIF in sorghum roots [Lynn and Chang, 1990]. DMBQ is a common constituent of plant cell walls [Handa et al., 1983] and is released into the rhizosphere as root exudates, as a breakdown product of lignin, or converted from phenolic acids by enzymatic digestion [Siqueira et al., 1991]. Various other quinones, flavonoids, *p*-hydroxy acids, and anthocyanidins induce haustoria when applied to parasite roots *in vitro* [Albrecht et al., 1998; Riopel and Timko, 1995; Smith et al., 1996]. The finding that active quinones have similar redox potentials while those with redox potentials outside a narrow, 300 mV window are inactive was critical to understanding how diverse structures can induce haustorium development [Smith et al., 1996]. HIFs are active if they have the appropriate electrophilic potential to complete a redox circuit with the parasite receptor. The study of HIF inhibitors such as tetrafluorbenzoquinone and cyclopropyl-*p*-benzoquinone established that one electron cycling between quinone and semiquinone forms is critical for HIF perception. These studies suggest that HIF perception and early signal transduction involves an oxidoreductase reaction between the inducing agent and parasite receptor.

There are similarities between HIF recognition and the induction of phase 2 xenobiotic




detoxification enzymes in mammalian cells. Phase 2 enzymes are induced by a variety of electrophilic and anticarcinogenic agents, all of which have the appropriate electrophilic potentials to serve as Michael reaction acceptors [Prestera et al., 1993]. Transient expression experiments identified electrophile-responsive/antioxidant-responsive elements (EpRE/ARE) present in phase II promoters that confer antioxidant inducibility [Prestera and Talalay, 1995].

We have developed an in vitro method for inducing haustoria in the parasitic plant *Triphysaria*. *Triphysaria* is a parasitic Scro-

phulariaceae that grows as a common annual throughout the Pacific Coast from Baja to British Columbia where it occupies diverse geographic regions [Hickman, 1993]. *Triphysaria* (previously *Orthocarpus*) is a small genus of cross-hybridizing diploids within the subtribe Castillejinae [Chuang and Heckard, 1991]. *Triphysaria* have perfect flowers each capable of setting about 50 seeds; a single plant will produce hundreds of seeds with a generation time of 3–4 months. Above ground *Triphysaria* looks like a typical grassland wildflower and only by examining its roots is its parasitic nature evident. Roots of field-grown *Triphysaria* can have hundreds of secondary haustoria, so named because they initiate proximal to the root tip in contrast to primary haustoria, which are terminal meristem differentiated [Kuijt 1969]. Like other facultative parasites, *Triphysaria* has a broad host range. Field studies showed that *Triphysaria* associates with members of at least 27 monocot and dicot genera, including *Arabidopsis* and *Zea* [Thurman, 1966]; [Estabrook and Yoder, 1998]. Indeed *Triphysaria* seems to parasitize most plants, even those it would not normally encounter in a natural setting. There is, however, an important exception: *Triphysaria* rarely parasitize other *Triphysaria*. Self and conspecific discrimination is manifested *in vitro* at the level of haustorium development and sibling *Triphysaria* almost never develop haustoria against each other [Yoder, 1997]. The mechanism by which *Triphysaria* discriminates itself from other plants is unknown.

When *T. versicolor* is exposed to maize root exudates or DMBQ, there is an almost immediate cessation of root elongation. Within 5 h, the initiation of haustorial hairs just behind the root tip can be observed. At about the same time, the region underlying the proliferating hairs begins to swell. Sections through the developing haustoria show the bulge is initially formed by an isodiametric expansion of cortical cells and to a lesser extent by new cortical cell divisions. After about 12 h, the root tip reverts from haustorium development to its typical growth program and a normal root grows out of the haustorium. This results because the roots acclimate to the continued presence of HIF and not because the HIF breaks down. The swelling and proliferation of haustorial hairs continues for about 24 h, during which time the haustorium is competent to attach to a host root. In *T. versicolor*, approximately 70–80% of the root tips form haustoria while virtually none develop when similarly treated with water.

In addition to serving as useful models of subterranean interactions, understanding how parasitic plants recognize host plant signals may have specific implications to worldwide agricultural pest management. Parasitic plants can be devastating agricultural pests. In Africa, over two-thirds of the 73 million hectares cultivated in cereals and legumes are infested with *Striga* [Lagoke et al., 1991]. The FAO estimates that the lives of over 300 million Africans in 25 countries are threatened by crop losses by *Striga*. *Orobanche*, a close relative of *Striga*, plagues agriculture in Mediterranean and Middle East regions and has repeatedly been introduced into the United States [Jain and Foy, 1989]. Dwarf mistletoe (*Arcethobium*) is estimated to destroy up to 3.2 billion board feet of lumber per year in western U.S. forests [Johnson et al., 1981]. Leafy mistletoes (*Phorodendron*) and dodders (*Cuscuta*) are also significant agricultural pests [Parker and Riches, 1993]. Understanding the genetic mechanisms governing host recognition and haustorium development should enable us to develop novel strategies for engineering host resistance against parasitic weeds.

