Identification and Mapping of Nucleotide Binding Site–Leucine-rich Repeat Resistance Gene Analogs in Bermudagrass

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ABSTRACT. Thirty-one partial bermudagrass (Cynodon spp.) disease-resistance gene analogs (BRGA) were cloned and sequenced from diploid, triploid, tetraploid, and hexaploid bermudagrass using degenerate primers to target the nucleotide binding site (NBS) of the NBS–leucine-rich repeat (LRR) resistance gene family. Alignment of deduced amino acid sequences revealed that the conserved motifs of the NBS are present and all sequences have non-Drosophilan melanogaster Toll and mammalian interleukin-1 receptor (TIR) motifs. Using a neighbor-joining algorithm, a dendrogram was created and nine groups of deduced amino acid sequences from bermudagrass could be identified from those sequences that span the NBS. Four BRGA markers and 15 bermudagrass expressed sequence tags (ESTs) with similarity to resistance genes or resistance gene analogs were placed on a bermudagrass genetic map. Multiple BRGA and EST markers mapped on T89 linkage groups 1a and 5a and clusters were seen on T89 19 and two linkage groups previously unidentified. In addition, three primers made from BRGA groups and ESTs with similarity to NBS-LRR resistance genes amplify NBS-LRR analogs in zoysiagrass (Zoysia japonica or Z. matrella) or seashore paspalum (Paspalum vaginatum). This gives evidence of conservation of NBS-LRR analogs among the subfamilies Chloridoideae and Panicoidae. Once disease resistance genes are identified, these BRGA and EST markers may be useful in marker-assisted selection for the improvement of disease resistance in bermudagrass.

Bermudagrass is a C4 perennial grass used widely for lawns, sports fields, parks, golf courses, and pastures, and to prevent soil erosion in most of the warmer environments across the world. Common bermudagrass (Cynodon dactylon, 2n = 4x = 36) can be a serious weed that is difficult to eradicate because of stolons that readily root at the nodes, deep rhizomes, and seed production (Webster et al., 2004). Another type of bermudagrass, African bermudagrass (Cynodon transvaalensis, 2n = 2x = 18), is a very fine-textured grass that has poor wear and pest tolerance compared with the improved triploid hybrids (Hanna, 1986; Wiecko, 2007). Improvement of bermudagrass pest tolerance compared with the improved triploid hybrids (2x = 18), is a very fine-textured grass that has poor wear and

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diseases or pests of interest in many plant species has been accomplished by use of traditional plant breeding, the use of marker-assisted selection (MAS) to transfer resistance genes from a resistant genotype to a cultivar, and by genetic transformation. In cases such as diseases that are difficult to phenotype or for which the resistance gene is recessive (noting that many bermudagrass clones are intolerant of selfing), MAS may be the preferred method to develop a product with resistance to a desired agent, as traditional plant breeding often takes years of selection and testing (Xu and Couch, 2008). Currently, transformation of resistance genes from an organism to a desired cultivar (genetic modification) is limited by challenges such as time to receive regulatory approval, public concern about genetic modification, and the specialized handling cost associated with these materials (Casler, 2006; McHughen and Smyth, 2008; Redenbaugh and McHughen, 2004; Sawahel, 1994).

Isolation and characterization of 40 plant resistance genes that confer resistance to a wide range of pathogens such as bacteria, viruses, fungi, oomycetes, and nematodes have been identified. The most abundant class of resistance genes (75%) encodes a centrally located nucleotide binding site (NBS) and a carboxyl terminal block of leucine-rich repeat (LRR) (Radwan et al., 2008). Two major subfamilies of NBS-LRR proteins exist. The Toll and mammalian interleukin-1 receptor (TIR)-NBS-LRR subfamily, found only in dicots, contain an amino-terminal–signaling domain with homology to the TIR (Collier and Moffett, 2009). The coiled-coil (CC)-NBS-LRR subfamily, found in monocots and dicots, contains a CC structure in the amino-terminal signal domain (DeYoung and Innes, 2006).

Studies using degenerate polymerase chain reaction (PCR) primers targeting conserved sequences within and adjacent to the NBS have enabled identification of NBS-LRR gene analogs from many plant species (for a review, see McHale et al., 2006). The conserved sequences of the NBS domain include the phosphate-binding loop (P-loop), kinase-2 motif, kinase-3a motif, as well as conserved blocks of unknown function that include RNBS-A, RNBS-C, Gly-Leu-Pro-Leu (GLPL), RNBS-D, and the hydrophobic domain (HD) (De Young and Innes, 2006). Similar to NBS-LRR cloned resistance genes, many NBS-LRR analogs are clustered in the genomes of various plant species (Brotman et al., 2002). Furthermore, several of these NBS-LRR analogs have been found to be linked to disease-resistance loci (Deng et al., 2000). For example, in potato (Solanum tuberosum), two NBS-LRR resistance gene analogs (RGAs) had very close linkage with the root cyst nematode resistance locus Gro1 (Leister et al., 1996).

Little is known about BRGA or their location in the bermudagrass genome. Therefore, the objective of this research was to identify RGAs in bermudagrass, to create markers tagging these NBS-LRR analogs, and to identify linkage groups where NBS-LRR analogs map. These goals may prove useful for identifying DNA markers for MAS once disease-resistance genes are identified and mapped. Furthermore, cross-species amplification and cloning of disease-resistance expressed sequence tag (EST) analogs were evaluated in other important turfgrass species to determine if the sequences are conserved among the subfamilies of Poaceae.

**Material and Methods**

**Plant material used for isolation of BRGA.** Clonally propagated plant material of T89 and PI 291586 (C. dactylon, 2n = 4x = 36), T574 (C. transvaalensis, 2n = 2x = 18), ‘TifGrand’, ‘Tifway’ (C. dactylon × C. transvaalensis 2n = 3x = 27), and ‘Tifton10’ (C. dactylon, 2n = 6x = 54) bermudagrass genotypes was obtained from the turfgrass breeding program at the University of Georgia, Tifton, except for PI 291586, which was obtained from the U.S. Department of Agriculture Southern Regional Plant Introduction (PI) Station in Griffin, GA. PI 291586 has resistance to tawny mole cricket (Reinert and Busey, 2001). ‘TifGrand’ has better tawny mole cricket resistance than ‘Tifway’ and ‘TifSport’, but is less resistant to the bermudagrass mite (Aceria cynodonensis) than the latter (Hanna et al., 2010). ‘Tifway’ has very high tolerance to the two-lined spittlebug (Hemiptera:Cercopidae) (Shortman et al., 2002) and has tolerance to sting nematodes (Giblin-Davis et al., 1992). ‘Tifton10’ is a highly preferred host to fall armyworm larvae (Chang et al., 1985), but is not preferred by fire ant (Solenopsis invicta) (Reinert, 2009).

**Bermudagrass mapping population.** F1 progeny of a cross between T574 (C. transvaalensis, 2x) and T89 (C. dactylon, 4x) were used to create a bermudagrass linkage map based on single-dose restriction fragments (Bethel et al., 2006). The mapping population was created by W. Hanna (University of Georgia, Tifton). DNA was extracted from 121 F1, field-grown individuals using a PureLink DNA Purification Kit (Invitrogen, Carlsbad, CA).

