USGA

Turfgrass and Environmental Research Online

...Using Science to Benefit Golf

Scientists at the University of Massachussets and the University of Illinois continue to identify gray leaf spot-resistance genes in ryegrass using the ryegrass genetic roadmap and annual x perennial ryegrass hybrids.

Volume 6, Number 2
January 15, 2007

PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 290 projects at a cost of $25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

Editor

Jeff Nus, Ph.D.
1032 Rogers Place
Lawrence, KS 66049
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

A Genetic Roadmap for Breeding Perennial Ryegrass Resistant to Gray Leaf Spot

Geunhwa Jung, Joe Curley, and Young-Ki Jo

SUMMARY

The fungus *Magnaporthe grisea* infects more than 50 graminaceous plants, as well as turfgrasses. This fungus causes blast on rice and gray leaf spot (GLS) on turfgrass. GLS has become an important disease on cool-season turfgrasses recently with destructive outbreaks on perennial ryegrass (*Lolium perenne*) occurring on golf courses and athletic fields in the Midwest and New England. Little is known about GLS resistance in perennial ryegrass, while a vast amount of genetic information about rice blast resistance in rice is available. In recent greenhouse evaluations, our lab identified partial GLS resistance in hybrids between annual and perennial ryegrass and found genetic markers (road signs) associated with the resistant traits on a ryegrass genetic roadmap. Knowing the genetics of the partial GLS resistance will allow the improvement of perennial ryegrass through breeding.

Plant breeding is a long integrated process which is accomplished by identifying, transferring, and fixing useful genes of interest (e.g., disease resistance) in elite cultivars. The genetic marker-assisted selection (MAS) can compliment the breeding process, which is conventionally based on visible phenotypic characteristics (i.e., appearance). MAS, on the other hand, is based on DNA fingerprinting, which facilitates the selection of traits of interest in individual plants for the next round of breeding. MAS improves the accuracy of selection and speeds the selection process by identifying plants with traits of interest at an earlier age. Therefore, genetic markers developed for GLS resistance in our study will significantly benefit turfgrass growers and practitioners in the U.S. This will eventually be very helpful in reducing dependence on costly fungicide applications for GLS. In brief, here are key research results.

- A high-density genetic roadmap of ryegrass was developed.
- Four partial GLS-resistance genes were detected in annual x perennial ryegrass hybrids. The partial resistance is thought to be much more durable, although the partial resistance is generally more difficult to manipulate using conventional breeding methods.
- The source of the GLS resistance appeared to originate from annual ryegrass cv. 'Floregon' since all perennial ryegrass plants tested, including grandparents, were very susceptible to GLS.
- The resistance to the ryegrass isolates and the rice isolate appeared to be controlled by different resistance genes.
- Some of the GLS resistance genes in ryegrass appeared similar to blast resistance genes in rice.
- Genetic markers tightly linked to the GLS resistance genes in the ryegrass hybrids were developed.

Perennial ryegrass is taxonomically closely related to oat, barley, and wheat, and somewhat distantly related to rice (6). It is a valuable cool-season turfgrass and is extensively used on golf course fairways and roughs, as well as athletic fields and home lawns. It is an outcrossing and self-incompatible species; only crossing between different parental plants can produce viable seed. Lately, many improved cultivars have become available, causing renewed interest in and more widespread use of this species.

GEUNHWA JUNG, Ph.D., Assistant Professor; YOUNG-KI JO, Ph.D., Post-doctoral Associate; Dept. of Plant, Soil, and Insect Sciences, University of Massachusetts, Amherst, MA; JOE CURLEY, Ph.D., Post-doctoral Associate, Dept. of Crop Sciences, University of Illinois, Urbana, IL

Gray leaf spot develops on the leaf blades of perennial ryegrass as small water-soaked lesions that subsequently turn into dark-colored, 1-3 mm necrotic spots.

