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ORIGINAL PAPER

# Mapping QTL for dollar spot resistance in creeping bentgrass (*Agrostis stolonifera* L.)

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**Abstract** Dollar spot caused by *Sclerotinia homoeocarpa* F. T. Bennett is the most economically important turf disease on golf courses in North America. Dollar spot resistance in a creeping bentgrass cultivar would greatly reduce the frequency, costs, and environmental impacts of fungicide application. Little work has been done to understand the genetics of resistance to dollar spot in creeping bentgrass. Therefore, QTL analysis was used to determine the location, number and effects of genomic regions associated with dollar spot resistance in the field. To meet this objective, field inoculations using a single isolate were performed over 2 years and multiple locations using progeny of a full sib mapping population '549 × 372'. Dollar spot resistance seems to be inherited quantitatively and broad sense heritability for resistance was estimated to be 0.88. We have detected one QTL with large effect on linkage group 7.1 with LOD values ranging from 3.4 to 8.6 and explaining 14–36% of the phenotypic variance. Several smaller effect QTL specific to rating dates, locations and years were also detected. The association of the tightly linked markers with the LG 7.1 QTL based on 106 progeny was further examined by single marker analysis on all 697 progeny. The high significance of the QTL on LG 7.1 at a sample size of 697 ($P < 0.0001$), along with its consistency across locations, years and ratings dates, indicated that it was stable over environments. Markers tightly linked to the QTL can be utilized for marker-assisted selection in future bentgrass breeding programs.

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## Introduction

Dollar spot caused by *Sclerotinia homoeocarpa* F. T. Bennett is a major disease of turfgrass throughout the world. It 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). This disease may occur at almost any time during the growing season.

Currently, stoloniferous, allotetraploid creeping bentgrass (*Agrostis stolonifera* L., $2n = 4x = 28$) is the most adapted species for use on golf course fairways and greens because of its high tolerance to low mowing height (Wipff and Fricker 2001). Dollar spot management, like other turf diseases, is highly dependent on chemical fungicide application. Intensive, repeated

application of fungicides resulted in the development of fungicide resistance to several important classes of fungicides (Burpee 1997). Moreover, registration of some fungicides has been discontinued due to environmental concerns. This has stimulated research into alternative disease management strategies such as host resistance. Dollar spot resistance in a creeping bentgrass cultivar would greatly reduce the frequency, costs, and environmental impacts of fungicide application.

Studies have shown that all creeping bentgrass cultivars were susceptible to dollar spot, but there were significant differences in their susceptibility (Baldwin and Newell 1992, Chakraborty et al. 2006). Bonos et al. (2004) have shown that resistant creeping bentgrass clones had smaller lesion diameter and larger trichomes compared to susceptible clones. However, turf density, stomata density and trichome number were not associated with dollar spot resistance (Bonos et al. 2004).

Studies have indicated that dollar spot resistance may be quantitatively inherited (Bonos et al. 2003). Moreover, high broad sense heritability estimates provided evidence that replication increased selection efficiency and that improvement in dollar spot resistance in creeping bentgrass should be possible. According to Bonos et al. (2003), a minimum of two to five effective factors or genes, depending on the cross, may be associated with dollar spot resistance. A mixture of three isolates was used in their study and it was unknown if these results were due to major resistance genes specific to each isolate or due to a large number of genes indicating quantitative disease resistance. Studies including effects of specific isolates and determining quantitative trait loci (QTL) associated with dollar spot resistance are imperative.

Based on previous studies (Chakraborty et al. 2006), it has been shown that, irrespective of the location of collection and vegetative compatibility groups (VCG), isolates of *S. homoeocarpa* were >40% genetically similar to each other and differed significantly in their aggressiveness. That study also demonstrated a lack of cultivar × isolate interaction. This is encouraging information for breeders, because isolate non-specific resistance is likely to be much more durable over a wide array of locations and environments in which creeping bentgrass is grown. Moreover, it also provided evidence that, for QTL analysis, we can choose one isolate for our field inoculation studies at several locations.

Mapping of QTL has been utilized to address the challenge of working with quantitatively inherited traits in other crops (Tanksley et al. 1993, Lespinasse et al. 2000, Wang et al. 2000, Curley et al. 2005, Portyanko et al. 2005). Analysis of QTL is used increasingly by breeders to locate the genomic regions associated with quantitative disease resistance (Young 1996), because some of the common limitations faced by phenotypic selection for quantitative traits can be overcome by this approach. Markers tightly linked to dollar spot resistance in creeping bentgrass would be identified by QTL analysis. Moreover, it is possible to pyramid these markers from different sources of resistance into one cultivar, and use these markers to screen germplasm for resistance to multiple diseases over several environments.

As a first step to detect QTL for dollar spot resistance, we have constructed the first linkage map in creeping bentgrass using 169 RAPD, 180 AFLP and 75 RFLP markers (Chakraborty et al. 2005). Analysis of QTL requires a linkage map with densely and uniformly distributed markers. Therefore, we mapped an additional 174 RFLP markers onto the existing map (Chakraborty et al. 2005) and then two separate linkage maps were constructed for parents '372' and '549'. These two separate maps were used to accomplish our main objective, which was to detect the number, location and effect of QTL for dollar spot resistance based on field inoculations over multiple years and locations. Moreover, we examined the effect of increased population size on the significance of detected QTL. This study reports the first QTL analysis for dollar spot resistance in creeping bentgrass. The future application of this research is to utilize molecular markers tightly linked with the large effect QTL for marker-assisted selection in bentgrass breeding programs.

## Materials and methods

### Plant materials and establishment of field plots for dollar spot evaluation

The full sib mapping population '549 × 372' was developed in the greenhouse at UW Madison. Detailed descriptions of the origin of the parents, selection criteria and development of the population are available in a previous publication (Chakraborty et al. 2005). The 697 progeny individuals have been vegetatively propagated in the greenhouse and only 106 progeny from this population were used to generate the first linkage map in creeping bentgrass (Chakraborty et al. 2005).

To evaluate the progeny population for resistance to dollar spot in the field, asexually propagated clones from each of the 697 progeny individuals of the '549 × 372' population were transplanted in a

randomized complete block design with four replicates in summer of 2003 at O. J. Noer Turfgrass Research and Educational Facility, Verona, WI, and at Gateway Golf Club at Land 'O Lakes in Northern WI (Table 1). Initially the plots were tilled and seeded to perennial ryegrass (*Lolium perenne* L.) at a rate of 18 kg/100 $m^2$. After the ryegrass stand was established, circles 0.45 m in diameter with spacing of 0.45 m from center to center were created by applying a solution of 4% Roundup (glyphosate) to the ryegrass. Transplanting of clones was performed 3 weeks after glyphosate application.

Additional plots, one at Gateway Golf Club and another at the turf research center at Urbana-Champaign, University of Illinois, were established in 2003. These plots consisted of 188 progeny, including 106 used for the linkage mapping, from the '549 × 372' population and they were under the United States Golf Association (USGA) creeping bentgrass breeding consortium study for dollar spot evaluation. Data from 188 progeny of the '549 × 372' population were included in our study. All plots were fertilized three times a year (37-0-0) and maintained as fairways with a mowing height of 1.5 cm.

## Preparation of *S. homoeocarpa* inoculum and inoculation method

*Sclerotinia homoeocarpa* isolate MNI belonging to VCG A was used for all the artificial inoculations in this study. This isolate was collected from a golf course in Minnesota (Chakraborty et al. 2006). Inoculum was prepared by growing the isolate on sterilized Kentucky bluegrass seed following a modified protocol of Bonos et al. (2003). In detail, 250 g of Kentucky bluegrass seeds were autoclaved for 25 min. at 121°C along with 200 ml of potato dextrose broth and 100 ml of water in 2 l Erlenmeyer flasks and allowed to sit overnight. The flasks were autoclaved again the next day and cooled to room temperature. The isolate was grown on potato dextrose agar plates for 5 days under artificial light. To each flask containing autoclaved seeds, a full plate of isolate was added after cutting into 1 × 1 cm pieces. Inoculated flasks of seeds were grown for 3 weeks in 12 h of artificial light at room temperature. The flasks were shaken daily to prevent clump formation.

After 21 days, the infested seeds were spread under a fume hood for 72 h until completely dried and then passed through a sieve (2 × 10 mm) and stored in plastic bags. A drop spreader (Scotts Pro Turf Professional Drop Spreader) was used to apply inoculum to each field plot at the rate of 1.75 g/$m^2$. In 2004, the O. J. Noer plot was inoculated on June 7. In Gateway, the plot consisting of 697 progeny was inoculated on July 13, and the USGA plot with 188 progeny was inoculated on August 3. In 2005, inoculation was performed on May 2 at the Illinois plot, on June 3 at the O. J. Noer plot and on June 30 at the Gateway plot. The second (USGA) plot at Gateway was not included in the 2005 study.