### Genomics of the Parasitic Plant *T. versicolor*

We constructed a cDNA library using subtractive hybridization with total RNA isolated from DMBQ induced and noninduced *T. versicolor* root tissue. We single-pass sequenced and assigned putative functions to a subset of DMBQ induced transcripts using BLAST searches on public databases. Over 100 different early DMBQ induced (EDI) cDNAs were identified that are one to two orders of magnitude more abundant in response to DMBQ exposure. We could assign functions to over 90% of the transcripts by homology comparisons to sequences in the public databases. Using this information, we have been able to identify candidate genes likely involved in HIF signal perception and transduction. One of these, TvQR1, has homology to a quinone oxidoreductase cloned from *E. coli*. The crystal structure of the *E. coli* enzyme has been determined [Thorn et al., 1995]. Using the Swiss Model program available through ExPASy (http://www.expasy.ch/), we have been able to thread most of the TvQR1

protein into the known structure [Guex and Peitsch, 1997]. This has allowed us to make very detailed predictions about the structural and biochemical properties of the *T. versicolor* enzyme. A homologous gene also encodes a crystallin in the eyes of some vertebrates [Rao et al., 1992]. The crystallin has been particularly well studied in guinea pigs because it is responsible for congenital cataracts [Rodriguez et al., 1992]. These studies suggest a regulatory role of the *T. versicolor* gene in haustorium development. Therefore sequence comparisons with genes from E. coli and guinea pigs allows us to generate very specific, testable hypotheses about mechanisms regulating plant-plant interactions in the soil. Such predictions would have been impossible to make in the pregenomics era.

We constructed a cDNA array on a nylon membrane and interrogated the array using normalized probes derived from parasite and nonparasite roots before and after DMBQ exposure. The results from these interrogations demonstrate that DMBQ induces the transcriptional activation of many genes differentially between the parasite and non-parasite species. We think it is unlikely that DMBQ directly induces hundreds of genes, but rather interacts with a regulator that coordinately activates gene expression. These studies together with the sequence comparisons allowed us to identify candidate regulators.

### The Role of Informatics

We are extending the sequence and transcription profiling to about 5,000 root cDNAs differentially expressed after exposure to rhizosphere signals. Clearly, informatics will be increasingly critical to these analyses. Informatics applications will be applied to three main areas to facilitate our research. We are developing a website that contains interactive DNA sequence analysis modules for use by our research group. We are developing a root transcript database accessible on the Internet. We are implementing existing software applications for cluster analysis and display of early DMBQ induced gene expression profiles. Using limited and existing resources for Informatics, we are developing utilities on three (and possibly four) different operating system platforms connected by a departmental LAN. Raw DNA sequence data files are trimmed by Macintosh Sequencher software that interfaces a Perkin-Elmer AB 377 sequencer. These files are transferred by ftp over the campus network to a UNIX partition on a SUN server and to a Windows PC.

We have implemented a local BLAST search engine on our UNIX partition and converted our root transcript collection to a BLAST-searchable format. We have performed a pairwise alignment of the transcript collection against itself in order to locally identify and remove redundancies in this and future subsets of root transcript sequences. We have also implemented intelligent clients, such as the BCM Search Launcher [Smith et al., 1996], that execute BLAST comparison searches of public databases over the Internet using multiple query sequences. Therefore we are able to monitor the most significant hits to the root transcript collection among the most current sequence submissions to public databases.

We are using the AceDB database framework to store EST sequences, annotate links to GenBank, and store images of gene expression arrays [Thierry-Mieg and Durbin, 1999]. To cluster the array data, we will evaluate the Cluster software [Eisen et al., 1998] and the GeneCluster package [Tamayo et al., 1999], both of which are Windows compatible and available for download over the Internet. By identifying genes that transcribe synchronously we believe we can dissect interacting genetic elements that underlie the developmental program of haustorium formation in the roots of parasitic plants.

## CONCLUSIONS

Our group is applying functional genomics to the identification of the genetic elements involved in subterranean plant-plant signaling. Since the phenotype we are investigating, haustorium development, does not occur in *Arabidopsis* or rice, we need to use non-model organisms for these studies. Our approach combines subtractive hybridization to identify a subset of interesting transcripts, homology searches with ESTs differentially abundant during distinct signal perception and transduction, and transcript profiling. This allows us to tap into existing database resources without large-scale sequencing of our experimental organism.

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