USGA Turfgrass and Environmental Research Online 6(2):1-7.
TGIF Record Number: 120895

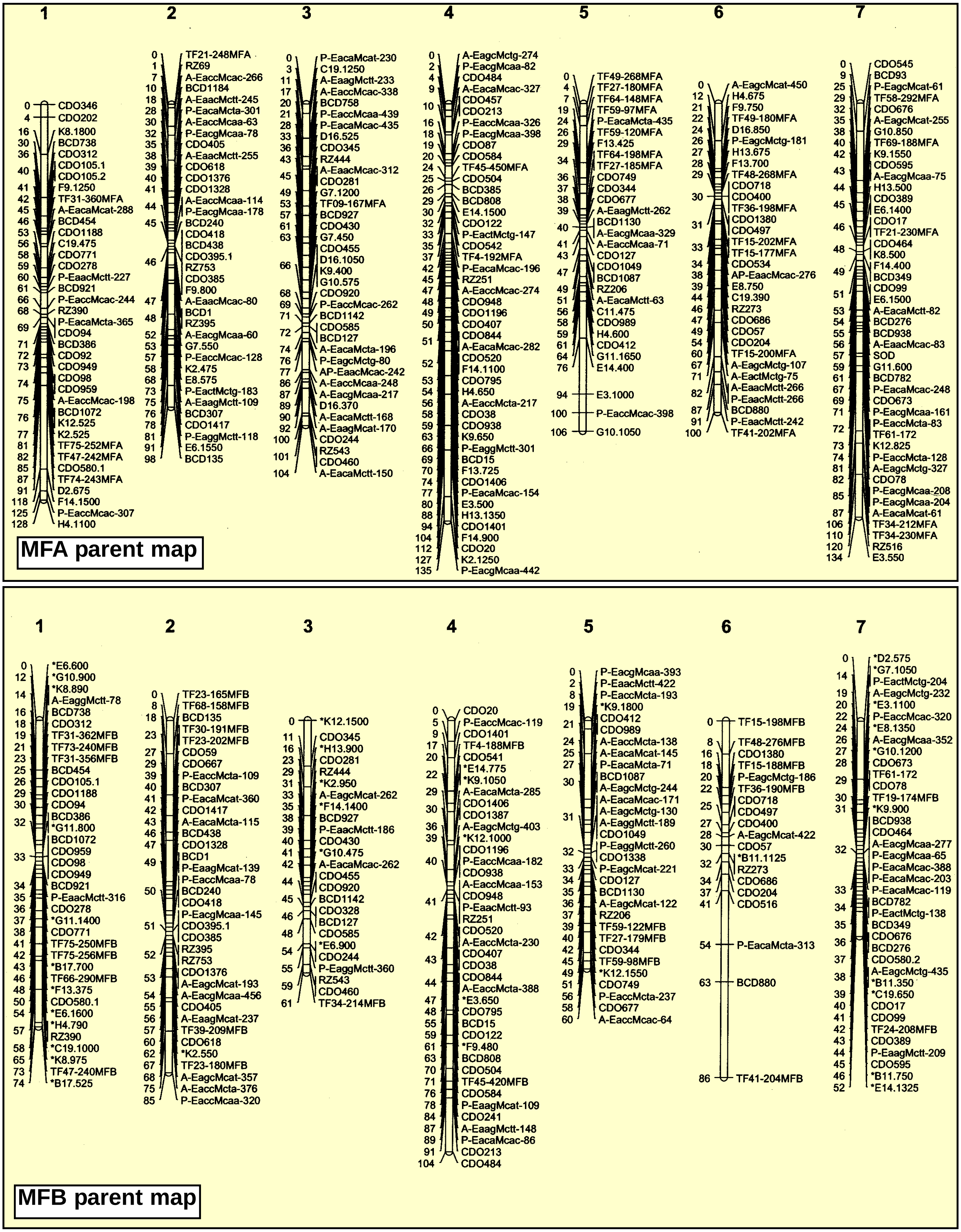

Figure 1. Genetic roadmaps of the MFA x MFB population used for locating gray leaf spot resistances in this study, constructed separately using various molecular marker data from Sim et al. (9). Linkage groups for both maps are numbered according to Warnke et al. (16). Top: MFA parent map. Bottom: MFB parent map.