After inoculation, the plots were irrigated twice a week to return 100% estimated evapotranspiration. Individual clones in all plots in both years were rated visually as disease severity in terms of percent diseased area over several rating dates as the disease progressed through the season. These rating dates, plots

**Table 1** The experimental plots at three locations used for evaluation of '549 × 372' population for dollar spot resistance in 2004 and 2005

| Year | Locations | | |
|---|---|---|---|
| | O. J. Noer Turfgrass Research and Educational Facility, Verona, WI[a] | Gateway Golf Club, Land O' Lakes, N. WI[b] | USGA turf research plot, University of Illinois, Urbana-Champaign, IL[c] |
| 2004 | Rating 1: July 17 (OJN-R1-04)<br>Rating 2: July 28 (OJN-R2-04)<br>Rating 3: August 9 (OJN-R3-04)<br>Rating 4: August 30 (OJN-R4-04) | Rating 1: September 14 (GW-R1-04)<br>Rating 2: October 12 (GW-R2-04)<br>Rating 1: USGA plot: September 15 | – |
| 2005 | Rating 1: July 1 (OJN-R1-05)<br>Rating 2: July 15 (OJN-R2-05) | Rating 1: August 19 (GW-R1-05)<br>Rating 2: September 16 (GW-R2-05) | Rating 1: June 24 (IL-R1-05)<br>Rating 2: July 12 (IL-R2-05) |

The rating dates at each plot are also presented. All the plots were artificially inoculated with a single *S. homoeocarpa* isolate MNI

[a] One plot consisting of 697 progeny from the '549 × 372' population was established in 2003. This plot was inoculated in 2004 and 2005

[b] Two plots were established in 2003, and one plot consisted of 697 progeny from the '549 × 372' population. The second plot consisted of 188 progeny from the '549 × 372' population and was part of the United States Golf Association creeping bentgrass breeding consortium. Both plots were inoculated in 2004, but the USGA plot was not inoculated in 2005

[c] One plot consisting of 188 progeny from the '549 × 372' population was established in 2003. This plot was part of the United States Golf Association creeping bentgrass breeding consortium, and was not inoculated in 2004

and locations are explained in Table 1. Only the USGA plot at Gateway in 2004 was rated by a different rater and a rating scale of 0–9 was used (0 = no disease, 9 = completely brown plant).

## Statistical analysis of data

Dollar spot severity data were analyzed by general linear models analysis of variance, assuming all factors to have random effects (SAS Institute 1999). Analysis was based on dollar spot data from 167 progeny clones of the '549 × 372'mapping population, rated on 17 July, 2004 and 1 July, 2005 at O. J. Noer, and 14 September, 2004 and 19 August, 2005 at Gateway. Initially, a modest sample size of 200 clones was chosen for this analysis, but due to missing data, the sample size was reduced to 167. Specifically, if more than two replicates of a clone within any of the four data sets were missing, it was necessary to omit that clone from all four data sets. The first rating dates at each location and year were chosen for the analysis, due to uniformity and very distinct nature of the disease symptoms. A random model for all effects was used, and broad sense heritability was calculated over 2 years using the variance components calculated from expected mean squares from the ANOVA (Bonos et al. 2003). In addition, phenotypic correlation of dollar spot severity was analyzed using 188 progeny over all the rating dates at two locations in 2004 and at three locations in 2005 (Table 3) using JMP software (SAS Institute, Cary, NC, USA).

For QTL analysis, linkage maps were constructed separately for each parent '372' and '549' using the double haploid (DH) population type option available in JoinMap (Van Ooijen and Voorips 2001). The marker data set consisted of all the markers (169 RAPD, 180 AFLP, 18 CDO, 3 BCD, 3 RZ and 36 'Ast' creeping bentgrass EST-RFLP markers) that were used to construct our first map (Chakraborty et al. 2005), and an additional 98 heterologous (64 CDO, 33 BCD and 4 RZ) and 76 homologous (Ast) EST-RFLP markers. Analysis of QTL for dollar spot resistance was performed using the two parental maps and the marker data sets separately, again using the DH population type option available in MapQTL software (Van Ooijen et al. 2002). Separate maps were used for QTL analysis due to unequal rates of recombination among markers in the two parents '372' and '549' as observed by previous researchers (Warnke et al. 2004, Curley et al. 2005).

Analysis of QTL was performed for all four ratings at O. J. Noer, two ratings of the plot at Gateway, as well as one rating of the USGA Gateway plot in 2004. In 2005, two ratings from each plot at three locations (Gateway, O. J. Noer and IL) were used for QTL analysis. Percent diseased area of individual clones, and the mean percent diseased area over four clonal replicates of 106 progeny was the phenotypic variable for all ratings except the USGA plot at Gateway (2004) in which a 0–9 scale was used. The consistency of a QTL was further tested by analyzing the QTL using the area under disease progress curve (AUDPC) values at all locations. The AUDPC value was calculated for each of the 106 progeny using the percent diseased area over all the rating dates (Table 1) as the disease progressed at each plot using the mid point rule method (Boulter et al. 2002). The map location, LOD score, and the percentage of phenotypic variance explained by the potential QTL were calculated using interval mapping (Lander and Botstein 1989, Curley et al. 2005).

Nonparametric, single-marker-based Kruskal–Wallis analyses were also performed to check if the QTL detected by interval mapping were significant when they are individually examined. Because there were 351 mapped loci in the '549' map and 321 loci in the '372' map, a genome-wide $P$ value of less than 0.00014 was chosen (Curley et al. 2005). Markers that were close to the potential QTL as detected by interval mapping and Kruskal–Wallis analyses, were chosen as cofactors and tested using automatic cofactor selection criterion in MapQTL. The $P$ value of 0.02, which is the default value in MapQTL, was used as a cutoff value for the elimination of a cofactor. The MQM option was then used in MapQTL for conducting the multiple QTL mapping to estimate the map location, LOD score and the percentage of phenotypic variance. The permutation test of MapQTL with 1,000 iterations was used to determine LOD significance thresholds. In general, a genome-wide 5% threshold and/or $P$ value of less than 0.00014 in the Kruskal–Wallis analysis was set as a LOD value benchmark to declare a QTL to be significant.

However, there were some QTL which did not meet the above criteria but were detected consistently over several ratings, locations and years. These QTL with $P$ value of 0.01 via Kruskal–Wallis analysis are also presented (Tables 4, 5). Any QTL with LOD value below 1.0 using interval mapping was considered not significant (NS) and reported as such. While a low significance threshold of 0.01 might lead to the detection of many false positives, these QTL with small effect that appeared consistently but had a low LOD, were reported in order to prevent Type II errors (Arahana et al. 2001, Curley et al. 2005).

In addition, the significant markers detected on various linkage groups were checked for possible epistatic interactions using JMP software. This was done using

markers (3.AW10.650, 3.Y10.1500 and Ast33) that were significantly associated with the QTL on LG 7.1, 6.2 and 3.2 that were detected over at least two ratings. After QTL were detected using only 106 progeny (Tables 4, 5), single marker analysis was executed using the Kruskal–Wallis method based on 200, 400 and 697 progeny.

## Results

### Disease assessment in field

A continuous frequency distribution for percent diseased area was observed based on 106 progeny, over three locations and all rating dates in both 2004 and 2005 (Fig. 2). No discrete phenotypic classes, such as total absence of lesions in some progeny and presence of lesions in others, were detected. This provided evidence that dollar spot resistance in creeping bentgrass was quantitatively inherited. Visual inspection revealed that the disease developed earlier in all the inoculated plots over 2 years than the non-inoculated fairways and greens adjacent to the research plots. Moreover the disease severity was much higher and more uniform over the artificially inoculated experimental plots compared to non-inoculated neighboring plots.

The first symptoms were tufts of bleached grass blades which rapidly coalesced to form distinct spots 3 to 5cm in diameter. In the resistant progeny clones, the number of bleached areas or spots were not only fewer but they did not rapidly increase in area. In susceptible clones, coalescence with neighboring spots often occurred, thereby blighting large areas ranging up to 90% diseased area of individual clones. At all the plots, after artificial inoculation, symptoms appeared rapidly, infecting almost all the progeny within 14–17 days. This was followed by an increase in disease severity over time, as shown by the increase in the mean diseased area over 106 progeny (Fig. 2).

Throughout all the rating dates and locations in 2004 and 2005, the two parents '372' and '549' were not significantly different from each other in susceptibility to dollar spot. Transgressive segregation was detected with the two parents being intermediate in resistance whereas the progeny showed a significant difference in resistance, ranging from 2 to 90% diseased area (Fig. 2). Even though the two parents had very similar percent diseased area, we did notice differences between them in the extent of bleaching on individual tillers or shoots. In the parent '372' the lesions or necrotic tissue appeared to be superficial extending only to the leaf blades, but in the parent '549' the necrosis extended further, blighting a major portion of each tiller. In some progeny, the tillers were completely killed, causing dead patches of turf to appear on individual clones as depressions or pits.

