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-*Drosophila 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 Panicoideae. 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 has occurred by selection for turfgrass adaptability and aesthetic traits from common bermudagrass such as ‘Tiflawn’ (Hein, 1953) and the release of sterile triploid hybrids ($2n = 3x = 27$) between *C. transvaalensis* and *C. dactylon* such as ‘Tifgreen’ and ‘Tifdwarf’ (Burton, 1966; Hein, 1961). Most

new releases are vegetatively propagated triploid hybrids that are more pest resistant, make a denser turf, tolerate close mowing, have fewer seedheads, and are more uniform than common bermudagrass (Webster et al., 2003).

Bermudagrass is susceptible to a wide range of fungi (Pratt, 2003), nematodes (Giblin-Davis et al., 1992), and insects (Chang et al., 1985; Reinert and Busey, 2001; Shortman et al., 2002), although few are considered to be serious issues when properly managed. The major fungal diseases of bermudagrass include spring dead spot caused by *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari*, brown patch caused by *Rhizoctonia* spp., and dollar spot caused by *Sclerotinia homoeocarpa* (Baird et al., 1998; Taliaferro, 2003). Serious insect pests include southern and tawny mole crickets (*Scapteriscus acletus* and *S. vicinus*), tropical sod webworm (*Herpetogramma phaeopteralis*), and fall armyworm (*Spodoptera frugiperda*) (Taliaferro, 2003). Nematodes, including sting (*Belonolaimus* spp.), lance (*Hoplolaimus* spp.), root-knot (*Meloidogyne* spp.), and spiral (*Helicotylenchus* spp.), also may cause serious injury to bermudagrass (Crow, 2002; White and Dickens, 1984).

Widespread monoculture of only a few cultivars such as Tifdwarf and Tifway could cause new resistant strains of diseases and pests to develop by selection pressure (Taliaferro, 1995). To date, the development of cultivars with resistance to

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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), 12.25 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 Terminator, 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

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

Protein region ^z	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	antiHD1	ARN GGI ARI CCY TTR CA	Brotman et al., 2002
	R11	AGI GC(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

^zP-loop = phosphate-binding loop, HD = hydrophobic domain.

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*) *Pib* (AB013449), *Xa1* (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* (AC116480) and *B149* (AAR29073) 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.

IDENTIFICATION OF DISEASE-RESISTANCE BERMUDAGRASS EST ANALOGS. ESTs with similarity to disease-resistance proteins

were mined from an Excel spreadsheet (Microsoft, Redmond, WA) provided in the supplemental material of a bermudagrass EST study that listed the strongest BLASTx hit to each EST (Kim et al., 2008). The keywords “NB-ARC,” “NBS-LRR,” “disease,” “disease resistance,” and “resistance” were used to identify those ESTs with similarity to disease-resistance proteins (Supplemental Table 1; see the online version of this article at ashs.org to view the table).

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. 1. Neighbor-joining tree with groupings based on an alignment of 44 deduced amino acid sequences of bermudagrass-resistance gene analogs (BRGA) and expressed sequence tag ES296578. Group assignments (A–I) and bootstrap values greater than 50% are shown. Scale bar represents the number of amino acid substitutions per site.

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.

(Promega), 1 μ L of 25 mM $MgCl_2$, 0.8 μ L of 2.5 mM dNTP mix, 0.5 μ L of M13-tagged forward primer at 1 μ M, 0.5 μ L of reverse primer at 1 μ M, 1.0 μ L of 1 μ M M13 primer (M13-TGTAACGACGCGCCAGT) fluorescently labeled with the IRDye® 800 CW fluorophore (Eurofins MWG Operon, Huntsville, AL), 0.04 μ L of GoTaq® DNA polymerase (Promega), 3.16 μ L of water, and 1 μ L of DNA at 2.5 ng· μ L⁻¹. Thermocycling conditions were as above except that a total of 39 cycles was used and the annealing temperature for each marker is stated in Supplemental Table 2. For some BRGA or ESTs, the polymorphism between T89 and T574 could be resolved on agarose and did not need to be fluorescently labeled. For these PCR products, PCR parameters were the same as for the cross-species amplification listed above, except that the annealing temperature listed in Supplemental Table 2 was used. Two microliters of PCR product was combined with 5 μ L of Blue Stop (LI-COR® Biosciences, Lincoln, NE), and 0.35 μ L of this mixture was loaded on a 6.5% acrylamide gel using a LI-COR® Biosciences 4300 DNA Analyzer. Amplicons that were monomorphic between T89 and T574 were digested with *HpaII*, *EcoRI*, *EcoRV*, and *AatII* following the manufacturer's protocols (New England Biolabs) to try to generate polymorphism between the parents. Ideal annealing temperatures to enhance the polymorphism between T89 and T574 were found by performing an annealing temperature gradient from 45 to 70 °C. Each individual of the genetic mapping population was loaded in duplicate and bands were scored visually, entered into Excel, and imported into Joinmap® 4 (Van Ooijen, 2006). BRGA or EST marker segregation

data was combined with the segregation data from the T89 and T574 maps previously created (Bethel et al., 2006) and markers were assigned to groups with a logarithm of the odds of at least five.