**DNA extraction and BRGA isolation.** Fresh tissue from the six genotypes was collected, cut into ~5-mm pieces, inserted into 2-mL microtubes with stainless steel beads, and placed in liquid nitrogen. Tissue was then ground using a vortexer, and tubes containing the tissue were replaced in liquid nitrogen to prevent thawing. DNA was isolated using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA). DNA was run on a 1% agarose gel and analyzed on a Nanodrop 2000c (Thermo Scientific, Wilmington, DE) to control for quality and quantity. BRGA were amplified using the previously published degenerate primers to amplify NBS-LRR RGAs (Table 1). Amplicons were generated in a 25-μL reaction volume. Each reaction contained 5 μL of Green GoTaq® reaction buffer (Promega, Madison, WI), 2.5 μL of 25 mM MgCl2, 2.5 μL of 2.5 mM dNTP mix, 0.25 μL of 100 μM degenerate primer pair, 0.25 μL of GoTaq® DNA polymerase (Promega), 12.5 μL of water, and 2 μL of DNA at 10 ng-μL−1. Thermocycling conditions began with 94 °C for 3 min, followed by 36 cycles of 94 °C for 30 s, 45 °C for 1 min, and 72 °C for 1 min and 10 s. A final extension was performed at 72 °C for 10 min. PCR products were run on a 1% agarose gel and bands of the expected size were excised and purified with a Cyclo-Pure agarose gel extraction kit (Amresco, Solon, OH). Fragments were ligated into a pGEM T-Easy vector (Promega) and transformed into Turbo cells (Genlantis, San Diego). Blue-white screening was performed and clones were grown overnight in Luria broth with ampicillin (100 μg·mL−1) and plasmids were purified using a Pure Yield Miniprep System (Promega). Purified plasmids were digested with EcoRI (New England Biolabs, Ipswich, MA) to select for those plasmids with inserts of the expected size. Inserts were sequenced using the Big Dye Terminators, version 3.1, cycle sequencing kit (Applied Biosystems, Carlsbad, CA) and were run on an ABI 3100 Genetic Analysis System at the Laboratory for Plant Genome Technologies (Texas A&M University, College Station).

**BRGA sequence analysis.** Nucleotide sequences were analyzed using Sequencer, version 4.9 (GeneCodes, Ann Arbor, MI).
Arbor, MI) and vector sequence was removed using VecScreen (National Center for Biotechnology Information, 2009). Sequence similarity queries were submitted to GenBank using the BLASTn and BLASTx algorithm from the National Center for Biotechnology Information. Those sequences with similarity to resistance genes or RGA were translated using ExPASy (Gasteiger et al., 2003). Deduced amino acid sequences were aligned using AliBee (Brodsky et al., 1992) in the CLUSTAL W (1.60) format and the Geneious version 4.7.4 software program (Drummond et al., 2009) (Fig. 1). Using the tree builder module of the Geneious software, the genetic distance was calculated between all amino acid sequences using the Jukes-Cantor model (Jukes and Cantor, 1969) and trees were created using the neighbor-joining algorithm. A bootstrap analysis was performed with 1000 repetitions, and branch support of at least 50% is shown on the dendrogram. BRGA were compared with known resistance genes by retrieving the amino acid sequences from GenBank for flax (Linum usitatissimum) L6 (U27081) and M (U73916); rice (Oryza sativa) Pit (AB013449), XAJ (AB002266.1), and Pit (AB379817); Arabidopsis thaliana RPS2 (U12860), RPM1 (NM_111584), RPP13 (AF209731–1), RFL1 (AF074916), and RPP8 (AF089710); tomato (Solanum lycopersicum) PRF (U65391), I2C-1 (AAB63274), and RPP8 (AF089710). Ornamental nightshade (Solanum bulbocastanum) genes Rpi-bt1 (ACI16480) and B149 (AAP29073) were also compared with BRGA as they had high BLASTx similarity to BRGA groups. These sequences were trimmed to include only the P-loop to the hydrophobic domain. Trees were created using the neighbor-joining algorithm as described above.

**Table 1. Degenerate primers corresponding to conserved domains of resistance genes that were used to amplify bermudagrass-resistance gene analogs.**

| Protein region | Primer | Primer sequence (5’–3’) | Reference |
|---------------|--------|------------------------|-----------|
| P-loop NBS-F1/LM638 | GGI GGI GTI GGI AAI ACI AC | Radwan et al., 2008 |
| 16409 | GGI GGI WSI GGI AAR ACI AC | Brotman et al., 2002 |
| PLP | GGI GGI RTI GGI AAR ACI AC | Brotman et al., 2002 |
| HD | IAG GCC(A/C/T) AGN GGN AGN CC | Deng et al., 2000 |
| antiHD1 | ARN GGI ARI CCY TTR CA | Brotman et al., 2002 |
| R11 | AGI GCC(A/C/T) AGN GGN AGN CC | Deng et al., 2000 |
| NBS-R1 | IAG IGC IAG IGG IAG ICC | Radwan et al., 2008 |
| LM637 | ARI GCT ARI GGI ARI CC | Kanazin et al., 1996 |

*P-loop = phosphate-binding loop, HD = hydrophobic domain.*

Genetic mapping of BRGA and disease-resistance EST analogs. Primers were developed from a member of each BRGA group (Fig. 2, list of groups): those BRGA that contain stop codons, and those ESTs that have similarity to genes involved with disease resistance (polymorphic markers listed in Supplemental Table 2; see the online version of this article at ashs.org to view the table). These BRGA and ESTs were amplified and fluorescently labeled for detection using a modified M13-tailed primer method (Boutin-Ganache et al., 2001). PCR reactions were in a 10-μL volume and contained 2 μL of 5× Clear GoTaq® reaction buffer.
Fig. 2. Neighbor-joining tree of deduced amino acid sequences from 44 bermudagrass-resistance gene analogs (BRGA) with known deduced amino acid sequences of nucleotide binding site–leucine-rich repeat resistance genes. Letters (A–I) by each BRGA or expressed sequence tag represent their Group from Fig. 1. Toll and mammalian interleukin-1 receptor (TIR) motifs are present in the deduced amino acid sequences of L6, M, and N. The scale at the bottom of the figure represents amino acid similarity. Bootstrap values greater than 50% are shown.

alignment of 31 BRGA deduced amino acid sequences revealed that conserved NBS motifs are present (Supplemental Fig. 1). The P-loop and hydrophobic domain (which served as degenerate primer sites), RNBS-A-non-TIR, kinase-2, and RNBS-B could be identified in all sequences. The consensus sequence for the P-loop was GG(V/R)GKTT, the RNBS-A-non-TIR was (F/L)(E/D/S/Q)(C/L/R/I)(A/K/R/I/P)(A/L/M/I)W (V/Y/L/H)(A/C)(V/I)/S/T(Q/K/E/D)(T/N/Y/E/D/S)(F/Y), the kinase-2 domain was (L/F)(V/L)(V/1)(L/LD(D/N/I/V/W, the RNBS-B domain was (G/R/N)(S/C/T/R)(R/T/S/N/L)(V/I/T)(V/I/L/P)(I/V/M)/Y(T/I/S)(R/Q)(S/L/K/D), and the hydrophobic domain was GLPLAL. Although one must note that the consensus sequence found in the P-loop and the HD domains is conditioned by the degenerate primers used in the experiment and sequences differing from these sequences may