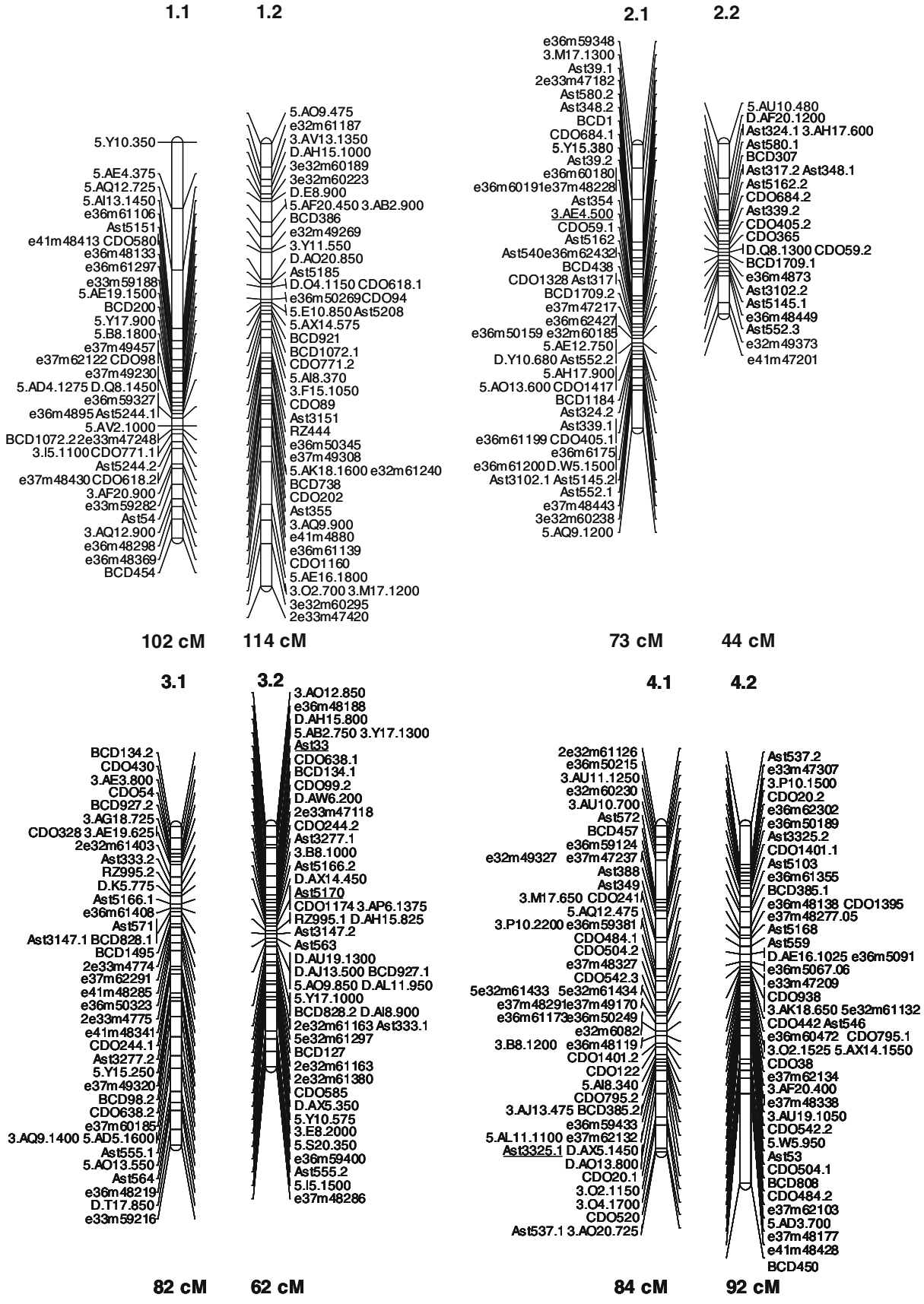
In 2004, there were three plots, one at O. J. Noer, and two at Gateway (Table 1). In 2005, there were three locations (Fig. 2g, h—Urbana-Champaign; i, j—O. J. Noer; k, l—Gateway) with one plot at each location and two rating dates at each plot (Table 1). In both 2004 and 2005, the dollar spot symptoms at the O. J. Noer plot started developing much earlier than the Gateway plots. However, we did notice differences in the time of symptom development in a particular location between years. In 2004, dollar spot symptoms first began to appear around the end of June at the O. J. Noer plot whereas in 2005, by July 1 the plot had sufficient disease for rating (Fig. 2). We found that the mean diseased area over 106 progeny at O. J. Noer on 15 July, 2005 was 48.7% compared to 17 July, 2004 when the mean disease was only 23.6%. Similarly, the disease symptoms at Gateway started to develop earlier in 2005 compared to 2004 (the mean percent diseased area was 52.0% on August 19, 2005 but only 22.6% on 14 September, 2004). In 2005, among three locations (Table 1), the Urbana-Champaign plot at Illinois was the first to be inoculated (May 2) and the symptoms first appeared at the beginning of June, much earlier than the other plots in Wisconsin.

At the O. J. Noer plot in both 2004 and 2005, and at the Urbana-Champaign plot in 2005, the disease severity in each clone increased as the disease progressed through the season. This was further illustrated by the increase in mean percent diseased area based on 106 progeny, from the first rating date to the last rating date at both of these locations (Fig. 2a–d, i, j).

The last rating date at Gateway plot in both years (Fig. 2f, l) showed a higher number of plants with less diseased area than the first rating date (Fig. 2e, k), contrary to the O. J. Noer and Urbana-Champaign plots. Moreover, at the Gateway plot in 2004, unlike the O. J. Noer and Urbana-Champaign plots, the mean disease remained the same as the disease progressed (Fig. 2e, f). Also, the mean percent diseased area at both rating dates at Gateway plot (Fig. 2e, f, Fig. 2k, l) in 2004 and 2005 were lower than the mean disease at the O. J. Noer plot.

### Analysis of variance, heritability and phenotypic correlation

The ANOVA (Table 2) showed significant clone, year, location and clone × environment interactions. The mean square value for clone effect was higher than the mean square values for the individual clone ×


1.1
1.2
5.Y10.350
5.AE4.375
5.AQ12.725
5.AI13.1450
e36m61106
Ast5151
e41m48413 CDO580
e36m48133
e36m61297
e33m59188
5.AE19.1500
BCD200
5.Y17.900
5.B8.1800
e37m49457
e37m62122 CDO98
e37m49230
5.AD4.1275 D.Q8.1450
e36m59327
e36m4895 Ast5244.1
5.AV2.1000
BCD1072.22e33m47248
3.I5.1100 CDO771.1
Ast5244.2
e37m48430 CDO618.2
3.AF20.900
e33m59282
Ast54
3.AQ12.900
e36m48298
e36m48369
BCD454
5.AO9.475
e32m61187
3.AV13.1350
D.AH15.1000
3e32m60189
3e32m60223
D.E8.900
5.AF20.450 3.AB2.900
BCD386
e32m49269
3.Y11.550
D.AO20.850
Ast5185
D.O4.1150 CDO618.1
e36m50269CDO94
5.E10.850 Ast5208
5.AX14.575
BCD921
BCD1072.1
CDO771.2
5.AI8.370
3.F15.1050
CDO89
Ast3151
RZ444
e36m50345
e37m49308
5.AK18.1600 e32m61240
BCD738
CDO202
Ast355
3.AQ9.900
e41m4880
e36m61139
CDO1160
5.AE16.1800
3.O2.700 3.M17.1200
3e32m60295
2e33m47420
102 cM
114 cM
2.1
2.2
e36m59348
3.M17.1300
Ast39.1
2e33m47182
Ast580.2
Ast348.2
BCD1
CDO684.1
5.Y15.380
Ast39.2
e36m60180
e36m60191e37m48228
Ast354
3.AE4.500
CDO59.1
Ast5162
Ast540e36m62432
BCD438
CDO1328 Ast317
BCD1709.2
e37m47217
e36m62427
e36m50159 e32m60185
5.AE12.750
D.Y10.680 Ast552.2
5.AH17.900
5.AO13.600 CDO1417
BCD1184
Ast324.2
Ast339.1
e36m61199 CDO405.1
e36m6175
e36m61200 D.W5.1500
Ast3102.1 Ast5145.2
Ast552.1
e37m48443
3e32m60238
5.AQ9.1200
5.AU10.480
D.AF20.1200
Ast324.1 3.AH17.600
Ast580.1
BCD307
Ast317.2 Ast348.1
Ast5162.2
CDO684.2
Ast339.2
CDO405.2
CDO365
D.Q8.1300 CDO59.2
BCD1709.1
e36m4873
Ast3102.2
Ast5145.1
e36m48449
Ast552.3
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e41m47201
73 cM
44 cM
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3.2
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CDO430
3.AE3.800
CDO54
BCD927.2
3.AG18.725
CDO328 3.AE19.625
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RZ995.2
D.K5.775
Ast5166.1
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Ast571
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BCD1495
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e37m62291
e41m48285
e36m50323
2e33m4775
e41m48341
CDO244.1
Ast3277.2
5.Y15.250
e37m49320
BCD98.2
CDO638.2
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3.AQ9.1400 5.AD5.1600
Ast555.1
5.AO13.550
Ast564
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D.T17.850
e33m59216
3.AO12.850
e36m48188
D.AH15.800
5.AB2.750 3.Y17.1300
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62 cM
4.1
4.2
2e32m61126
e36m50215
3.AU11.1250
e32m60230
3.AU10.700
Ast572
BCD457
e36m59124
e32m49327 e37m47237
Ast388
Ast349
3.M17.650 CDO241
5.AQ12.475
3.P10.2200 e36m59381
CDO484.1
CDO504.2
e37m48327
CDO542.3
5e32m61433 5e32m61434
e37m48291e37m49170
e36m61173e36m50249
e32m6082
3.B8.1200 e36m48119
CDO1401.2
CDO122
5.AI8.340
CDO795.2
3.AJ13.475 BCD385.2
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Ast3325.1 D.AX5.1450
D.AO13.800
CDO20.1
3.O2.1150
3.O4.1700
CDO520
Ast537.1 3.AQ20.725
Ast537.2
e33m47307
3.P10.1500
CDO20.2
e36m62302
e36m50189
Ast3325.2
CDO1401.1
Ast5103
e36m61355
BCD385.1
e36m48138 CDO1395
e37m48277.05
Ast5168
Ast559
D.AE16.1025 e36m5091
e36m5067.06
e33m47209
CDO938
3.AK18.650 5e32m61132
CDO442 Ast546
e36m60472 CDO795.1
3.O2.1525 5.AX14.1550
CDO38
e37m62134
3.AF20.400
e37m48338
3.AU19.1050
CDO542.2
5.W5.950
Ast53
CDO504.1
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e37m48177
e41m48428
BCD450
84 cM
92 cM