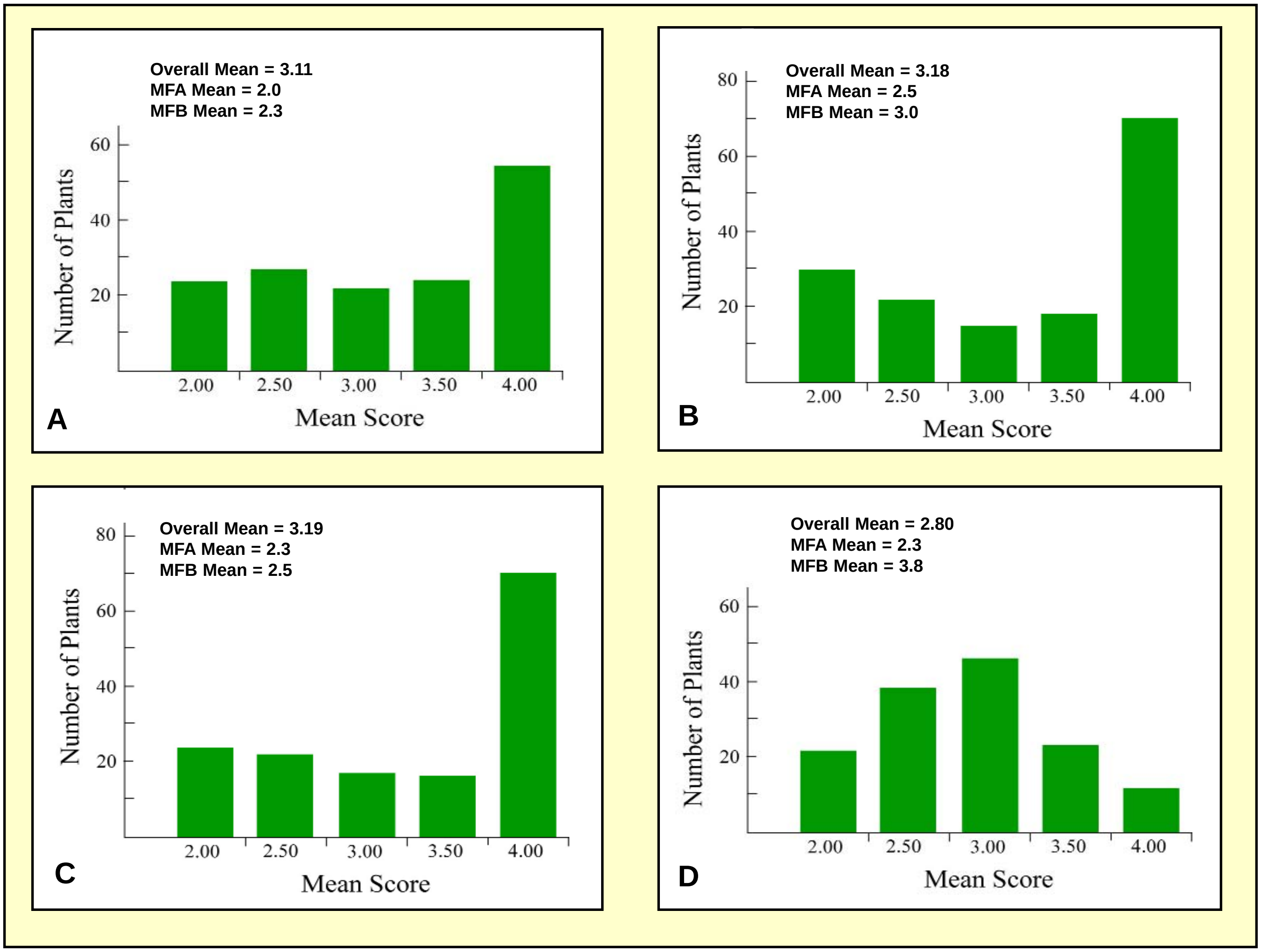

Figure 2. Frequency distributions of GLS phenotypic data from four greenhouse inoculations, based on the mean over four clonal replicates of each progeny genotype. For each experiment, the mean GLS reaction for each of the two mapping parents, MFA and MFB, is given. A: GG9, mist chamber experiment. B: GG9, first growth chamber experiment. C: GG9 second growth chamber experiment. D: 6082, growth chamber experiment

