Development of Gray Leaf Spot Resistant Perennial Ryegrass Through Breeding and Biotechnological Approaches

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Objectives:

1. To evaluate Pi-CO39, a resistance gene from rice, for effectiveness against gray leaf spot.
2. Introduce gray leaf spot resistance into perennial ryegrass.

Start Date: 2000
Project Duration: 3 years
Total Funding: $75,000

Gray leaf spot is an emerging and devastating disease of perennial ryegrass. The disease is caused by the fungus *Pyricularia grisea*, which has an extremely broad host range among the graminae. *Pyricularia grisea* infects over 50 species of grass, including crops such as rice, wheat, barley, oats, rye and millet. However, the fungus displays considerable host specificity and any given strain is usually only capable of infecting one or two host species. This is true also of *P. grisea* strains infecting perennial ryegrass.

DNA fingerprinting studies revealed that *P. grisea* strains found on rice, crabgrass or foxtails are not responsible for gray leaf spot outbreaks on perennial ryegrass. Instead, the disease is caused by a very specific subpopulation of *P. grisea*. The limited genetic diversity that was revealed by the DNA fingerprinting studies indicate that it should be possible to control gray leaf spot effectively using disease resistance genes.

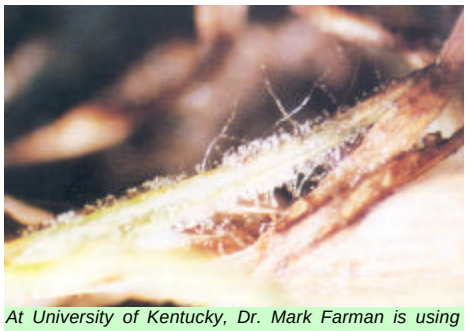
Unfortunately, there appears to be little or no natural resistance to gray leaf spot in currently perennial ryegrass cultivars. For breeding, it is necessary to seek resistance in grasses that are closely related to perennial ryegrass. Also available at our disposal, however, are methods for genetic transformation of perennial ryegrass. This opens up the possibility of finding resistance in more exotic sources, which may then be used for genetic engineering of disease-resistant perennial ryegrass.

Gray leaf spot destroys perennial ryegrass fairways and rough by infecting stem and crown tissues.

One of our objectives is to transfer gray leaf spot resistance from an annual ryegrass x tall fescue hybrid (H#9) into a perennial ryegrass background through a series of crosses. However, given the known pathogenic variability of *P. grisea*, it is unlikely that any one resistance will last forever. Therefore, a second objective will be to continue efforts to identify new sources of resistance, which may allow us to pyramid genes. This will be done in two ways. First, we will continue to evaluate germplasm in a current tall fescue breeding program. Second, we are attempting to use genetic transformation to transfer into perennial ryegrass a gray leaf spot resistance gene found in rice.

Previous experiments indicated that crossing of H#9 with perennial ryegrass (Linn) failed to result in resistant progeny in the F1 generation. This result suggested that the resistance may be recessive. To address this possibility, we performed crosses between susceptible F1 progeny. We also performed a number of crosses between H#9 and other perennial ryegrass cultivars. Seed has been produced, but we have not yet germinated these to evaluate resistance in the progeny.

We focused most of our efforts this year on establishing routine methods for transformation of perennial ryegrass. This will enable us to introduce Pi-C039(t), a disease-resistance gene from rice. We have shown that this gene prevents infection of rice by *P. grisea* strains that are found on



At University of Kentucky, Dr. Mark Farman is using conventional and molecular genetics to help identify resistance to gray leaf spot that infects perennial ryegrass.

perennial ryegrass. Production of tissue cultures was achieved by germinating seeds on tissue culture medium containing 2,4-D. The resulting callus tissues were maintained by serial subculture on the same medium, and whole plants were then regenerated by transferring calli to medium containing kinetin. After the successful establishment of conditions enabling routine production and regeneration of callus tissue, we sought to introduce DNA into perennial ryegrass tissues using biolistic transformation.

Callus tissues were bombarded with gold particles coated with vector DNA. We have been successful in obtaining expression of foreign DNA in perennial ryegrass. Bombarded calli have now been transferred to regeneration medium in an attempt to recover stable, transformed plants. In the coming project year, we expect to try introducing the Pi-C039(t) resistance gene into perennial ryegrass.

Summary Points

- Researchers focused most of their efforts this year on establishing routine methods for transformation of perennial ryegrass. This will enable them to introduce into prg, Pi-C039(t), a disease resistance gene from rice.
- Researchers have been successful in obtaining expression of foreign DNA in perennial ryegrass.