**Fig. 1**

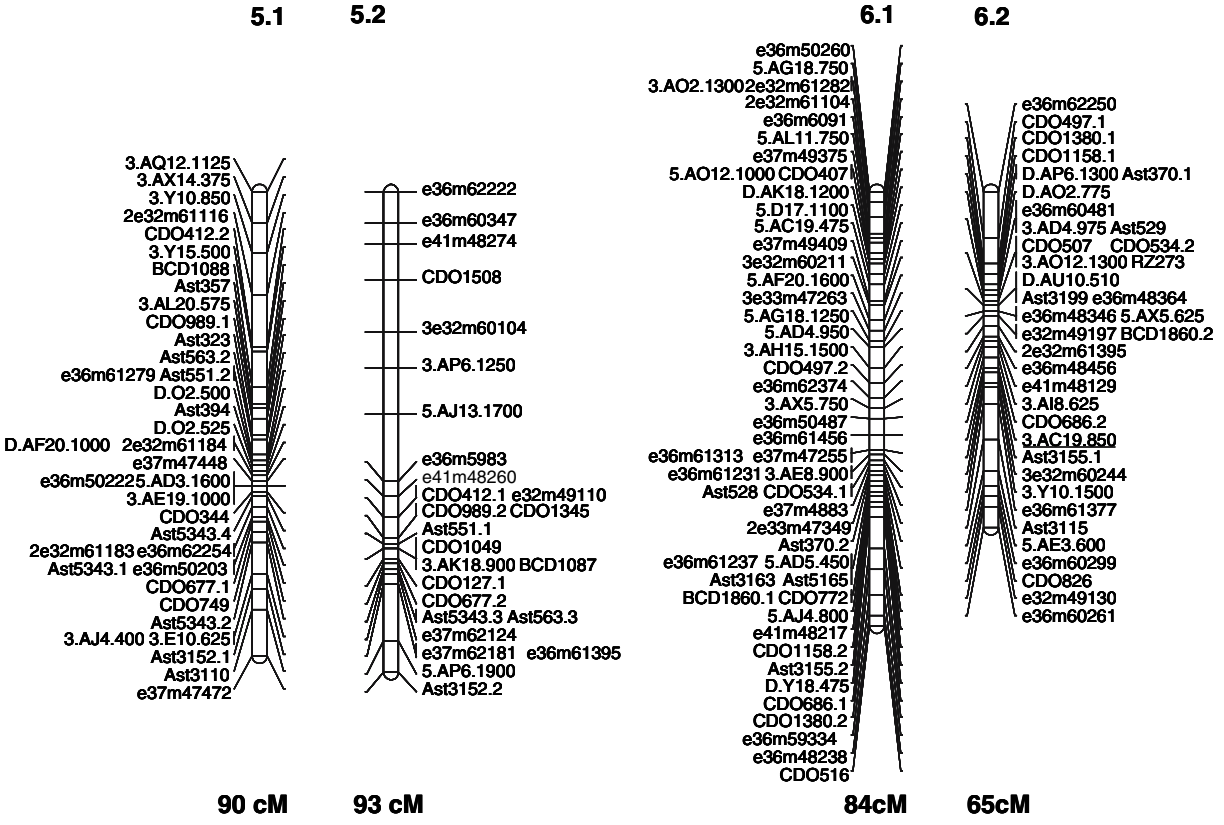


**Fig. 1** Enhanced linkage map of the '549 × 372' population, constructed using RFLP, RAPD and AFLP marker data from Chakraborty et al. (2005) and additional 98 heterologous (BCD, CDO and RZ) and 76 homologous creeping bentgrass Ast EST-RFLP markers. The numbering of all the linkage groups and the naming of the markers were according to Chakraborty et al. (2005). Genetic length in cM of each homologue is indicated below each group. The markers linked to dollar spot resistance QTL with LOD above 2.0 are underlined

environment interactions. The broad sense heritability based on genetic variance values in Table 2 over 2 years and two locations was estimated to be 0.88.

The phenotypic pairwise correlation coefficient values for percent diseased area are presented in Table 3. In general, the O. J. Noer plot ratings in both 2004 and 2005 were highly correlated to each other and also to ratings from Gateway and Urbana-Champaign plots, with coefficients ranging from 0.5 to 0.9. Similarly, the ratings from the Gateway plot were highly correlated to each other over both years, with coefficients ranging from 0.4 to 0.8. Interestingly, the second rating at Gateway was very poorly correlated with both Urbana-Champaign ratings in 2005, and was not significantly correlated ($P > 0.05$) with the rating date of 12 July, 2005 at Urbana-Champaign.

## Enhanced linkage map of the creeping bentgrass population '549 × 372'

An additional 98 heterologous (64 CDO, 33 BCD and 4 RZ) and 76 homologous (Ast) EST-RFLP markers were mapped onto the existing creeping bentgrass linkage map (Chakraborty et al. 2005) (Fig. 1). As expected, 14 linkage groups were detected based on common RFLP markers mapped in previously published linkage map (Chakraborty et al. 2005) and there were 578 mapped loci consisting of 169 AFLP, 170 RAPD and 239 EST-RFLP markers. The average distance between markers was 1.4 cM and the length of each LG varied from 44 cM to 114 cM. Similar to our previous findings (Chakraborty et al. 2005), seven pairs of homoeologous chromosomes have been identified based on RFLP loci duplicated between two LG (Chakraborty et al. 2005). Moreover, two linkage maps that were constructed separately for each parent '372' and '549', had 351 loci mapped in the '549' map and 321 loci in the '372' map. These two separate maps were used for QTL analysis.

## QTL analysis

Quantitative trait loci for dollar spot resistance, measured as percent diseased area, were analyzed for each rating separately using Kruskal–Wallis, interval map-

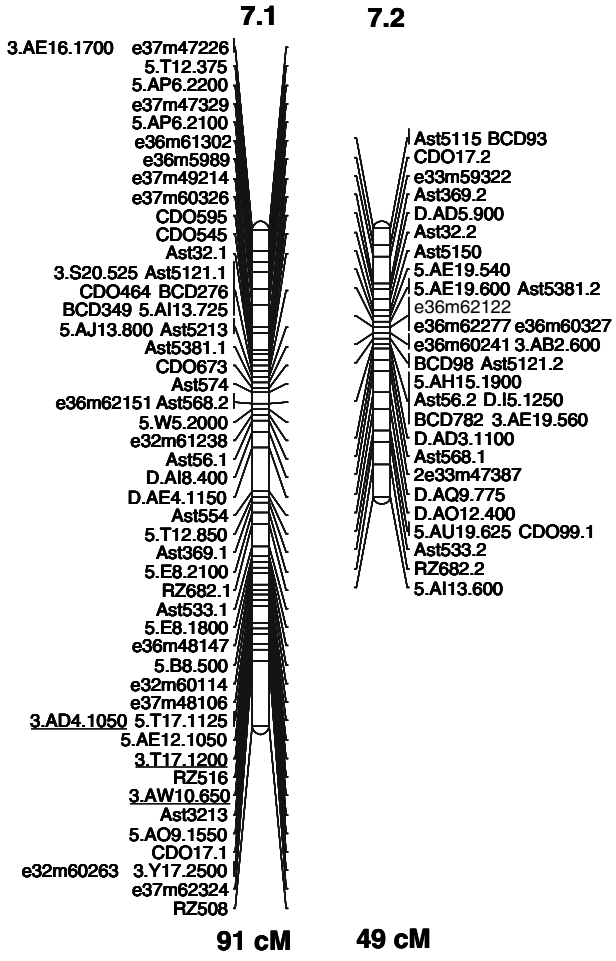


**Fig. 1** continued

ping and MQM analysis (Tables 4, 5), using 106 progeny. In 2004 and 2005, at O. J. Noer, Gateway and Urbana-Champaign, one QTL was detected on LG 7.1 over all rating dates irrespective of rater or rating scale (Tables 4, 5). It showed a very high significance of $P < 0.0001$ to 0.01, LOD ranging from 3.4 to 8.6 according to interval mapping (IM), whereas LOD ranged from 3.7 to 8.8 by MQM analysis. This genomic region explained 14.1–36.0% of the phenotypic variance. The RAPD marker 3.AW10.650 was significantly associated with this QTL over several rating dates in 2004 and 2005 and the 'b' allele (presence of the RAPD band) in parent '372' was linked with reduced percent diseased area.