In recent years, an emerging disease known as gray leaf spot (GLS) has caused a serious problem on intensively managed perennial ryegrass (14). The causal fungus is *Magnaporthe grisea*. This fungus also causes blast disease on rice and various foliar diseases on a wide range of plants in the grass family. The outbreak of GLS on perennial ryegrass was first reported in Pennsylvania in 1992 (7). Since then, spontaneous, destructive outbreaks have been reported from the transition zone through Mid-Atlantic States to the Midwest and New England.

Perennial ryegrass is generally susceptible to GLS and can be completely destroyed in a matter of days under warm and humid conditions (7). Despite the use of cultural management techniques (e.g., reducing the period of leaf wetness and lowering the nitrogen fertility level) to protect valuable perennial ryegrass turf on golf courses, preventative fungicide applications are necessary. Due to the high genetic variability of *M. grisea* (15), the rapid development of fungal isolates insensitive to fungicides has increased the concerns of turfgrass managers.

There is, therefore, a pressing need for alternative ways of managing GLS. Use of host resistance is a very attractive, environmentally sound control strategy. For example, rice blast disease has been controlled by host resistance. Major resistance genes conferring complete resistance to specific fungal races and minor genes associated with partial resistance to various races have been identified and used for improving host resistance in rice.

Major genes are relatively easy for genetic manipulation and so their incorporation into rice cultivars is easy. However, they can potentially break down within a few years as the pathogen population shifts to new virulent races (3, 10). On the other hand, partial resistance is difficult for breeders to manipulate but is more durable to successfully prevent rice blast outbreaks for a longer time (11). Therefore, the improvement of rice blast resistance in rice cultivars has been achieved by incorporating many minor resistance genes instead of a few major genes.

Similarly, the identification of many major and minor resistance genes to GLS in perennial ryegrass would be very valuable in future systematic breeding of this plant. An essential tool for studying the genetics of GLS resistance is a genetic roadmap using genetic markers which helps us to locate resistance genes. Genetic roadmaps have been widely used to study blast resistance in rice (4, 11). The genetic roadmap facilitates the identification and manipulation of individual genetic factors associated with complex traits such as partial disease resistance. Further, the roadmap can be used for comparative genetic analyses between different plants. Simply, different genetic roadmaps (e.g., ryegrass maps and rice maps) can be aligned and genetic locations of interest (e.g., disease resistance genes) can be compared.

Although resistance to *M. grisea* is well studied in other hosts, it has received little attention in perennial ryegrass. Little resistance has been reported in available perennial ryegrass cultivars, although some exotic plant introductions seem to show some level of resistance (5). Further, there is almost no knowledge of the genetics of resistance in turfgrass. This information would be important in providing resistant ryegrass cultivars, by giving an indication of the durability of the resistance, as well as the amount of work required to transfer the resistance into new perennial ryegrass cultivars.