CROSS-SPECIES AMPLIFICATION OF BRGA OR DISEASE-RESISTANCE EST ANALOGS. Zoysia grass (Z. japonica) cultivars Empire, JaMur, and Meyer; Z. matrella cultivars Zeon, Emerald, and Diamond; st. augustinegrass (Stenotaphrum secundatum) cultivars Sapphire, FX-10, Mercedes, Raleigh, Palmetto, Captiva, Delta Shade, and Classic; seashore paspalum (P. vaginatum) cultivars Sealsle 1, Sealsle 2000, and UGA31), bentgrass (Agrostis palustris, collected by E. Elsner of the University of Georgia, Athens), and centipedegrass (Eremochloa ophiuroides) cultivars TifBlair and Common) samples were obtained to test for BRGA conservation among varying turf species. Amplicons were generated in a 20-μL reaction volume. Each reaction contained 4 μL of 5× Green GoTaq® reaction buffer (Promega), 2 μL of 25 mM MgCl2, 1.6 μL of 2.5 mM dNTP mix, 2 μL of 1 μM forward and reverse primer pair, 0.08 μL of GoTaq® DNA polymerase (Promega), 8.32 μL of water, and 2 μL of DNA at 2.5 ng·μL⁻¹. Thermocycling conditions were the same as listed above for BRGA isolation. PCR amplicons were gel extracted, cloned, and sequenced as previously described above. Sequences were translated and aligned using the same methods as described for the BRGA sequence analysis.

Results

ISOLATION AND IDENTIFICATION OF BRGA SEQUENCES. NBS-LRR analogs in bermudagrass were amplified by the use of degenerate primer pairs NBSF1/NBSR1, PLP/antiHD1, PLP/NBSR1, LM637/LM638, and 16409/R11 (Table 1) from six genotypes representing diploid, triploid, tetraploid, and hexaploid bermudagrass. Amplicons of the expected size (≈500 bp) were gel extracted and cloned. Sixty-four clones were sequenced, of which 49 clones had similarity to resistance genes. Fifteen clones had sequences with similarity to retrotransposons, secretion proteins, and hypothetical proteins. The 49 BRGA (accession numbers GU246998–GU247046) were translated and the deduced amino acid sequences of five BRGA (BRGA38, BRGA81, BRGA 201, BRGA220, and BRGA229) contained stop codons. These five BRGA, along with 13 sequences that were nearly identical to other BRGA sequences, were excluded from the amino acid alignment (Supplemental Fig. 1; see the online version of this article at ashs.org to view the figure).
not be amplified. The presence of a RNBS-A-non-TIR domain and a tryptophan at the end of the kinase-2 domain indicates that all sequences have motifs of non-TIR-NBS-LRR resistance genes (Meyers et al., 1999).

Using the neighbor-joining algorithm, a dendrogram was created from all 44 BRGA, from which eight groups were identified (Fig. 1, A and C–I). The largest cluster, Group A, contains 26 BRGA with sequence similarity to a barley (Hordeum vulgare)-resistance protein analog (CAD45036). Group A sequences were identified from all degenerate primer pairs tested (Table 2). Degenerate primer pairs LM637/LM638 and PLP/NBSR1 amplified BRGA clustering in Groups C and D, respectively. The primer pair PLP/antiHD1 amplified BRGA that clustered into five groups (Table 2, Groups E–I). BRGA were amplified from all six genotypes, with the largest number of BRGA groups amplified from ‘TifGrand’ (Table 2, five groups), followed by ‘Tifton10’ and T89 (Table 2, four groups). BRGA were isolated from ‘Tifway’ and T574, which contained BRGA that clustered in only one group (Table 2, ‘Tifway’ Group A and T574 Group 1). All BRGA had strong BLASTx similarity to NBS-LRR protein analogs from the monocots barley, rice, sugarcane (Saccharum officinarum), and finger millet (Eleusine coracana), except those BRGA clustered in Group C, which have similarity to a characterized rice NBS-LRR resistance gene, *Put*, that enables race-specific resistance against the fungal pathogen Magnaporthe grisea (Hayashi and Yoshida, 2009). From the amino acid alignment, a distance matrix was created displaying the number of substitutions per site between BRGA. Distance values ranged from 0 (identical sequences) to 1.38 for BRGA202 and BRGA216 when compared with BRGA34. BRGA202 and BRGA216 were assigned to Group C whereas BRGA34 was assigned to Group A. The nucleotide sequence of the BRGA spanned from 503 to 538 bp with similarly sized BRGA tending to have similar amino acid sequences. BRGA nucleotide sequences ranges were 536 to 538 bp for Group A, 503 and 509 bp for Group C, 504 bp for Group D, 503 and 504 bp for Group E, 509 bp for Group F, 512 and 514 bp for Group G, 515 bp for Group H, and 518 bp for Group I. After excluding the P-loop and HD, which served as degenerate primer sites, many of the groups of BRGA have different motifs for the RNBS-A-non-TIR, kinase-2, and RNBS-B (Supplemental Fig. 1). For example, each BRGA group contains a different amino acid sequence for the RNBS-B and RNBS-A-non-TIR motifs.

Deduced amino acid sequences spanning the P-loop to the HD from 44 BRGA and 13 known NBS-LRR resistance genes from flax, rice, *A. thaliana*, ornamental nightshade, and tomato were compared using the neighbor-joining algorithm on aligned sequences (Fig. 2, overlapping labels excluded). A representative sequence from each BRGA can be seen for all groups except Group A, which contained three sequences and may suggest that this group could be further subdivided. Genes classified as TIR-NBS-LRRs clustered within one group (Fig. 2, L6, M, and N). Furthermore, the *Put* gene from rice, which confers race-specific resistance against *M. grisea* (Hayashi and Yoshida, 2009), was similar to Group C sequences (Fig. 2). This similarity is in agreement with Group C sequence BLASTx data (Table 2). The ornamental nightshade genes *B149* and *Rpi-bt1*, which confer broad spectrum resistance to *Phytophthora infestans* in transgenic cultivated potato and tomato (Oosumi et al., 2009; van der Vassen et al., 2003), had weak similarity to Group F-I sequences.

**Identification of bermudagrass disease-resistance EST analogs.** A recent bermudagrass EST study using genotype T89 identified 9414 unigenes (Kim et al., 2008). From these, 24 unigenes were identified with similarity to genes involved in disease resistance (Supplemental Table 1). Five of these 24 unigenes, ES304944, ES303684, ES296578, ES295859, and ES296552, could be translated without stop codons and could be aligned to the BRGA sequences. Only one EST, ES296578, was found to contain the entire P-loop to the HD sequence. ES296578 was compared with the BRGA as well as known R genes (Figs. 1B and 2) and formed its own group.