Two additional RAPD markers (3.T17.1200 and 3.AD4.1050) were also tightly linked to the QTL on LG 7.1 at other rating dates (Table 4). For these two markers, the 'a' allele (absence of the RAPD band) in parent '372' was linked with reduced percent diseased area. These three RAPD markers are less than 10 cM from one another on the LG 7.1 (Fig. 1). Specifically, marker 3.AW10.650 was 4 cM away from 3.T17.1200, whereas it was 8 cM away from 3.AD4.1050. Markers 3.T17.1200 and 3.AD4.1050 are 4 cM away from each other (Fig. 1). When this QTL was analyzed using AUDPC values as the phenotypic variable, the significance ranged from $P < 0.0001$ to 0.01 based on Kruskal–Wallis analysis (Tables 4, 5). The LOD plots for this QTL, calculated using AUDPC values over all the rating dates, are shown in Fig. 3.

Moreover, we detected QTL on LG 2.1, 3.2, 4.1, 4.2, 6.2, and 7.2 (Tables 4, 5). Some of these QTL with smaller effect were only detected at specific rating dates, years and locations. These QTL had a weaker significance ($P = 0.005$–0.01) with a lower LOD value (1.2–3.2) based on interval mapping and explained a much smaller portion of the phenotypic variance (5.9–15.0%).

In 2004, at O. J. Noer, the QTL on LG 4.2 was detected on two rating dates (July 28 and Aug. 9), and the QTL on LG 6.2 was consistently detected on three rating dates (July 28, Aug. 9, and Aug. 30). These two QTL were also detected using AUDPC values. The QTL on LG 6.2 was also detected at the first Gateway plot on the 12 October, 2004 rating date, as well as the Gateway USGA plot (Table 4). Interestingly, the QTL on LG 6.2 was not significant at any locations in 2005.

However, in 2005, QTL with smaller effect on LG 3.2 and 7.2 were detected at several rating dates and different locations (Table 5). The QTL on LG 3.2 was not significant at any rating date in 2004, but in 2005 it was detected consistently at both ratings in the Gateway and O. J. Noer plots. The QTL on LG 7.2 was detected at the Urbana-Champaign plot at both rating dates in 2005, unlike in 2004 when it was detected only at the first Gateway plot on September 14 (Tables 4, 5).

We also detected a smaller effect QTL on LG 3.2, with LOD ranging from 1.4 to 2.5, and this was the only QTL in which the allele associated with reduced disease severity seemed to be contributed by the parent '549' (Table 5). This QTL was reported irrespective of its low LOD based on interval mapping, because it was consistently detected at O. J. Noer in both ratings of 2005, and also at the Aug. 19 rating at Gateway. This QTL was also detected at O. J. Noer in 2005 with an LOD score of 2.1 when the AUDPC values over two rating dates were used for QTL analysis. However, it was not detected in 2004. This QTL was closely linked to RFLP markers CDO1174 and Ast5170, which were both very close to each other (<1 cM in Fig. 1).

The QTL on LG 7.1 always exceeded the genome-wide significance threshold (except the rating at Gateway on October 12, 2004). The smaller effect QTL generally failed to exceed the genome-wide significance threshold, but were reported since they appeared consistently either over rating dates, years or locations, or all of the above.

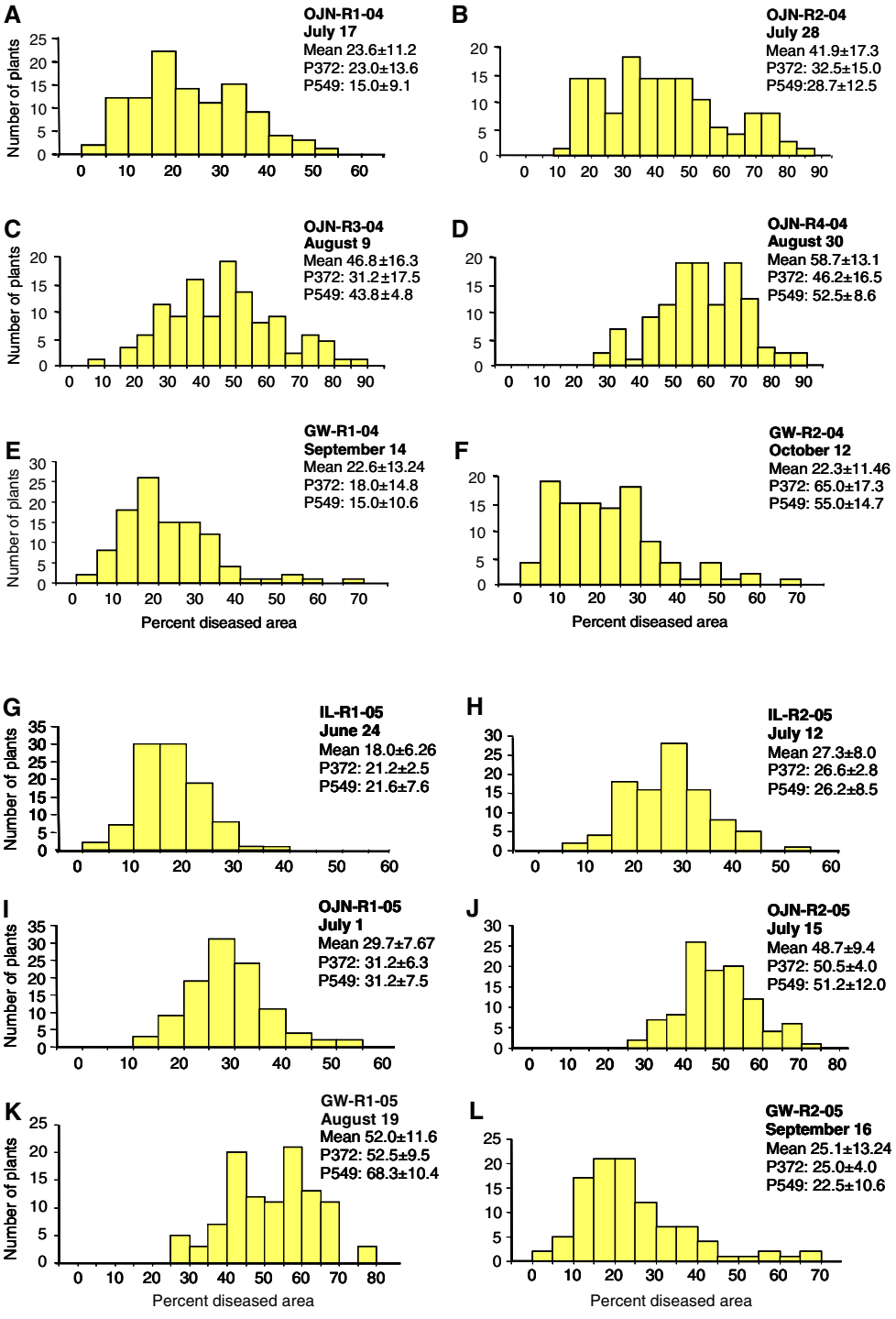


**Fig. 2** Frequency distribution of dollar spot severity (percent diseased area) of 106 progeny from '549 × 372' population in 2004 at two locations: A-D at O. J. Noer Turfgrass Research and Educational Facility (OJN), WI, and E and F at Gateway Golf Club (GW), WI and in 2005 at three locations: G and H at Urbana-Champaign, IL, I and J at O. J. Noer and K and L at Gateway, WI. Mean percent diseased area, percent diseased area of the parents ('372' and '549'), and standard deviation over 106 progeny and two parents are shown. Each rating is denoted by the location (OJN, GW or IL) followed by the order of rating as R1, R2 etc., and the year

For the validation of the associated markers, we have examined the significance of several QTL (LG 7.1, 6.2 and 3.2), with both large and small effects, by performing single marker analysis using RAPD markers (3.AW10.650, 3.Y10.1500 and Ast33) and various sample sizes of 200, 400, and 697 progeny individuals. The QTL on LG 6.2 was significant ($P = 0.005$) in 2004 at O. J. Noer but not at Gateway, at a sample size of 200. However, this QTL was not detected at sample sizes of 400 or 697, and was not detected at all in 2005 (data not shown). The LG 3.2 QTL was not detected at any location or year using a sample size of more than 106. However, unlike the smaller effect QTL, the QTL on LG 7.1 showed very high significance ($P < 0.0001$) even at a sample size of 697 at all locations and years.

