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.

Previously we showed that *P. grisea* isolates from perennial ryegrass possess a homolog of the avirulence gene, AVR1-CO39. Fungal isolates that contain a functional copy of this gene are unable to infect rice cultivar CO39. They retain the ability to colonize another cultivar, 51583. Genetic analysis of the resistance in CO39 led to the identification of a single, dominant gene that confers a resistant phenotype against *P. grisea* isolates with AVR1-CO39.

We hypothesize that this resistance gene, named Pi CO39(t), encodes a receptor protein that binds to the AVR1 CO39 gene product, causing activation of a signal transduction pathway, leading ultimately to a resistance response. If we are correct in this prediction, it may be possible to transfer this receptor into perennial ryegrass, thereby conferring an ability to recognize the AVR1 CO39Lp gene product present in the *P. grisea* isolates causing gray leaf spot. In turn, this may enable the perennial ryegrass to signal a resistance response against these isolates.

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

This approach will work only if the Pi CO39(t) gene product is involved in the resistance against the AVR1 CO39Lp allele in the isolates from perennial ryegrass. This was tested by introducing a copy of AVR1 CO39Lp into a rice pathogenic isolate of *P. grisea*, and using the resulting transformant to inoculate F3 progeny families derived from a cross between CO39 and 51583.

The results of this experiment were consistent with the hypothesis that resistance conferred by Pi-CO39(t) is also effective against the AVR1 CO39Lp allele. Filial generation 3 (F3) families that showed non-segregating resistance/susceptibility to AVR1 CO39, showed either no segregation or minimal segregation for resistance/susceptibility to AVR1 CO39Lp; families that showed segregation with AVR1 CO39, also showed segregation when AVR1 CO39Lp was present.

Although, there was not a perfect correspondence between the F3 segregation patterns for AVR1 CO39 and AVR1 CO39Lp, the data were inconsistent with any models invoking the existence of a second resistance gene. Therefore, we conclude that resistance to AVR1 CO39Lp is controlled by Pi CO39(t) and the discrepancies in segregation patterns are attributed to a difference in the genetic background between

At University of Kentucky, conventional and molecular genetics are being used to help identify resistance to gray leaf spot that infects perennial ryegrass.

P. grisea strains used for the inoculation test.

In preparation for transformation of perennial ryegrass with the Pi CO39(t) gene from rice cultivar CO39, we have initiated tissue cultures of perennial ryegrass. At first, microbial contamination of seed was a significant barrier to these procedures. However, we established a very effective sanitization protocol that virtually eliminates contamination.

Experiments were performed to determine appropriate conditions for efficient production and stable growth of callus from cultivars Derby, Supreme, and Manhattan III. Also established were protocols for regeneration of whole plants from these callus cultures. Finally, we performed experiments to determine optimal conditions for introduction and stable expression of DNA in callus cultures. Thus far, we have only been able to obtain transient expression of a GUS reporter gene present on the transformation vector.

Summary Points

- Significance progress has been made to identify a resistance gene, Pi Co39(t), and efforts are being focused on transferring that gene into perennial ryegrass.