**Mapping of BRGA and ESTs with similarity to disease-resistance genes or disease RGA.** Six primer pairs (BRGA131, BRGA163, BRGA167, BRGA179, BRGA208, and ES296578) generated from the eight BRGA groups and ES296578, each of which had an open reading frame that spanned the P-loop to the HD, generated six polymorphic markers between T89 and T574 (Supplemental Table 2). Of these six markers, only BRGA179, BRGA208, and ES296578 were found to segregate among the F1 progeny (note that only polymorphisms that are heterozygous in diploid T574, or single/double dose in autotetraploid T89, would be expected to segregate in the F1 progeny). Of those markers designed from five BRGA containing stop codons in the NBS (BRGA38, BRGA81, BRGA220, BRGA201, and BRGA229), five markers were polymorphic, and two BRGA segregated at three

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Table 2. Degenerate primers used to amplify each bermudagrass-resistance gene analog (BRGA) group, genotypes of sequences generated that were assigned to each BRGA group, and BLASTx similarity of each BRGA group with top hit (GenBank accession number, organism, e-value range) are shown.

| Group | Primers | Genotypes | Homology | GenBank accession no. | Organism | e-value (range) |
|-------|---------|-----------|----------|-----------------------|---------|-----------------|
| A (26) | All | All but T574 | NBS-LRR–like protein | CAD45036 | *Hordeum vulgare* | 2e–26 to 5e–52 |
| C (5) | LM637/LM638 | TifGrand, T89, and Tifton10 | NBS-LRR protein *Put* | BAH20861 | *Oryza sativa* | 3e–52 to 5e–58 |
| D (1) | PLP/NBSR1 | PI 291586 | NBS-LRR–like protein | ABK57113 | *Saccharum officinarum* | 2e–69 |
| E (3) | PLP/antiHD1 | T89 and Tifton10 | NB-ARC domain | ABA93733 | *O. sativa* | 2e–50 to 2e–41 |
| F (1) | PLP/antiHD1 | TifGrand | NBS-LRR–like protein | BAD08985 | *O. sativa* | 2e–69 |
| G (2) | PLP/antiHD1 | Tifton10 | NBS-LRR–like protein | AAQ16577 | *Saccharum hybrid* | 1e–64 to 5e–66 |
| H (1) | PLP/antiHD1 | Tifton10 | NBS-LRR–like protein | ABW04964 | *Eleusine coracana* | 2e–56 |
| I (5) | PLP/antiHD1 | T574, TifGrand, and T89 | NBS-LRR–like protein | ABW04965 | *E. coracana* | 3e–62 to 4e–62 |

Numbers shown in parentheses represent the number of BRGA assigned to each group.

NBS1/NBSR1, PLP/antiHD1, PLP/NBSR1, LM637/LM638, and 16409/R11.
loci (BRGA201 and BRGA229a and b) in the F₁ progeny (Supplementary Table 2). Primers were developed for the remaining ESTs that had similarity to disease-resistance genes or disease-resistance analogs (Supplemental Tables 2 and 3; see online version of this article at ashs.org to view the tables). Excluding ES296578, 20 primer pairs designed for each ESTs were polymorphic between the mapping parents, forming 23 markers (three EST markers, ES292682, ES299682, and ES304457, had two alleles), and yet only 15 EST markers were segregating in the F₁ progeny. Of the 21 total polymorphic markers segregating in the F₁ progeny, 13 could be placed on reported bermudagrass linkage groups (Table 3), noting that the present map is estimated to be only about 61% complete (Bethel et al., 2006): Two EST markers mapped on each of T89 linkage groups 1a and 5a; one EST marker each on T574 1a, T89 3c, T89 6a; one BRGA marker on T89 12, one EST marker on T89 13, and three EST or BRGA markers on T89 linkage group 19. Markers that grouped together, but not on the previously identified linkage groups, include ES296578 with ES302976 (4.4 cM) and ES303684 with ES303694 (15 cM).

**Conservation of BRGA Among Turf Species.** Primers were made (Supplemental Table 3) for each BRGA group (Groups A, C–I) and to five ESTs possessing open reading frames with similarity to NBS-LRR proteins to determine if RGAs in Table 3. Twenty-one bermudagrass-resistance gene analogs (BRGA) or expressed sequence tags (labeled “ES”) containing similarity to disease-resistance genes or disease-resistance analogs that were segregating in the T89 × T574 F₁ population.

| GenBank/BRGA | cM  | Left marker | Right marker | Map Linkage group |
|--------------|-----|-------------|--------------|------------------|
| ES297666     | 7.9 | T5748B02b   | T5741E11c    | T89, 1a          |
| ES292682B    | 31.1| PCD068      | —            | T89, 1a          |
| ES306149     | 29.1| PCD128      | T5741C03a    | T574, 1a         |
| ES304077     | 17.3| T5745A04d   | PAP07C04b    | T89, 3c          |
| ES295859     | 8.7 | T5742D09    | PCD065a      | T89, 4c          |
| ES302322     | 0   | —           | T5742C08a    | T89, 5a          |
| ES298588     | 39  | PAP03E08    | T5741G07     | T89, 5a          |
| ES296282A    | 17.7| PCD137c     | T5746B02b    | T89, 6a          |
| BRGA208      | 37.6| T5748F06a   | RZ543b       | T89, 12          |
| ES304457–273 | 19  | T5741H04b   | —            | T89, 13          |
| ES304457–268 | 24.6| T5742G03A   | BRGA229A     | T89, 19          |
| BRGA229A     | 45.5| ES304457    | BRGA201      | T89, 19          |
| BRGA201      | 50.6| BRGA229A    | —            | T89, 19          |
| ES299682B    | 0   | —           | T5743D12b    | T574*            |
| ES296578     | 0   | —           | ES299276     | T89*            |
| ES299276     | 4.4 | ES296578    | —            | T89*            |
| ES303684     | 15  | T5741E07c   | ES303694     | T89*            |
| ES303694     | 30  | ES303684    | PCD108       | T89*            |
| ES307377     | —   | Unlinked    | —            | —               |
| BRGA229B     | —   | Unlinked    | —            | —               |
| BRGA179      | —   | Unlinked    | —            | —               |

*Left and right are markers that flank the EST or BRGA.  
†Markers found on this linkage group were not mapped by Bethel et al. (2006).  
‡These markers group together on a linkage group not previously identified by Bethel et al. (2006).  
§These markers group together on a linkage group not previously identified by Bethel et al. (2006).