**Table 2** Analysis of variance of dollar spot severity (percent diseased area) of 167 progeny from '549 × 372' creeping bentgrass population

| Sources of variation | *df* | Mean square | *F* value | $P > F$ | $\sigma^2$ |
|---|---|---|---|---|---|
| Year | 1 | 15,410 | 106.2 | <0.0001 | |
| Location | 1 | 239,288 | 1648.7 | <0.0001 | |
| Replication (location) | 6 | 1,888 | 13.0 | <0.0001 | |
| Year × rep (loc) | 6 | 1,186 | 8.2 | <0.0001 | |
| Year × loc | 1 | 1,506 | 10.4 | 0.0013 | |
| Clone | 166 | 2,123 | 7.03 | <0.0001 | 113.8 |
| Clone × loc | 166 | 228 | 0.90 | 0.7987 | 0.0 |
| Clone × rep (loc) | 979 | 182 | 1.26 | 0.0003 | 18.5 |
| Clone × year | 166 | 219 | 1.01 | 0.4647 | 0.4 |
| Clone × year × loc | 166 | 216 | 1.49 | 0.0002 | 17.8 |
| Error | 890 | 145 | | | 145.0 |

Data from one rating at each of the two locations (O. J. Noer Turfgrass Research and Educational Facility, and Gateway Golf Club, WI) in 2004 and 2005 were used for the analysis

## Discussion

### Disease assessment and statistical analysis

A continuous frequency distribution for dollar spot severity was observed (Fig. 2), which was very similar to previous research (Bonos et al. 2003) and provided evidence that dollar spot resistance in creeping bentgrass was quantitatively inherited. In the resistant progeny clones, the number of necrotic spots was fewer, did not coalesce to form larger areas and the lesions were restricted to leaves. The mechanisms of dollar spot resistance are unknown but researchers (Bonos et al. 2003, 2004) have made similar observations where resistant creeping bentgrass clones maintained smaller lesion diameter sizes and the lesions rarely coalesced to form large dollar spots compared to susceptible clones. This might indicate the formation of induced fungal inhibiting compounds in the resistant clones, which might play a role in reducing the spread of the pathogen horizontally to neighboring healthy tissues as well as vertical damage in each tiller. However, future studies identifying preformed and induced defense response compounds might resolve the mechanism of dollar spot resistance in creeping bentgrass.

Analysis of variance of percent diseased area ratings for dollar spot resistance (Table 2) detected significant effects of clone, year and location, and significant clone × environment interaction. The significant year effect can be explained by the hotter, drier summer in 2005 at the O. J. Noer and Gateway locations compared to summer of 2004, which was much cooler with higher amounts of rainfall. Studies have shown that

**Table 3** Phenotypic pairwise correlations for dollar spot severity (percent diseased area) based on 2 years (2004 and 2005) and several rating dates (denoted by R) at three locations: O. J. Noer Turfgrass Research and Educational Facility (OJN), Gateway Golf Club (GW), WI, and Urbana-Champaign (IL), IL, USA

| | GW-R2-04 | OJN-R1-04 | OJN-R2-04 | OJN-R3-04 | OJN-R4-04 | OJN-R1-05 | OJN-R2-05 | GW-R1-05 | GW-R2-05 | IL-R1-05 | IL-R2-05 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GW-R1-04 | 0.84 | 0.66 | 0.69 | 0.72 | 0.69 | 0.65 | 0.65 | 0.73 | 0.52 | 0.47 | 0.40 |
| GW-R2-04 | | 0.56 | 0.60 | 0.62 | 0.62 | 0.58 | 0.63 | 0.71 | 0.47 | 0.44 | 0.42 |
| OJN-R1-04 | | | 0.91 | 0.90 | 0.80 | 0.68 | 0.67 | 0.65 | 0.45 | 0.51 | 0.42 |
| OJN-R2-04 | | | | 0.94 | 0.85 | 0.70 | 0.71 | 0.64 | 0.46 | 0.57 | 0.42 |
| OJN-R3-04 | | | | | 0.89 | 0.69 | 0.72 | 0.66 | 0.44 | 0.60 | 0.43 |
| OJN-R4-04 | | | | | | 0.68 | 0.75 | 0.67 | 0.50 | 0.61 | 0.43 |
| OJN-R1-05 | | | | | | | 0.90 | 0.61 | 0.40 | 0.44 | 0.41 |
| OJN-R2-05 | | | | | | | | 0.70 | 0.45 | 0.50 | 0.40 |
| GW-R1-05 | | | | | | | | | 0.57 | 0.49 | 0.35 |
| GW-R2-05 | | | | | | | | | | 0.30 | 0.15[a] |
| IL-R1-05 | | | | | | | | | | | 0.46 |

[a] All correlation coefficients significant at $P < 0.00001$ except this value, which was not significant at $P = 0.05$

**Table 4** Summary of the QTL for dollar spot severity (percent diseased area) at O. J. Noer Turfgrass Research and Educational Facility over four ratings and Gateway Golf Club over two ratings in WI in 2004, after artificial inoculation with *S. homoeocarpa* isolate MNI

| Trait[a] | LG[b] | Marker[c] | Kruskal–Wallis[d] | | | IM[e] | | MQM[f] | | LOD thresholds[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *P* value | Allele means | | | | | | |
| | | | | a | b | LOD | Variance (%) | LOD | Variance (%) | $\alpha_g$ |
| O. J. Noer-2004 | | | | | | | | | | |
| Rating-1-July 17 | 372-7.1 | 3.AW10.650 | 0.0001 | 30.6 | 18.2 | 5.8 | 22.0 | 5.8 | 22.5 | 3.0 |
| Rating-2-July 28 | 372-4.2 | Ast53 | 0.005 | 42.3 | 31.5 | NS[h] | | NS | | 3.1 |
| | 372-6.2 | 3.Y10.1500 | 0.005 | 39.9 | 31.0 | 1.2 | 5.9 | NS | | |
| | 372-7.1 | 3.AW10.650 | 0.0001 | 44.0 | 28.0 | 4.7 | 18.4 | 4.7 | 18.4 | |
| Rating-3-August 9 | 372-4.2 | BCD808 | 0.01 | 48.1 | 37.9 | NS | | NS | | 3.0 |
| | 372-6.2 | 3.Y10.1500 | 0.01 | 46.2 | 37.4 | 1.4 | 6.1 | NS | | |
| | 372-7.1 | 3.AW10.650 | 0.0001 | 52.3 | 33.4 | 5.7 | 22.3 | 5.6 | 20.5 | |
| Rating-4-August 30 | 372-4.1 | Ast3325 | 0.01 | 43.1 | 53.2 | 1.9 | 7.8 | 3.6 | 11.3 | 3.0 |
| | 372-6.2 | 3.AC19.850 | 0.005 | 51.0 | 41.1 | 1.9 | 8.0 | 1.5 | 4.5 | |
| | 372-7.1 | 3.AW10.650 | 0.0001 | 56.5 | 39.0 | 5.3 | 21.0 | 7.3 | 24.0 | |
| AUDPC | 372-4.2 | BCD808 | 0.005 | 2902 | 2131 | 1.8 | 8.1 | 1.5 | 5.7 | 3.0 |
| | 372-6.2 | 3.Y10.1500 | 0.005 | 2671 | 2132 | NS | | NS | | |
| | 372-7.1 | 3.AW10.650 | 0.0001 | 3017 | 1902 | 4.3 | 17.4 | 4.0 | 15.0 | |
| Gateway-2004 | | | | | | | | | | |
| Rating-1-September 14 | 372-2.1 | 3.AE4.500 | 0.0005 | 26.7 | 18.4 | 1.8 | 8.0 | 2.8 | 8.8 | 2.9 |
| | 372-7.2 | RZ682.2 | 0.01 | 27.8 | 18.2 | 1.6 | 8.2 | 1.3 | 5.1 | |
| | 372-7.1 | 3.AD4.1050 | 0.0001 | 18.3 | 25.9 | 5.8 | 22.5 | 5.8 | 22.5 | |
| Rating-2-October 12 | 372-6.2 | 3.AC19.850 | 0.01 | 24.5 | 18.1 | 1.4 | 6.0 | 1.7 | 6.3 | 3.0 |
| | 372-7.1 | 3.T17.1200 | 0.01 | 17.9 | 25.4 | 3.4 | 14.1 | 3.4 | 14.1 | |
| AUDPC | 372-7.1 | 3.AD4.1050 | 0.0005 | 503 | 747 | 5.0 | 19.9 | 5.0 | 19.9 | 2.9 |
| USGA September 15 | 372-6.2 | 3.AC19.850 | 0.005 | 4.8 | 4.0 | 2.4 | 10.0 | 2.2 | 7.9 | 3.1 |
| | 372-7.1 | 3.AW10.650 | 0.0001 | 5.0 | 4.0 | 4.5 | 18.3 | 4.5 | 18.3 | |