Recently, ryegrass genetic roadmaps based on genetic markers have been developed (9, 16) using annual and perennial ryegrass hybrids (Figure 1). Ryegrass hybrid plants for the genetic roadmap construction were originally developed by Dr. R. Barker (USDA-ARS, Corvallis, OR) and consisted of 156 progenies derived from the cross between two ryegrass parent clones, MFA

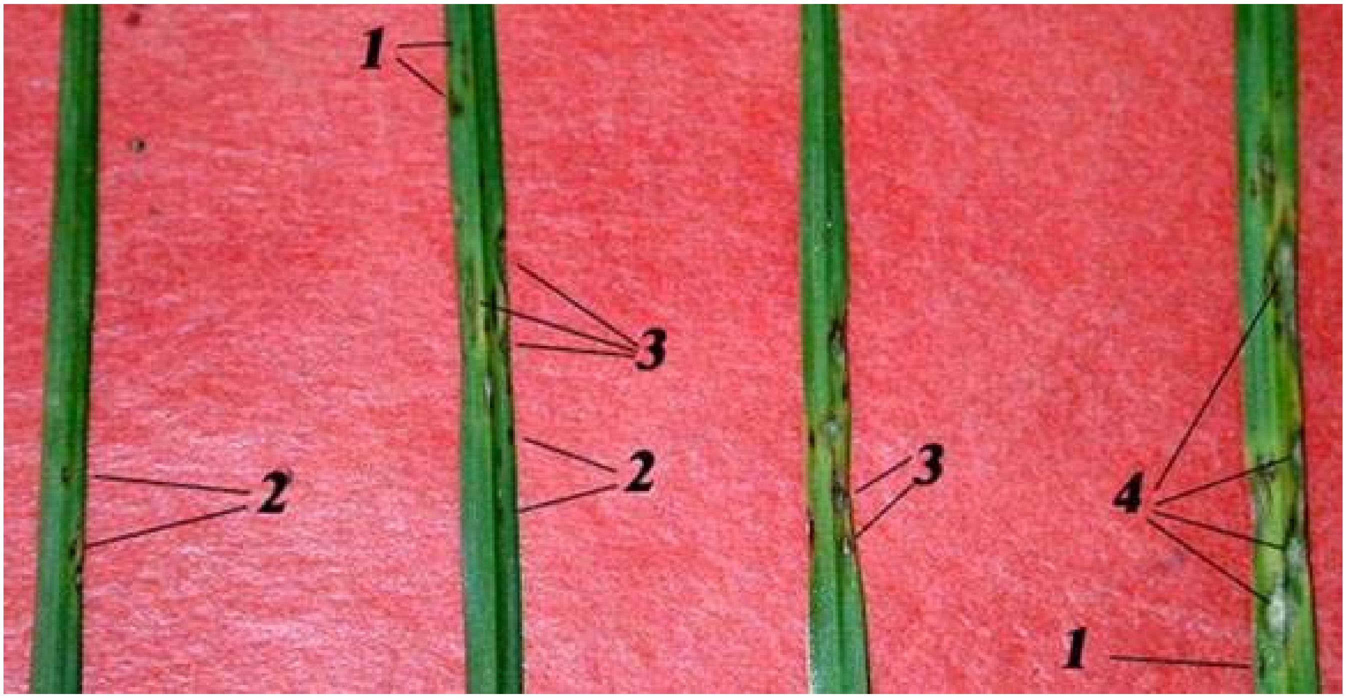

Figure 3. Rating scale for gray leaf spot severity. Types 1 and 2 cause little damage to the plant or turf except some browning of leaves in very high numbers, and are considered resistant reactions. Type 3 is an intermediate reaction, and type 4 is the most severe reaction, causing leaf discoloration, blighting, and in high numbers, tiller death.

and MFB (9). Both of these parental clones are annual x perennial ryegrass hybrids, derived from crosses between perennial ryegrass cultivar `Manhattan', termed Manh-1 and Manh-3, and two different grandparental clones of the annual ryegrass cultivar 'Floregon'.

Our lab initiated pilot projects to identify GLS resistance genes in ryegrass using the ryegrass genetic roadmap and annual x perennial ryegrass hybrids. An MFA x MFB mapping population consisting of 156 ryegrass plants was prepared for artificial GLS inoculations in the greenhouse. *M. grisea* inocula included one lab rice isolate, 6082, which is capable of infecting both rice and ryegrass, and four field isolates collected from diseased perennial ryegrass.