**Discussion**

**Isolation of BRGA.** Thirty-one unique BRGA and EST that formed nine groups were identified from diploid, triploid, and hexaploid bermudagrass using degenerate primers previously used to amplify RGAs in sunflower (Helianthus spp.), melon (Cucumis melo), soybean (Glycine max), and citrus (hybrid of Poncirus trifoliata and Citrus grandis) (Radwan et al., 2008; Brotman et al., 2002; Kanazin et al., 1996; Deng et al., 2000). Although it might be expected that more RGAs would be isolated from the hexaploid bermudagrass ‘TifGrand’, this is likely due to preferential amplification of ‘TifGrand’ with the degenerate primer pairs used. In contrast, only one degenerate primer amplified BRGA from T574 (Table 2), as all other degenerate primers amplified retrotransposon or hypothetical protein analogs from T574. It is possible that the BRGA fragments from T574 are not ≈500 bp and the incorrect fragment was captured or, alternatively, that the degenerate primers used (excluding PL/antiHD1) do not have enough sequence similarity to amplify BRGA from T574.

The similarity of the amino acid sequence (68% identity) spanning the P-loop to the HD domain between the Pit gene from rice and BRGA215 (Fig. 2) holds promise. The Pit gene confers race-specific resistance against M. grisea in rice (Hayashi and Yoshida, 2009). This fungus is the same fungus that causes gray leaf spot (blast) primarily on st. augustinagrass, but may also cause severe damage to turfgrasses in the genera Cynodon, Eremochloa, and Paspalum (Smiley et al., 1992). BRGA215
would be a candidate RGA to test if linkage exists between BRGA215 and the gray leaf spot resistance gene in a bermudagrass population segregating for gray leaf spot resistance.

**Mapping of BRGA.** Although the polymorphism rate was high between the mapping population parents T89 and T574 (30 of 38 primer pairs amplified a polymorphic fragment), only seven primer pairs amplified RGA where the dominant allele was derived from T574 (Supplemental Table 2). This lack of amplification of T574 alleles could be because of the small number of BRGA amplified from T574 using degenerate primers and that the EST library was generated using the genotype T89. Of the 30 primer pairs that amplified polymorphic markers, only 21 markers were segregating in the F1 genotype T89. Of the 30 primer pairs that amplified polymorphic markers, only 21 markers were segregating in the F1 mapping population (Supplemental Tables 2 and 3). The high percentage of non-segregating polymorphic markers could be due to the homozygosity of alleles in T574 or to the presence of the allele at three or all four homologous chromosomes in T89 causing all progeny to contain the allele, or possibly due to segregation distortion.

Of the 18 markers that could be placed on the T89 or T574 map (noting that the genetic map was created using single-dose restriction fragment mapping and necessitates the building of separate maps for each of the parental genomes), multiple BRGA or ESTs mapped on T89 linkage groups 1a and 5a and three markers with similarity to NBS-LRR proteins mapped to a 26-cM region on T89 linkage group 19. Furthermore, clustering of markers could be seen on linkage groups that were not previously identified (Table 3). ES296578 and ES2999276, which have similarity to NBS-LRR, formed their own linkage group and are 4.4 cM apart. ES303684 and ES303694, which have similarity to a NBS-LRR and a dirigent protein, respectively, form a linkage group of four markers. This clustering of RGA has been seen in many other plant species (Bakker et al., 2003; Brotman et al., 2002; Harris et al., 2009; McHale et al., 2006), and RGAs have been found to be linked to disease-resistance genes in several species (Brotman et al., 2002; Deng et al., 2000; Irigoyen et al., 2004; Kanazin et al., 1996; Xu et al., 2005).

**Conservation of BRGA.** Species within the grass family, Poaceae, contain genomes that are syntenic, i.e., the gene number and order are conserved (Gale and Devos, 1998). This synteny frequently does not extend to RGAs in rice, barley, and foxtail millet (Setaria spp.) and indicates a rapid evolution of resistance genes in each of these species (Leister et al., 1998). Many analogs were conserved across the three species, although this was not always the case (Leister et al., 1998). Although NBS-LRR loci frequently rearrange and evolve through recombination, unequal crossing-over, gene conversion, insertion-deletion, and point mutations (Radwan et al., 2008), and may have a rapid evolutionary rate compared with the rest of the monocot genome (Leister et al., 1998), three RGAs could be amplified from zoysiagrass or seashore paspalum using BRGA or EST primers. These RGA in zoysiagrass or seashore paspalum may map closely to resistance gene loci that are identified in these species. Furthermore, the amplification of RGA from turf species belonging to the subfamily Chloridoideae and Panicoideae indicates that these BRGA or ESTs were present in a common ancestor. Chloridoideae and Panicoideae subfamilies are thought to have diverged about 34.6 to 38.5 million years ago (Kim et al., 2009).

**SSRs.** Four unique clones with no similarity to BRGA contained SSRs (GU170365–GU170368) and may be useful in adding markers to the bermudagrass genetic map (Bethel et al., 2006) that could potentially link homologous linkage groups for the T89 genetic map and for comparative mapping with other grass species. SSR discovery has been limited in bermudagrass. Five SSR markers were identified in a bermudagrass genomic library study (Williams, 2003), and 10 chloroplast-specific SSR length polymorphisms were amplified in bermudagrass (Karaca et al., 2002). Also, 143 EST-SSRs that contained two- to five-nucleotide repeats of a minimum repeat length of five were identified from a bermudagrass EST study, but were not empirically tested (Kim et al., 2008). In many RGA studies, it is not uncommon that the majority of sequences generated have no similarity to RGA (Brotman et al., 2002; Chen et al., 2006; Glynn et al., 2008). Examination of these sequences for SSRs may be useful in many plant species for the generation of SSR markers in data that would likely be discarded.

In conclusion, in this study, nine groups of BRGA or ESTs were identified from diploid, triploid, tetraploid, and hexaploid bermudagrass that displayed disease resistance to a wide range of phytopathogens or were used to create the F1 map. The NBS sequences examined contained non-TIR-NBS-LRR motifs, and the mapping of BRGA and ESTs identified regions where these markers cluster. The creation and placement of these markers on the bermudagrass genetic maps is an important step in the study of disease resistance in bermudagrass. Phenotyping a population segregating for a disease of interest and determination of whether these markers are linked to disease-resistance genes are needed to use these markers for MAS.

**Literature Cited**

Baird, J.H., D.L. Martin, C.M. Taliaferro, M.E. Payton, and N.A. Tisserat. 1998. Bermudagrass resistance to spring dead spot caused by Ophiophaerella herpotricha. Plant Dis. 82:771–774.

Bakker, E., P. Butterbach, J. Roupe van der Voort, E. van der Vossen, J. van Vliet, J. Bakker, and A. Goverse. 2003. Genetic and physical mapping of homologues of the virus resistance gene Rx1 and the cyst nematode resistance gene Gpa2 in potato. Theor. Appl. Genet. 106:1524–1531.

Bethel, C.M., E.B. Sciara, J.C. Estill, J.E. Bowers, W. Hanna, and A.H. Paterson. 2006. A framework linkage map of bermudagrass (Cynodon dactylon ×transvaalensis) based on single-dose restriction fragments. Theor. Appl. Genet. 112:727–737.

Boutin-Ganache, I., M. Raposo, M. Raymond, and C.F. Deschepper. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31:25–28.