Results from one rating at the USGA plot at Gateway Golf Club are also presented. QTL were analyzed using percent diseased area and area under disease progress curve (AUDPC) as the phenotypic variables

[a] Trait: disease severity in terms of percent diseased area and rating date, and AUDPC

[b] Linkage group and parental map ('372' or '549') in which each QTL was detected

[c] Name of the marker most closely linked to the QTL in question. Naming of RAPD, AFLP and RFLP markers were described in Chakraborty et al. (2005)

[d] For Kruskal–Wallis analysis, the *P* value at the indicated marker is presented, along with mean phenotypic score of progeny carrying the 'a' or 'b' allele of each marker

[e] For interval mapping (IM), the LOD score and percentage of phenotypic variance explained at each of the named markers are given

[f] For multiple QTL mapping (MQM), the LOD score and percentage of phenotypic variance explained at each of the named markers are given. Also, the named markers were used as cofactors, after being selected for use as cofactors in the automatic cofactor selection test ($P < 0.02$)

[g] LOD thresholds derived from permutation analysis, for each combination of trait and parent linkage map, are given. The $\alpha g$ indicates the 5% LOD threshold for all linkage groups of the parental map in question

[h] QTL that appeared at multiple ratings but with LOD less than 1.0 are presented as not significant (NS)

drought may play a role in predisposing turf to dollar spot infection (Couch 1995, Bonos et al. 2003). In 2005, the disease developed earlier than in 2004 at both locations (Fig. 2). Higher summer temperatures along with drought may have resulted in low moisture microclimate in the field, which may have influenced disease development as seen in other studies such as QTL controlling sheath blight resistance in rice (Zou et al. 2000).

The location effect can be explained by the geographical location of the plots. The Gateway plot is located at Land O' Lakes, WI (USDA hardiness zone 3), which is 350 km north of Verona (USDA hardiness zone 4) where O. J. Noer is situated. Land O' Lakes area has much cooler, and shorter summers compared to south central Wisconsin. Additional differences between the locations are soil type, pH, and management practices, such as irrigation and fertilizer application, all of which may affect the growth and vigor of the host plants. Creeping bentgrass has been shown to be more susceptible to dollar spot when it is stressed, possibly due to a higher amount of senescent tissue (Walsh et al. 1999). Interestingly, the phenotypic correlations among the rating dates

**Table 5** Summary of the QTL for dollar spot severity (percent diseased area) at O. J. Noer Turfgrass Research and Educational Facility and Gateway Golf Club, WI and Urbana-Champaign, IL over two ratings in 2005, after artificial inoculation with *S. homoeocarpa*isolate MNI

| Trait[a] | LG[b] | Marker[c] | Kruskal–Wallis[d] | | | IM[e] | | MQM[f] | | LOD thresholds[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *P* value | Allele means | | | | | | |
| | | | | a | b | LOD | Variance (%) | LOD | Variance (%) | $\alpha_g$ |
| O. J. Noer | 549-3.2 | CDO1174 | 0.01 | 32.3 | 27.9 | 1.4 | 6.4 | NS[h] | | |
| Rating-1-July 1 | 372-3.2 | Ast33 | 0.005 | 27.7 | 33.2 | 2.1 | 9.0 | 1.5 | 5.2 | |
| | 372-7.1 | 3.AD4.1050 | 0.0001 | 26.4 | 34.3 | 7.3 | 28.0 | 6.4 | 22.9 | 2.9 |
| Rating-2-July 15 | 549-3.2 | Ast5170 | 0.005 | 45.1 | 54.1 | 2.5 | 11.8 | 2.5 | 11.8 | |
| | 372-3.2 | Ast33 | 0.005 | 45.6 | 53.2 | 2.7 | 11.6 | 1.7 | 5.4 | |
| | 372-7.1 | 3.T17.1200 | 0.0001 | 44.3 | 53.3 | 7.6 | 29.0 | 6.6 | 23.0 | 3.0 |
| Gateway | 549-3.2 | Ast5170 | 0.01 | 22.1 | 33.2 | 1.5 | 7.0 | NS | | |
| Rating-1-August 19 | 372-3.2 | Ast33 | 0.01 | 22.0 | 31.5 | 2.6 | 11.3 | NS | | |
| | 372-7.1 | 3.AD4.1050 | 0.001 | 20.6 | 30.7 | 3.8 | 16.1 | 3.4 | 12.2 | 2.9 |
| Rating-2-September 16 | 372-3.2 | Ast33 | 0.01 | 48.0 | 54.0 | 1.8 | 8.0 | NS | | |
| | 372-7.1 | 3.AW10.650 | 0.0001 | 57.0 | 47.0 | 5.0 | 20.0 | 5.0 | 19.0 | 3.1 |
| Urb. Champaign | 372-7.2 | 3.AI13.1000 | 0.005 | 19.3 | 15.5 | 2.3 | 10.9 | 2.1 | 8.2 | |
| Rating-1-June 24 | 372-7.1 | 3.AD4.1050 | 0.0001 | 15.6 | 20.2 | 3.7 | 17.7 | 3.7 | 16.1 | 2.8 |
| | 372-7.2 | 3.AI13.1000 | 0.05 | 28.7 | 24.9 | 1.8 | 9.0 | 1.6 | 5.7 | |
| Rating-2-July 12 | 372-7.1 | 3.AD4.1050 | 0.0001 | 23.3 | 31.6 | 6.3 | 26.6 | 6.0 | 23.4 | 3.0 |
| AUDPC | 549-3.2 | Ast5170 | 0.005 | 19.0 | 30.0 | 2.1 | 10.0 | 2.0 | 10.0 | |
| O. J. Noer | 372-3.2 | Ast33 | 0.001 | 32.0 | 50.0 | 2.6 | 11.0 | 2.0 | 6.3 | |
| | 372-7.1 | 3.AW10.650 | 0.0001 | 64.0 | 35.0 | 8.4 | 32.0 | 8.0 | 27.0 | 3.0 |
| Gateway | 549-3.2 | Ast5170 | 0.01 | 18.3 | 28.0 | 1.5 | 7.0 | NS | | |
| | 372-3.2 | Ast33 | 0.01 | 32.1 | 46.0 | 2.8 | 12.0 | 1.8 | 6.0 | |
| | 372-7.1 | 3.T17.1200 | 0.0001 | 37.0 | 57.0 | 4.7 | 20.0 | 4.2 | 15.0 | 3.0 |
| Urbana-Champaign | 372-7.2 | 3.AI13.1000 | 0.005 | 50.0 | 33.0 | 3.2 | 15.0 | 3.8 | 11.0 | |
| | 372-7.1 | 3.AD4.1050 | 0.0001 | 29.0 | 57.0 | 8.6 | 36.0 | 8.8 | 28.0 | 3.1 |

QTL were analyzed using percent diseased area and area under disease progress curve (AUDPC) as the phenotypic variables

[a] Trait: disease severity in terms of percent diseased area and rating date, and AUDPC

[b] Linkage group and parental map ('372' or '549') in which each QTL was detected

[c] Name of the marker most closely linked to the QTL in question. Naming of RAPD, AFLP and RFLP markers were described in Chakraborty et al. (2005)

[d] For Kruskal–Wallis analysis, the *P* value at the indicated marker is presented, along with mean phenotypic score of progeny carrying the 'a' or 'b' allele of each marker

[e] For interval mapping (IM), the LOD score and percentage of phenotypic variance explained at each of the named markers are given

[f] For multiple QTL mapping (MQM), the LOD score and percentage of phenotypic variance explained at each of the named markers are given. Also, the named markers were used as cofactors, after being selected for use as cofactors in the automatic cofactor selection test ($P < 0.02$)

[g] LOD thresholds derived from permutation analysis, for each combination of trait and parent linkage map, are given. The αg indicates the 5% LOD threshold for all linkage groups of the parental map in question

[h] QTL that appeared at multiple ratings but with LOD less than 1.0 are presented as not significant (NS)

were usually high (Table 3), but we did notice low correlation coefficient between second rating date at Gateway and both rating dates in 2005 at Urbana-Champaign, which is 710 km south of Land O' Lakes and in USDA hardiness zone 5. These exceptions could be explained by the previously mentioned climatic differences between the two locations.

In both years, the dollar spot symptoms at the O. J. Noer plot started developing much earlier than the Gateway plots and also the mean percent diseased area at Gateway were lower than the mean disease at the O. J. Noer plot (Fig. 2e, f, k, l). This indicated that at Gateway, the temperatures needed for maximum pathogenicity did not occur over an extended time period. As a result, the pathogen got a very narrow opportunity to cause a level of disease severity similar to that observed at O. J. Noer.