The result from the greenhouse inoculation experiments suggested that the annual ryegrass is a donor of the source of GLS resistance. Grandparent annual ryegrass 'Floregon' was resistant to GLS. In contrast, grandparent perennial ryegrass plants Manh-1 and Manh-3 were very susceptible to GLS, and parental clones MFA and MFB, maintained intermediate resistance to GLS.

An in-depth analysis of GLS resistance (called trait mapping) in the MFA x MFB population uncovered three genomic regions (road signs) associated with GLS resistance on the ryegrass genetic roadmap. Two of these were uniquely associated with resistance to a perennial ryegrass field isolate, while one was uniquely associated with resistance to the rice strain, 6082. Results suggest either that the population has at least two different sets of resistance genes that are differentially effective against the two isolates, or that there is only one set of genes against which the different isolates can cause differing amounts of disease.

The GLS resistance identified in this study is related to partial resistance genes, involving differences in lesion size rather than presence or absence of lesions (Figure 3). Resistant plants often show few small lesions, with a reduced amount of pathogen reproduction compared to that of large, expanding lesions on susceptible progenies. Also, the disease reaction was sensitive to the vigor of the youngest leaves, as might be modified by nitrogen fertilization, optimum light intensity, and temperature and soil conditions. In rice, blast disease severity has been shown to be increased by nitrogen fertilizer application. Partial resistance to *M. grisea* has been reported in other species, such as tall fescue (12), rice (17), and barley (8).

Gray leaf spot normally develops from early August to mid-October. Environmental conditions prevailing during this late summer period and availability of inoculum are major determinants in the development of gray leaf spot epidemics.

Based on high genomic similarity among graminaceous plants, our comparative mapping study further revealed that the GLS resistance we identified has a close relationship with blast resistance in rice. Since the whole rice genome has been sequenced, rice chromosomal regions similarly with ryegrass regions containing the GLS resistance provide information about potential candidate resistance genes (1). Once these candidate genes are confirmed to be present and function similarly in ryegrass, the candidate genes can be used for the marker-assisted breeding for GLS resistance in ryegrass.

Little information about GLS resistance is available in perennial ryegrass germplasm in the U.S. Some perennial ryegrass collections from Canada, Japan, New Zealand and Europe show a moderate level of resistance (5). Bonos et al. (2), using open-pollinated single-plant progeny evaluations, have revealed some partially resistant perennial ryegrass selections. Further, they reported that the resistance is under strong genetic control, with evidence for a single major gene, in contrast with the current study which suggests that multiple, partial genes are important. Similarly, annual ryegrass has shown variation in reaction to GLS in field and greenhouse tests, as well (13). Further, the annual ryegrass cultivar 'Floregon' was subjected to unintentional selection for resistance due to a natural gray leaf spot outbreak in Florida during a breeding cycle. Results of this study also provide evidence for GLS resistance in annual ryegrass, as well as its potential as a genetic resource in this respect.

In conclusion, the GLS resistance identified in the annual and perennial ryegrass hybrids represents a different source of GLS resistance from previously determined ones. The new source of resistance will allow us to incorporate various resistance genes into a perennial ryegrass cultivar. In the current study, the GLS resistance was characterized as four potential genetic regions conferring partial resistance. Such a characterization is important for estimating transferability of resistance into perennial ryegrass. Genetic markers tightly linked to GLS resistance will enable us to perform the marker-assisted breeding in perennial ryegrass. The marker-assisted selection will be enhanced as more sources of GLS resistance are discovered and become available for breeding programs.

Acknowledgements

The authors wish to express thanks to the USGA Turfgrass and Environmental Research Program for its partial support of this project.