Brodsky, L.I., A.V. Vasiliev, Y.L. Kalaidzidis, Y.S. Osipov, R.L. Tatuzov, and S.I. Feranchuk. 1992. GeneBee: The program package for biopolymer structure analysis. Dimacs 8:127–139.

Brotman, Y., L. Silberstein, I. Kovalski, C. Perin, C. Dogimont, M. Boutin-Ganache, I., M. Raposo, M. Raymond, and C.F. Deschepper. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31:25–28.

Burton, G.W. 1966. Registration of crop varieties: Tifdwarf bermudagrass. Crop Sci. 6:93–94.

Casler, M.D. 2006. Perennial grasses for turf, sport and amenity uses: Evolution of form, function and fitness for human benefit. J. Agr. Sci. 144:189–203.

Chang, N.T., B.R. Wiseman, R.E. Lynch, and D.H. Habeck. 1985. Fall armyworm (Lepidoptera: Noctuidae) orientation and preference for selected grasses. Fla. Entomol. 68:296–303.

Chen, Y., L. Long, X. Lin, W. Guo, and B. Liu. 2006. Isolation and characterization of a set of disease resistance-gene analogs (RGAs)
from wild rice, *Zizania latifolia* Griseb. I. Introggression, copy number lability, sequence change, and DNA methylation alteration in several rice-*Zizania* introgression lines. Genome 49:150–158.

Collier, S.M. and P. Moffett. 2009. NB-LRRs work a “baits and switch” on pathogens. Trends Plant Sci. 14:521–529.

Crow, W.T. 2002. Nematode, where is thy sting? Golf Course Mgt. 70:103–106.

Deng, Z., S. Huang, P. Ling, C. Chen, C. Yu, C.A. Weber, G.A. Moore, and F.G. Gmitter. 2000. Cloning and characterization of NBS-LRR class resistance-gene candidate sequences in citrus. Theor. Appl. Genet. 101:814–822.

DeYoung, B.J. and R.W. Innes. 2006. Plant NBS-LRR proteins in pathogen sensing and host defense. Nat. Immunol. 7:1243–1249.

Drummond, A.J., B. Ashton, M. Cheung, J. Heled, M. Kearse, R. Moir, P. Ling, C. Chen, C. Yu, C.A. Weber, G.A. Moore, C. Deng, Z., S. Huang, P. Cynodon dactylon. 1961. [Cynodon dactylon (L.) Rich], p. 235–245. In: M.A. Hein. 1953. [Cynodon dactylon (L.) Pers.] Registration of varieties and strains of Bermuda-grass. U.S. Golf Assn. Green Section Record 24:11–13.

Flintoff, J. and F.G. Gmitter. 2000. Cloning and characterization of NBS-LRR class resistance-gene candidate sequences in citrus. Theor. Appl. Genet. 101:814–822.

Hayashi, K. and H. Yoshida. 2009. Refunctionalization of the ancient NB-LRR repeat and kinase resistance gene analogues from sugarcane (*Saccharum* spp.). Pest Manag. Sci. 65:48–56.

Hanna, W.W. 1986. A bermudagrass primer and the Tifton bermudagrass. U.S. Golf Assn. Green Section Record 42:11–13.

Hayashi, K. and H. Yoshida. 2009. Refunctionalization of the ancient rice blast disease resistance gene *Pit* by the recruitment of a retrotransposon as a promoter. Plant J. 57:413–425.

Hein, M.A. 1953. [Cynodon dactylon (L.) Pers.] Registration of varieties and strains of bermudagrass: II. Agron. J. 45:572–573.

Hein, M.A. 1961. [Cynodon dactylon (L.) Pers.] Registration of varieties and strains of bermudagrass: III. Agron. J. 53:276.

Irigoyen, M.L., Y. Loare, A. Fominyah, and E. Ferrer. 2004. Isolation and mapping of resistance gene analogs from the *Avena strigosa* genome. Theor. Appl. Genet. 109:713–724.

Jukes, T.H. and C.R. Cantor. 1969. Evolution of protein molecules, p. 21–132. In: H.N. Munro (ed.). Mammalian protein metabolism. Academic Press, New York.

Kanazin, V., L.F. Marek, and R.C. Shoemaker. 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. USA 93:11746–11750.

Karaca, M., S. Saha, A. Zipf, J. Jenkins, and D. Lang. 2002. Genetic diversity among forage bermudagrass (*Cynodon* spp.). Pest Manag. Sci. 58:106. 18 Jan. 2010. <http://ucce.ucdavis.edu/files/repositoryfiles/c5a802p106-69110.pdf>.

Kanazin, V., L.F. Marek, and R.C. Shoemaker. 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. USA 93:11746–11750.
White, R.H. and R. Dickens. 1984. Plant-parasitic nematode populations in bermudagrass as influenced by cultural practices. Agron. J. 76:41–43.
Wiecko, G. 2007. Management of tropical turfgrasses, p. 116–137. In: M. Pessarakli (ed.). Handbook of turfgrass management and physiology. CRC Press, Boca Raton, FL.
Williams, N.R. 2003. PCR-based polymorphism in bermudagrass (Cynodon spp.). MS Thesis, Univ. Florida, Gainesville.
Xu, Q., X. Wen, and X. Deng. 2005. Isolation of TIR and non-TIR NBS-LRR resistance gene analogues and identification of molecular markers linked to a powdery mildew resistance locus in chestnut rose (Rosa roxburghii Tratt). Theor. Appl. Genet. 111:819–830.
Xu, Y. and J.H. Couch. 2008. Marker-assisted selection in plant breeding: From publications to practice. Crop Sci. 48:391–407.
### Supplemental Table 1

Bermudagrass expressed sequence tags (EST) with BLASTx homology to resistance genes or resistance gene analogs (the top BLASTx hit, including GenBank accession, organism, and e-value, is shown for each EST query) that were used for cross-species amplification or were polymorphic between T89 and T574, the parents of the F1 genetic mapping population.