The last rating date at Gateway plot in both years (Fig. 2f, l) showed a higher number of plants with less diseased area than the first rating date (Fig. 2e, k), contrary to what had been observed at the O. J. Noer and Urbana-Champaign plots. This indicated that the disease pressure was low due to less favorable temperatures for the pathogen in September and October at Gateway, and also that the plants have started recovering from the dollar spot damage. Creeping bentgrass is

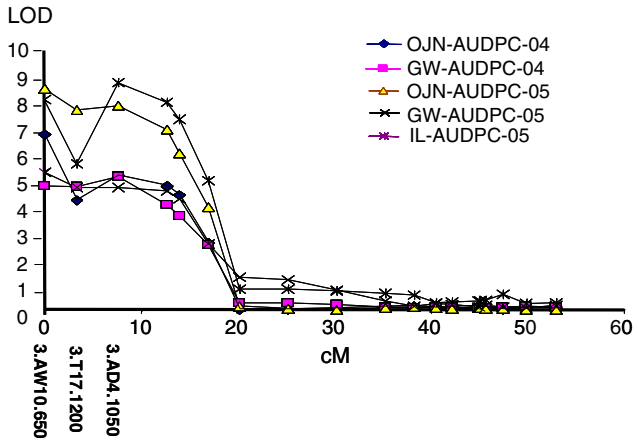


**Fig. 3** Plots of LOD scores for QTL on linkage group 7.1 calculated using the area under the disease progress curve (AUDPC) of dollar spot severity (percent diseased area) based on 106 progeny of the '549 × 372' mapping population. The disease severity data from all rating dates at the three locations, O. J. Noer Turfgrass Research and Educational Facility, WI, Gateway Golf Club, WI, and Urbana-Champaign, IL in both 2004 and 2005, were used to calculate AUDPC. In 2004, AUDPC was calculated at two locations, and it was calculated at three locations in 2005. All plots were artificially inoculated with a single *Sclerotinia homoeocarpa* isolate, MNI. Positions of significant markers (3.AW10.650, 3.T17.1200, and 3.AD4.1050) associated with percent diseased area are indicated. Each LOD plot is denoted by the location (OJN, GW or IL) followed by the year of data collection

a cool season grass and the optimal temperature for growth is around 13–18°C (Warnke 2003). Due to a short summer, the clones at Gateway started to regrow and recover as the temperature decreased much earlier in fall compared to the other locations. We noticed a high standard deviation and a replicate effect over all locations and years (Fig. 2; Table 2). This might have resulted from variation within a plot due to microclimate caused by presence of forests bordering certain edges of the field.

We also noted significant interactions between clones and environment (Table 2). However, the MS values for all four of these interactions were much lower than the MS value for the genotype effect, indicating that even though disease severity differed over years and locations, the clones generally maintained the similar rank order. It also suggested a large genetic component in the resistance. The magnitude of this component can be estimated using broad sense heritability calculations. Heritability is important to plant breeders since traits with high heritability can be improved through selection and breeding more rapidly than traits with lower heritabilities. Even though broad sense heritability includes the variance due to dominance and epistatic effects, we detected a high heritability estimate (0.88) in our study, which was also very similar to previous estimates of 0.90 that were based on clonal mean (Bonos et al. 2003). This indicated sufficient total genetic variation present to enable the improvement of dollar spot resistance in creeping bentgrass through selection and breeding.

## QTL analysis

We have detected fifteen markers with significant association to dollar spot resistance in the '549 × 372' creeping bentgrass population. The significant markers were located on eight linkage groups (Fig. 1) and they were linked to eight QTL. One QTL was found with large effect on LG 7.1 explaining 14.1–36.0% of the phenotypic variance, whereas the QTL with smaller effects each explained less than 15%. The remaining phenotypic variation, which could not be explained by the detected QTL might come from other sources such as (1) measurement error, (2) environmental variation within the field, (3) QTL with effects too small to be detected with confidence in this experiment, and (4) interactions between QTL which were too small to be detected in this experiment (Paterson et al. 1991). The significance threshold of 0.01 was low and might have led to the detection of many false positives, but this relaxed threshold allowed us to avoid Type II errors (rejection of weak but valid QTL). This approach has been undertaken by many studies especially in the exploratory phase of research, since reported false positives will undergo further studies (Arahana et al. 2001, Portyanko et al. 2005) which will help to resolve the importance of these genomic regions to dollar spot resistance.

Of all the QTL we detected, it was noted that only the QTL on LG 7.1 was robust over all locations, years, rating dates, raters and rating scales (Table 4). We also detected this QTL when AUDPC values were used as phenotypic variables based on all the ratings at a particular plot. AUDPC takes into account all the rating dates and gives a better understanding of the disease severity over time as the disease progressed. Therefore, detection of the QTL on LG 7.1 provided evidence that this particular genomic region might not be very sensitive to changes in environmental conditions. Several QTL studies (Paterson et al. 1991, Zou et al. 2000, and Portyanko et al. 2005) have identified QTL with large effects which are consistent over environments, while QTL with smaller effects seemed to be environment sensitive.

One possible explanation for detecting certain location or rating date specific QTL might be due to certain isolates which are endemic to particular areas. Isolate specific QTL for disease resistance have been detected

in several systems, such as rice blast (Sallaud et al. 2003) and barley leaf rust (Qi et al. 1999). However, a greenhouse pathogenicity study in our lab (Chakraborty et al. 2006) has demonstrated lack of *S. homoeocarpa* isolates by bentgrass cultivars interactions. Since our study was based in the greenhouse and only on 23 isolates, further field evaluations with artificial inoculation using different isolates will provide additional information on effect of these isolates on the QTL detected in this study.

Another more likely explanation for these location, year, and rating date specific QTL might be due to the previously mentioned soil and climatic differences between the years and locations. These differences were manifested by the clone × environment interactions, and even though they were small compared to the clone effect, both the host and the pathogen may have been influenced to an extent sufficient to affect QTL significance in the different ratings. Similar results have been found previously (Paterson et al. 1991, Zou et al. 2000, and Portyanko et al. 2005). We did not detect any interaction between QTL of both large and small effects located on different linkage groups. This provided evidence of lack of epistatic interaction and suggested that the most of the effects were likely to be additive.

Analyzing the significance of several QTL, with both large and small effects, using various sample sizes of 200, 400, and 697 progeny individuals showed that the smaller effect QTL were significant at lower sample size only in particular locations or years. One explanation of detecting these QTL might be due to overestimating their effect at a small sample size, resulting in their disappearance with increase in sample size. The importance of these smaller effect QTL might be examined by increasing the number of progeny with marker data, in order to increase the power of QTL detection (Melchinger et al. 1998). However, unlike the smaller effect QTL, the QTL on LG 7.1 showed very high significance ($P < 0.0001$) even at a sample size of 697.

The robustness of the QTL on LG 7.1 clearly provided evidence that this particular genomic region played a key role in dollar spot resistance. The RAPD markers linked to this QTL will be excellent candidates for developing PCR based markers which will be cost effective in marker assisted selection for creeping bentgrass breeding. However, we still do not know if this QTL is influencing the physiological resistance or escape mechanism in the host as seen in other diseases such as white mold in common bean (Miklas et al. 2001) and Sclerotinia stem rot in soybeans (Arahana et al. 2001).

Other studies have examined synteny between significant QTLs affecting other traits within a species or across species (Curley et al. 2005). Therefore, the Gramene website was used to search for QTLs for other traits in rice chromosome 6, which is syntenic with the genomic region containing the large effect QTL on LG 7.1 in creeping bentgrass. Several DTHD (days to heading QTL) have been detected in rice, located at a range of 5–30 cM from marker RZ516. In creeping bentgrass, RZ516 mapped 3 cM away from AW10.650, which was tightly linked to the major QTL for dollar spot resistance. Also, the Hd3b gene controlling heading date has been reported to be located at marker R1952 on rice chromosome 6. Comparative mapping reveals that Hd3b is located 7 cM away from RZ516. Moreover QTL for plant height, leaf length and seedling vigor are also located close to RZ516. However the relevance of these QTLs to dollar spot resistance is not yet known.

Additionally, several QTLs for disease resistance have been reported on rice chromosome 6. Therefore, comparative analyses of genomic locations for disease resistance in rice chromosome 6 with creeping bentgrass LG 7 was also conducted. A QTL for BPHRS (brown planthopper resistance) is located 5 cM away from RZ516 (Xu et al. 2002), and a blast resistance QTL is located close to RG213 (Talukder et al. 2005), 30 cM away from RZ516. However, QTL for BBRS (bacterial blight disease resistance) and SHBTRS (sheath blight disease resistance) have not been reported on chromosome 6. It would have been interesting if QTL for SHBTRS caused by *Rhizoctonia solani* Kuhn had been detected close to the genomic region for dollar spot resistance, since *S. homoeocarpa* and *R. solani* may have similar pathogenicity. Again the significance of this syntenic QTL information is unclear although it serves to indicate relevant areas for further work.

In conclusion, this was the first study involving QTL mapping for field resistance to dollar spot in creeping bentgrass. We have demonstrated that dollar spot resistance was quantitatively inherited and that resistance was highly heritable. Further, we have identified QTL, one with large effect and seven with smaller effects. The large effect QTL was robust over all locations, years, rating dates, raters, rating scales, and different population sizes. The RAPD markers tightly linked to this QTL have potential application for marker-assisted selection of dollar spot resistance in creeping bentgrass breeding programs.

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