Genetic Markers Identify Dollar-Spot Resistance

By Nanda Chakraborty and Geunhwa Jung

All modern bentgrass cultivars are susceptible to dollar spot, but there are significant differences in their susceptibility. Bentgrass is a large genus of more than 100 species. Only about four species are used for turfgrass in the United States. These are colonial, velvet, highland and creeping.

These species are perennial, outcrossing cool-season grasses and are used for golf courses. Currently, the stoloniferous, allotetraploid creeping bentgrass is the most adapted species for use on fairways and greens (Wipff and Fricker, 2001).

Dollar spot, caused by a fungus, is a major disease of turfgrass throughout the world and is the most prevalent and economically important turf disease in North America, particularly on intensively managed golf course putting greens and closely mown fairways (Couch, 1995; Vargas, 1994).

The disease occurs from spring through fall, but is most active during humid periods of warm days with cool nights in spring, early summer and fall. The optimum temperature for disease development is 70 degrees F to 80 degrees F, though the fungus will grow over a wider range, 50 degrees F to 90 degrees F.

On low-height turf, such as greens or fairways, the symptoms appear as round or irregularly shaped sunken, straw- to brown-colored patches approximately 1.2 inches in diameter (or about the same size as a silver dollar). When disease pressure is high, isolated spots grow together to form large, irregular patches. Since this fungus is not known to produce conidia or a sexual stage in the United States, the organism most likely spreads through mycelia or transport of infected leaf tissue by wind, water, machinery such as mowers, or by human traffic (Baldwin and Newell, 1992).

Why the interest in this disease?

Different current research strategies can be used to improve management strategies to combat this disease (Hensler, 2002). For example, research indicates that dollar spot severity can be reduced in a blended population by mixing a resistant cultivar with a susceptible cultivar (Abernathy et al., 2001).

Significant reductions have also been reported after morning mowing or poling treatment or both (Williams et al., 1996). According to some extension services, late-spring dollar spot severity can be minimized by the late-spring application nitrogen fertilizer, which will induce growth during early summer when dollar spot infections begin.

Two creeping bentgrass clones with significant difference in disease response were identified from more than 300 clones collected.

Some researchers have also shown that multiple applications of composts were effective in reducing disease incidence and severity (Boulter et al., 2002).

Dollar spot management, like other turf diseases, is highly dependent on chemical fungicide application. The causal fungus has, however, developed resistance to several important classes of fungicides such as benzimidazoles, dicarboximides and demethylation inhibitors (DMI) (Cole et al., 1968; Warren et al., 1974).

Fluazinam, a nonsystemic, pyridylaniline compound was able to suppress dollar spot caused by two benzimidazole and DMI resistant strains of the fungus to a threshold of less than 5 percent disease for more than 21 days (Burpee, 1997). Some fungicides have not been reregistered due to environmental concerns. This has stimulated research into alternative disease management strategies such as host resistance.

How this work differs

Previous reports indicate variability among bentgrass cultivars in their susceptibility to

dollar spot. Two greenhouse inoculation experiments performed in our lab detected large genetic variation at the species, cultivar and clone level.

Eighty-one clones of 10 cultivars of the creeping, colonial, highland and velvet bentgrass species were inoculated with the dollar-spot isolate MN1.

Two creeping bentgrass clones with significant difference in disease response were identified from more than 300 clones collected from fairways and greens in golf courses throughout Wisconsin.

The clone 372 is highly resistant while 549 is susceptible to dollar spot. It has been well known that the colonial bentgrass species is naturally more resistant to dollar spot than creeping bentgrass. Our previous results indicated that the level of resistance detected in the 372 clone was approximately the same as that of cultivars of the colonial species.

When a differential response was noted between the clones using only one isolate, we were interested to determine the disease response of these clones using multiple isolates belonging to different Vegetative Compatibility Groups (VCGs). Sixteen new fungal isolates were provided to us by Jon Powell from the University of Minnesota.

We studied the genetic relatedness of the isolates to find if there is a correlation between the genetic distance and the VCGs. We found that some of the isolates belonging to the same VCGs are genetically similar but others are genetically distant from one another even though belonging to same VCGs.

The next step was to find the correlation between VCGs and virulence. We used eight isolates belonging to five VCGs to inoculate the two creeping bentgrass clones. There was an overall clone and isolate effect. Some of the isolates did not show significant difference in disease response between the two clones but two isolates, MN1-VCG A and Les Bolstead-VCG J, showed a significant clone effect.

The clone 372 was more resistant than the clone 549 for all the eight isolates. There was no race-specific interaction noted. We will further study the race-specific interaction and virulence by including 16 more isolates belonging to 11 VCGs.

Since we noticed a significant difference in disease response between the two creeping bentgrass isolates 372 and 549, that fact suggested that a mapping population created from the cross will segregate for dollar-spot resistance.

So a mapping population was created by crossing clone 372 by 549 in 2002. We will use the resulting progeny segregating for dollar spot resistance to analyze locations, numbers, and effect of dollar spot resistance genes using molecular markers and Quantitative

Molecular markers for resistance to dollar spot are currently unavailable.

Trait Locus (QTL) analysis. Currently more than 200 barley, oat and rice Restriction Fragment Length Polymorphism (RFLP) anchor probes are being screened using the two parental DNA, in order to use these probes as molecular markers (Lespinasse et al., 2000; VanDeynze et al., 1998; Wang et al., 2000).