| EST         | BLASTx similarity                | GenBank accession no. | Organism       | e-value |
|-------------|----------------------------------|-----------------------|----------------|---------|
| ES292237    | Similar to RPM1                  | AAX96326              | *Oryza sativa* | 9e–58   |
| ES292254    | NBS-LRR–like protein             | AF456245              | *O. sativa*    | 7e–15   |
| ES292682    | NBS-LRR disease-resistance protein| ABB88855             | *O. sativa*    | 6e–98   |
| ES293253    | Putative NBS-LRR–resistance protein| AAM47598             | *Capsicum annuum* | 2e–97   |
| ES295852    | NB-ARC domain containing         | ACX8754               | *O. sativa*    | 9e–34   |
| ES295859    | NB-ARC domain containing         | ACX8900               | *O. sativa*    | 2e–85   |
| ES296552    | Putative NB-ARC domain           | XP_002456000          | *Sorghum bicolore* | 2e–88 |
| ES296578    | Putative NB-ARC domain           | ACX8912               | *O. sativa*    | 9e–99   |
| ES297666    | Putative NBS-LRR protein         | CAD45027              | *Hordeum vulgare* | 7e–20 |
| ES298588    | Putative protein kinase Xa21 D   | BAD19603              | *O. sativa*    | 4e–49   |
| ES299276    | P-loop NTPase                    | XM_002457736          | *S. bicolor*   | 1e–21   |
| ES299682    | Serine-threonine protein kinase   | XP_002512112          | *Ricinus communis* | 2e–86 |
| ES299920    | Putative disease response protein 206 | NP_001151705       | *O. sativa*    | 1e–59   |
| ES302322    | Plant-hopper–induced resistance protein | AAO54305            | *O. sativa*    | 5e–66   |
| ES303684    | Putative NBS-LRR                 | CA26375               | *Brachypodium sylvaticum* | 1e–28 |
| ES303694    | Putative disease-response protein 206 | NP_001149580       | *Zea mays*     | 7e–24   |
| ES304077    | NB-ARC domain containing         | ABA91338              | *O. sativa*    | 4e–11   |
| ES304457    | NB-ARC domain containing         | ABB22544              | *O. sativa*    | 5e–13   |
| ES304944    | NB-ARC domain containing         | ACX89999              | *O. sativa*    | 1e–16   |
| ES305038    | LRR19                            | AAK20736              | *Triticum aestivum* | 3e–35 |
| ES306059    | Putative blight-associated protein p12 | BAD09315          | *O. sativa*    | 1e–32   |
| ES306149    | Putative disease-resistance protein RPM1 | AB97409          | *O. sativa*    | 2e–05   |
| ES307083    | Putative disease-resistance protein | NP_001064537       | *O. sativa*    | 4e–46   |
| ES307377    | NB-ARC domain containing         | ACX69175              | *O. sativa*    | 4e–35   |

*BLASTx hit, as no similarity to other GenBank accessions was seen using BLASTn or BLASTx.

### Supplemental Table 2

Sequences of 30 primer pairs used to generate 34 polymorphic markers used for genetic mapping in a T89 × T574 F1 cross (*Cynodon dactylon × C. transvaalensis*).

| Primer | Sequence                  | Ta (°C) | Amplicon size (bp) (-Dominant parent) | SEG* |
|--------|---------------------------|---------|---------------------------------------|------|
| BRGA38-F | M13^*-AAATCTAGCTTGTCCTTCTGACGA | 50      | 151*,50-T574                           | No   |
| BRGA38-R | AGGTCCATGTCCTCAGCGAC     |         |                                       |      |
| BRGA81-F | M13-GCAATTCTGGAGACGATGTTA | 56.7    | 156-T574                              | No   |
| BRGA81-R | TGCGTGTGGTATGGTTGATG     |         |                                       |      |
| BRGA131-F | TTTGGAAGCTGCTGTTTGTG    | 63.4    | 375-T89                               | No   |
| BRGA131-R | GGGACCAAATCTGTTTGTCT    |         |                                       |      |
| BRGA163-F | TGGCCTGTGGTGACAGATGA | 45      | 402-T89                               | No   |
| BRGA163-R | AGCTTCCCCATTTTTCACCA    |         |                                       |      |
| BRGA167-F | CCGTTGGGCCAGGTAGGTAT    | 45      | 500*,400-T574                         | No   |
| BRGA167-R | TCTACAAAATGTTCGTTTCG   |         |                                       |      |
| BRGA179-F | GCAAGGCTTTGGTTCCTCA    | 45      | 328-T89                               | Yes  |
| BRGA179-R | ACAAGCTCCCCATCTCACTCG  |         |                                       |      |
| BRGA201-F | M13-GGACCATATGGCTGTTTG | 63.4    | 124-T89                               | Yes  |
| BRGA201-R | GGCGCAGAGGGTGTGACCTT   |         |                                       |      |
| BRGA208-F | CATGACAGAGGTGACAGAGA   | 45      | 367*,300-T89                         | Yes  |
| BRGA208-R | TCAACAGGTCTAGGCGCATC   |         |                                       |      |
| BRGA229-F | M13-GCTGAGGAGAGAGATGATAAAT | 45      | 108-T574,126-T89                     | Yes* |
| BRGA229-R | TCCTATGACCACCGGACATC   |         |                                       |      |
| ES292237-F | M13-AGCTCAGAGGACCTGTCTCA | 64.6    | 160-T89                              | No   |
| ES292237-R | GGGTCAATTGTTTTCAGC    |         |                                       |      |
| ES29282-F | M13-GGTGAGCTAGCTGTGATGA | 50      | 122,140-T89                           | Yes* |
| ES29282-R | CACCGAGGTAGGTCAGTT    |         |                                       |      |

*continued next page*
| Primer       | Sequence                              | Ta (°C) | Amplicon size (bp)                                      | SEG  |
|-------------|---------------------------------------|---------|--------------------------------------------------------|------|
| ES293253-F  | M13-AGAGCTGTGGAGGCGACACTTT            | 45      | 150*, 148-T89                                          | No   |
| ES293253-R  | TCCTCAAGCTCTGGCGGCTT                 |         |                                                        |      |
| ES295859-F  | M13-ACCAAACTGTTCCATGTCGTCG          | 45      | 94-T89                                                 | Yes  |
| ES295859-R  | GCTTTCCGTCGCCATGTCGCTG               |         |                                                        |      |
| ES296552-F  | M13-CTCTCGTCGTGGAGGAGATGAG           | 63.2    | 96-T89                                                 | No   |
| ES296552-R  | GAGAAGAGGAGGCTGAAATGCA               |         |                                                        |      |
| ES296578-F  | AGTAACCCAGGCCAGTCACAG                | 45      | 576-T89                                                | Yes  |
| ES296578-R  | GCCTGTCACAGGCAGATGAG                 |         |                                                        |      |
| ES297666-F  | M13- GCAGGTCCATCGACTGAAAT            | 45      | 126-T89                                                | Yes  |
| ES297666-R  | GCGCGCAGATACATGCTGTCG               |         |                                                        |      |
| ES298588-F  | M13-CCCCACAATAGTTCTCTTC              | 50      | 134-T89                                                | Yes  |
| ES298588-R  | AACGAGGATGCTGCTGCTGTCG               |         |                                                        |      |
| ES299276-F  | M13-CGAGCTTTGGCTCTGTTGAG            | 50      | 146-T89                                                | Yes  |
| ES299276-R  | GTGTGCTGCTGGAGATGAG                   |         |                                                        |      |
| ES299682-F  | M13-CAAGATGTGCCTCCACCA              | 50      | 158*, 156-T574                                          | Yes' |
| ES299682-R  | GCGTAAACGGAGATTCAT                   |         | 156*, 154-T89                                          |      |
| ES299920-F  | M13-ACCTACATGTCTGGGTTCCAT            | 40      | 260-T574                                               | No   |
| ES299920-R  | CTCTAAACTCTCCCGAGCTG                |         |                                                        |      |
| ES302322-F  | M13-CTCGCGATCCATCTCCAGTTC            | 45      | 148-T89                                                | Yes  |
| ES302322-R  | GACGGCCACATGCTGTTCTGCA              |         |                                                        |      |
| ES303684-F  | GGGGTATTGGGCGTGTTACTT                | 45      | 523-T89                                                | Yes  |
| ES303684-R  | CCCATTCCTTCACAAAGGCGCTG             |         |                                                        |      |
| ES303694-F  | M13-AGCTCTTCTGGCCTTCTTCAT            | 61.8    | 122-T89                                                | Yes  |
| ES303694-R  | GTAGTGCTGCTGGTGTCAGG                |         |                                                        |      |
| ES304077-F  | M13-GTTGGCGAACAGCACTTCGTT           | 50      | 150-T89                                                | Yes  |
| ES304077-R  | CCAATTCCTGTGAGGCGCTGAC              |         |                                                        |      |
| ES304457-F  | M13-TCCTTCATCTCCACCAAGG             | 50      | 268,270-T89                                            | Yes' |
| ES304457-R  | CAGAAAGCTCTACGTGCGCTG               |         |                                                        |      |
| ES305038-F  | M13-GGTGGCGTGGGACTGGCAGTGTCT         | 50      | 130-T89                                                | No   |
| ES305038-R  | GCCGCCCACTACACTCTCTAC               |         |                                                        |      |
| ES306059-F  | M13-GTTCAGCTGATGCCGTCA              | 50      | 167-T89                                                | No   |
| ES306059-R  | CGTGCTGCTTCATGCTGCGA               |         |                                                        |      |
| ES306149-F  | M13-AGGAAAATGGTGTTGCGTCA            | 50      | 138-T574                                               | Yes  |
| ES306149-R  | TAATACGAGCTCGTTACGCAATTC            |         |                                                        |      |
| ES307083-F  | M13-GGTTACACGTGGCAGCAAGAT           | 66.8    | 122-T89                                                | No   |
| ES307083-R  | GAAGAGGTGAGGACGACGTCG               |         |                                                        |      |
| ES307377-F  | M13-AACGGTGGGCAAGAACAGAAGAACAC      | 61.8    | 129-T89                                                | Yes  |
| ES307377-R  | TCCATTGGTCGTGATCCTG                  |         |                                                        |      |