Literature Cited

1. Armstead, I.P., L.B. Turner, M. Farrell, L. Skøt, P. Gomez, T. Montoya, I.S. Donnison, I.P. King, and M.O. Humphreys. 2004. Synteny between a major heading-date QTL in perennial ryegrass (*Lolium perenne* L.) and the Hd3 heading-date locus in rice. *Theor. Appl. Genet.* 108:822-828. (TGIF Record 120311)

2. Bonos, S.A., C. Kubik, B. B. Clarke, and W. A. Meyer. 2004. Breeding perennial ryegrass for resistance to gray leaf spot. *Crop Sci.* 44:575-580. (TGIF Record 93831)

3. Chauhan, R., M. L. Farman, H-B Zhang, and S. A. Leong. 2002. Genetic and physical mapping of a rice blast resistance locus, Pi-CO39(t), that corresponds to the avirulence gene AVR1-CO39 of *Magnaporthe grisea*. *Mol. Gen. Genomics*. 267:603-612.

4. Fukuoka, S., and K. Okuno. 2001. QTL analysis and mapping of pi21, a recessive gene for field resistance to rice blast in Japanese upland rice. *Theor. Appl. Genet.* 103:185-190.

5. Hoffman, N., and A. Hamblin. 2001. Reaction of perennial ryegrass to gray leaf spot following inoculation in the field, 2000. *Biological and Cultural Tests* 16:T56. (TGIF Record 91468)

6. Kellogg, E. 2000. The grasses: A case study in macroevolution. *Ann. Rev. Ecol. Syst.* 31:217-238. (TGIF Record 120301)

7. Landschoot, P., and E. Hoyland. 1992. Gray leaf spot of perennial ryegrass turf in Pennsylvania. *Plant Dis.* 76:1280-1282. (TGIF Record 24955)

8. Sato, K., T. Inukai, and P. M. Hayes. 2001. QTL analysis of resistance to the rice blast pathogen in barley (*Hordeum vulgare*). *Theor. Appl. Genet.* 102:916-920.

9. Sim, S., T. Chang, J. Curley, S. Warnke, R.

Barker, and G. Jung. 2005. Chromosomal rearrangements differentiating the ryegrass genome from the Triticeae, oat, and rice genomes using common heterologous RFLP probes. *Theor. Appl. Genet.* 110:1011-1019. (TGIF Record 104479)

10. Tabien, R.E., Z. Li, A. H. Paterson, M. A. Marchetti, J. W. Stansel, and S. R. M. Pinson. 2000. Mapping of four major rice blast resistance genes from 'Lemont' and 'Teqing' and evaluation of their combinatorial effect for field resistance. *Theor. Appl. Genet.* 101:1215-1225.

11. Tabien, R., Z. Li, A.H. Paterson, M.A. Marchetti, J.W. Stansel, and S.R.M. Pinson. 2002. Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. *Theor. Appl. Genet.* 105:313-324.

12. Tredway, L. P., K. L. Stevenson, and L. L. Burpee. 2003. Components of resistance to *Magnaporthe grisea* in 'Coyote' and 'Coronado' tall fescue. *Plant Dis.* 87:906-912. (TGIF Record 89670)

13. Trevathan, L.E., M. A. Moss, and D. Blasingame. 1994. Ryegrass blast. *Plant Dis.* 78:113-117. (TGIF Record 29930)

14. Viji, G., B. Wu, S. Kang, W. Uddin, and D. R. Huff. 2001. *Pyricularia grisea* causing gray leaf spot of perennial ryegrass turf: population structure and host specificity. *Plant Dis.* 85:817-826. (TGIF Record 74504)

15. Vincelli, P., and E. Dixon. 2002. Resistance to QoI (Strobilurin-like) fungicides in isolates of *Pyricularia grisea* from perennial ryegrass. *Plant Dis.* 86:235-240. (TGIF Record 79029)

16. Warnke, S.E., R. E. Barker, G. Jung, S. Sim, M. A. Rouf Mian, M. C. Saha, L. A. Brilman, M. P. Dupal, and J. W. Forster. 2004. Genetic linkage mapping of an annual x perennial ryegrass population. *Theor. Appl. Genet.* 109:294-304. (TGIF Record 103681)

17. Zenbayashi, K., T. Ashizawa, T. Tani, and S. Koisumi. 2002. Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32. *Theor. Appl. Genet.* 104:547-552.