The probes tested so far hybridized with and detected abundant polymorphisms in both bentgrass DNA. Moreover RAPD (Random Amplified Polymorphic DNA) primers are currently being tested to collect molecular markers about the polymorphic nature of 94 progenies and the two parental clones.

We are going to score the bands that are polymorphic between the two parents and segregate among progenies. The polymorphism, as indicated by arrows, is created due to recombination and segregation during the event of crossing.

The polymorphic data will be used to create a linkage map with the help of a statistical program. The linkage map is a hypothetical map of the chromosomes, which shows the position of the markers, depending on their percentage of crossovers.

Thus all the necessary tools and expertise are already in place to successfully create the linkage map in bentgrass. This linkage map can be used to create durably resistant cultivars using multiple-resistance gene markers obtained through QTL analysis.

No other groups have yet studied race-specific interactions in clones, cultivars and species using multiple isolates of the dollar spot pathogen.

If any race-specific interactions are noted, results from the experiment using multiple isolates from 11 VCGs can be used to analyze whether the QTLs for disease resistance are similar or different for different isolates.

Potential benefit of this work

The proposed research program incorporates molecular and genetic methods, plant pathology, plant breeding and quantitative genetics. Every year, public health concerns due to synthetic chemicals applied on golf courses and other grassy areas are significantly increasing worldwide.

Late spring dollar-spot severity can be minimized by the application of late-spring nitrogen fertilizer.

Dollar spot resistance in bentgrass would considerably decrease the need for fungicide applications on bentgrass. In many cases, molecular markers are available for marker-assisted selection of novel genes. However, markers for resistance to dollar spot are currently unavailable. By pairing markers with novel resistance genes, producers and private industry will be able to access the germplasm diversity held within the public sector in improved forms well into the 21st century. This marker-assisted selection can result in higher gain from selection than phenotypic selection, thus expediting the disease resistance breeding progress.

The linkage map will also be useful to other researchers on this topic in the future, as our map will be the reference map, and they will be able to compare dollar spot resistance QTLs with our QTLs.

Help for breeders, superintendents

These research results will help turfgrass breeders to speed the selection process by using molecular markers, with the ultimate goal of developing disease-resistant breeding lines and cultivars.

Ultimately, superintendents, private seed companies and anyone using bentgrass will receive nearly immediate benefit from the development of disease-resistant cultivars. In the end, the amount of fungicides used for the control of dollar spot will be dramatically reduced.

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REFERENCES

Abernathy, S. D., R. H. White, P. F. Colbaugh, M. C. Engelke, G. R. Taylor, II, and T. C. Hale. 2001. "Dollar spot resistance among blends of creeping bentgrass cultivars." *Crop Science.* 41:806-809.

Baldwin, N.A., and A.J. Newell. 1992. "Field production of fertile apothecia by *Sclerotinia homoeocarpa* in *Festuca* turf." *J. Sports Turf Res. Inst.* 68:73-76.

Boulter, J. I., G. J. Boland, and J. T. Trevors. 2002. "Evaluation of compost for suppression of dollar spot (*Sclerotinia homoeocarpa*) of turfgrass." *Plant Disease.* 86: 405-410.

Burpee L. L. 1997. "Control of dollar spot of creeping bentgrass caused by an isolate of *Scleretonia homoeocarpa* resistant to benzimidazole and demethylation inhibitor fungicides." *Plant Disease.* 81:1259-1263.

Cole, H., B. Taylor, and J. Duich. 1968. "Evidence of differing tolerances to fungicides among isolates of *Sclerotinia homoeocarpa.*" *Phytopathology.* 58:683-686.

Couch, H.B. 1995. *Disease of turfgrasses. 3rd ed.* Krieger Publ., Malabar, Fla.

Hensler, K. L. 2002. "Dollar Spot." *The Grass Roots.* 2002. July/August. 43-47.

Lespinasse D., L. Grivet, V. Troispoux, M. Rodier-Goud, F. Pinard, and M. Seguin. 2000. "Identification of QTLs involved in the resistance to South American leaf blight (*Microcyclus ulei*) in the rubber tree." *Theor. Appl. Genet.* 100:975-984.

Vargas, J.M., Jr . 1994. *Management of turfgrass diseases. 2nd ed.* Lewis Publ., Boca Raton, Fla.

Van Deynze, A.E., M.E. Sorrells, W.D. Park, N.M. Ayres, H. Fu, S.W. Cartinhour, E. Paul, and S.R. McCouch. 1998. "Anchor probes for comparative mapping of grass genera." *Theor. Appl. Genet.* 97:356-369.

Wang, D., R. Karle, A. F. Iezzoni. 2000. "QTL analysis of flower and fruit traits in sour cherry." *Theor. Appl. Genet.* 100:535-544.

Warren, C.G., P.L. Sanders, and H. Cole, Jr. 1974. "*Sclerotinia homoeocarpa* tolerance to benzimidazole configuration fungicides." *Phytopathology* 64:1139-1142.

Williams, D. W., D. W. Powell Jr., P. Vincelli, C. T. Dougherty. 1996. "Dollar spot on bentgrass influenced by displacement of leaf surface moisture, nitrogen and clipping removal." *Crop Science.*36:1304-1309.

Wipff, J. K., and C. Fricker. 2001. "Gene flow from transgenic creeping bentgrass (*Agrostis stolonifera*) in the Willamette Valley in Oregon." *International Turfgrass Society Research Journal* 9:224-242.