Ta was the annealing temperature used.

SEG was whether the polymorphic marker was segregating in the F1 population.

Forward primers were labeled with a 5’ M13 Tag (TGTAAGACGGACGCACTTT) where indicated (M13) and were resolved on 6.5% acrylamide gels on a LI-COR® Biosciences 4300 DNA Analyzer (LI-COR, Lincoln, NE). Those primers without M13 labels were resolved on 1% agarose gels.

Amplicon was digested with HpaII to generate a polymorphism between the mapping parents T89 and T574.

Both amplicons from T89 were segregating.

Amplicon was digested with EcoRV to generate a polymorphism between the mapping parents T89 and T574.

Only the T574 band segregated in the F1 population.
Supplemental Table 3. Primers designed from the eight bermudagrass-resistance gene analogs (BRGA) groups and five expressed sequence tags (ESTs) with similarity to nucleotide binding site–leucine-rich repeat (NBS-LRR) proteins for cross genera amplification.

| Primer   | Sequence                        | Size of amplicon (bp) |
|----------|---------------------------------|-----------------------|
| BRGA131F | TTTCGAAGCTGCTGTTGTTG            | 375                   |
| BRGA131R | GCGACAAACTCTGTTTGTGC            | 431                   |
| BRGA136F | CTCGCAGACCTTCACAATCA            | 431                   |
| BRGA136R | GCCATTTCTTGCAGCATCTG            | 393                   |
| BRGA162F | ACTTCAGAGCCACCGATTT            | 402                   |
| BRGA162R | TGACGACACACTACCGATCA            | 402                   |
| BRGA163F | TGGCTGTGTTGACAGATGA            | 411                   |
| BRGA163R | ACACCTCCAATTCTCACA             | 411                   |
| BRGA167F | CGCTTTGAGGGAGTGTAT              | 257                   |
| BRGA167R | TCTCAAACAAATGCTCCTG            | 257                   |
| BRGA173F | GCCGGAAAAAGGTTCTACCT            | 328                   |
| BRGA173R | TGTTTGCTCTTGACTCTCCCT          | 328                   |
| BRGA179F | GCATGGCTTTGGTTTCTCA            | 367                   |
| BRGA179R | ACAGCTCCCATCTCATCG             | 367                   |
| BRGA208F | CATGAGCAGAGGATAAGAGA           | 367                   |
| BRGA208R | TCAACAGCTTAGGCCATCC            | 367                   |
| ES295859F | TCCAGGCATTACCAACTTC            | 660                   |
| ES295859R | AGAGCCCTTCTGAAACCT            | 660                   |
| ES296552F | AGTACCCAGCCCAGTCACAG           | 576                   |
| ES296552R | CACGTGTCAACAGGATCC            | 576                   |
| ES296578F | GCTCCATCGTGAGCTGAC            | 587                   |
| ES296578R | CTGTGTGCTGGGAGTCCTGA         | 587                   |
| ES303684F | GGAGTTATTTGCTGTTACTT          | 523                   |
| ES303684R | CCCATCTCTCAAGCCTTG            | 523                   |
| ES304944F | ATCGAAACACGGAGCTTATT          | 510                   |
| ES304944R | TCTTGTCCTCGGAATCGT            | 510                   |

*The annealing temperature used to amplify these fragments among the turf genera was 45 °C.*
Supplemental Fig. 1. Deduced amino acid alignment of 31 bermudagrass-resistance gene analogs (BRGA) that contain nucleotide binding site–leucine-rich repeats. Motifs are highlighted and named as indicated at the top of the sequence. HD = hydrophobic domain, TIR-Toll, and mammalian interleukin-1 receptor.
Supplemental Fig. 1. (Continued).
| TRGA   | Primer        | Genotype         | analog (BLASTx)        | Accession | Organism            | e-value |
|--------|--------------|------------------|------------------------|-----------|---------------------|---------|
| TRGA1  | ES295859     | Empire           | putative disease resistance protein | BAC15497  | *Oryza sativa*      | 60\(^6\) |
| TRGA2  | ES296552     | Empire           | putative NBS-LRR protein | ACD70335  | *Saccharum arundinaceum* | 76\(^5\) |
| TRGA3  | BRGA173      | SI1              | NBS-LRR like protein    | ABW04965  | *Eleusine coracana*  | 56\(^3\) |

Supplemental Fig. 2. Turfgrass-resistance gene analogs (TRGA) successfully amplified from zoysiagrass (TRGA1 and TRGA2) and seashore paspalum cultivars (TRGA3) using primers designed from bermudagrass-resistance gene analogs (BRGA) and expressed sequence tag (EST) sequences. Conserved amino acids (highlighted light blue) and conserved domains of the nucleotide binding site (NBS) or leucine-rich repeat (highlighted in green) between the TRGA and BRGA/ESTs are shown. TRGA1–3 are listed as GenBank accession numbers GU247047–GU247049. SI1 is ‘Sea Isle 1’. TRGA1 spans the end of the leucine-rich repeat to the C-terminal region. Underlined amino acids represent the primer sites.