

# Research Thrusts

## ➤ Molecular Technologies for Plant Resistance to Nematode Pests

Soybean Cyst Nematode (SCN) is the most important pest of soybeans in the U.S., with losses ranging from 3-45% in infested fields. Annual losses to SCN in U.S. soybean fields have been estimated at \$267M (Doupnik [1993] Plant Disease 77:1170-71). Other estimates are three times that figure. Our guiding principle in managing this pest has been to provide our farmer clientele with rapid assistance by deploying highly adapted resistant germ plasm of immediate benefit to the industry. Simultaneously, we are doing molecular research geared toward eventual exploitation of cloned genes using plant transformation and other state-of-the-art techniques.

- Virulence varies among geographically separated SCN soil populations, and none of the currently available resistant varieties provides satisfactory protection across wide geographic areas. MPRINT nematologists in Entomology have collaborated with MPRINT molecular geneticist R. A. Vierling to use molecular markers plus unique and replicated protocols for phenotypic screens in order to incorporate superior broad-based SCN resistance (derived from the agronomically undesirable 'Hartwig' cultivar) into adapted resistant germ plasm. Our unique germ plasm has good agronomic characteristics as well as resistance to SCN that is effective against all field populations known in the U.S. No other laboratory has accomplished this feat.

- Our utility patent-protected resistant germ plasm, with the name and logo CystX, has been deployed via an Indiana seed broker firm, Access Plant Technology, Inc. It is currently in use by many breeders in Indiana and elsewhere,

and will also be licensed as a branded cultivar, owing to pressure from seed companies and growers who have seen its performance in commercial test plots. The CEO of Access Inc., Mr. John Gerard, stated in an open letter dated 12/31/97, "The effort by commercial soybean breeders to capture the Hartwig cyst resistance has been extensive and fruitless. . . . The Purdue/ICIA cyst technology is crucial in obtaining elite, high yielding varieties with Hartwig SCN resistance." Use of our adapted resistant germ plasm has the potential for increasing farmer profit \$21-\$35/A.

- Work is underway to characterize and clone the particular SCN resistance genes that are in our adapted germ plasm. In collaboration with researchers at the Los Alamos National Laboratory, we have undertaken a cloning effort using novel "gene mining" techniques that utilize software developed at Los Alamos. With Dr. Paul Erhardt and Dr. Steve Goldman, at the Medical College of Ohio, we are using HPLC-MS to look at differences in constitutive and induced small molecules in proteins. A novel patented technique for tagging proteins that employs vegetative tissue in insertional mutagenesis is being explored with Dr. Randy Nelson, who owns an Arizona company, Intrinsic Bioprobes. Dr. Nelson has used the method to study mammalian proteins in functional genomic analysis and is willing to work with us in plant systems. The technique is analogous to transposon tagging (but can be used for heterologous hosts) and uses *Agrobacterium tumefaciens* to deliver DNA into plant cells. The reading-frame-independent sequence can be inserted into the bacterium, which is then injected into rapidly growing

meristematic tissue of the plant. Proteins can be targeted by infecting different meristems of a plant, and mutant proteins can be captured using antibody techniques.

Vierling, R. A., J. M. Ferris, V. R. Ferris, and J. Faghihi. Methods for conferring broad-based soybean cyst nematode resistance to a soybean line. (Patent pending)

Vierling, R. A., J. Faghihi, V. R. Ferris, and J. M. Ferris. (1996) Association of RFLP markers with loci conferring broad-based resistance to the soybean cyst nematode (*Heterodera glycines*). Theor. Appl. Genet. 92:83-86.

Faghihi, J., R. A. Vierling, J. M. Halbrendt, V. R. Ferris, and J. M. Ferris. (1995) Resistance in a 'Williams 82' X 'Hartwig' soybean cross to an inbred line of *Heterodera glycines*. J. Nematol. 27:418-421.