CROSS-SPECIES AMPLIFICATION OF BRGA OR DISEASE-RESISTANCE EST ANALOGS. Zoysiagrass (*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 SeaIsle 1, SeaIsle 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- μ L reaction volume. Each reaction contained 4 μ L of 5 $\times$ Green GoTaq® reaction buffer (Promega), 2 μ L of 25 mM $MgCl_2$, 1.6 μ L of 2.5 mM dNTP mix, 2 μ L of 1 μ M forward and reverse primer pair, 0.08 μ L of GoTaq® DNA polymerase (Promega), 8.32 μ L of water, and 2 μ L of DNA at 2.5 ng· μ L⁻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 ($\approx$ 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).

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/I)LD(D/N)(I/V)W, the RNBS-B domain was (G/R/N)(S/C/T/R)(R/T/S/V/L)(V/I/T)(V/I/L/P)(I/V/M)T(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

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 I). 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, *Pit*, 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 *Pit* 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 *Bl49* and *Rpi-bl1*, which confer broad spectrum resistance to *Phytophthora infestans* in transgenic cultivated potato and tomato (Oosumi et al., 2009; van der Vossen 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

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 ^z	Primers	Genotypes	Homology	GenBank accession no.	Organism	e-value (range)
A (26)	All ^y	All but T574	NBS-LRR-like protein	CAD45036	<i>Hordeum vulgare</i>	2e ⁻²⁶ to 5e ⁻⁵²
C (5)	LM637/LM638	TifGrand, T89, and Tifton10	NBS-LRR protein <i>Pit</i>	BAH20861	<i>Oryza sativa</i>	3e ⁻⁵³ to 5e ⁻⁵⁸
D (1)	PLP/NBSR1	PI 291586	NBS-LRR-like protein	ABK57113	<i>Saccharum officinarum</i>	2e ⁻⁶⁹
E (3)	PLP/antiHD1	T89 and Tifton10	NB-ARC domain	ABA93733	<i>O. sativa</i>	2e ⁻⁵⁰ to 2e ⁻⁴¹
F (1)	PLP/antiHD1	TifGrand	NBS-LRR-like protein	BAD08985	<i>O. sativa</i>	2e ⁻⁶⁹
G (2)	PLP/antiHD1	TifGrand	NBS-LRR-like protein	AAQ16577	<i>Saccharum hybrid</i>	1e ⁻⁶⁴ to 5e ⁻⁶⁶
H (1)	PLP/antiHD1	Tifton10	NBS-LRR-like protein	ABW04964	<i>Eleusine coracana</i>	2e ⁻⁵⁶
I (5)	PLP/antiHD1	T574, TifGrand, and T89	NBS-LRR-like protein	ABW04965	<i>E. coracana</i>	3e ⁻⁶² to 4e ⁻⁶²

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

^yNBSF1/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 the 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 4c, 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 ES299276 (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

zoysiagrass, st. augustinegrass, seashore paspalum, bentgrass, and centipedegrass amplify. Seven primer pairs (BRGA131, BRGA167, BRGA173, BRGA179, ES304944, ES295859, and ES296552) amplified a product among turf genera, whereas six primer pairs (BRGA136, BRGA162, BRGA163, BRGA208, ES303684, and ES296578) failed to amplify a product among turf genera. Primer pairs designed from the ES296552 and ES295859 sequences amplified only in zoysiagrass cultivars, primer pairs designed from the ES304944 and BRGA179 sequences amplified only in st. augustinegrass cultivars, and primer pairs designed from the BRGA167 sequence amplified only seashore paspalum cultivars. Primer pairs designed from the BRGA173 sequence amplified only seashore paspalum and centipedegrass cultivars and primer pairs designed from the BRGA131 sequence amplified in st. augustinegrass, zoysiagrass, bentgrass, centipedegrass, and seashore paspalum cultivars. PCR products generated from cross-species amplifications were cloned and sequenced. Only primer pairs ES295859 and ES296552 amplified RGAs in the zoysiagrass cultivar Empire, whereas primer pair BRGA173 amplified a RGA from seashore paspalum cultivar Sea Isle 1. The deduced amino acid sequence of these turfgrass-resistance gene analogs (TRGA) is shown in Supplemental Fig. 2 (see the online version of this article at ashs.org to view the figure). TRGA2 and TRGA3 contained the conserved motifs of the NBS (Supplemental Fig. 2, amino acids highlighted green), and when all three TRGA were compared with the bermudagrass sequence, shared regions of amino acid identity could be identified (Supplemental Fig. 2, amino acids not highlighted or highlighted green). The TRGA1 sequence spanned the end of the LRR motif to the C-terminal region of a NBS-LRR analog.

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 ^a	Right marker ^a	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
ES292682A	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 ^y
ES296578	0	—	ES299276	T89 ^{y,x}
ES299276	4.4	ES296578	—	T89 ^{y,x}
ES303684	15	T5741E07c	ES303694	T89 ^{y,w}
ES303694	30	ES303684	PCD108	T89 ^{y,w}
ES307377				Unlinked
BRGA229B				Unlinked
BRGA179				Unlinked

^aLeft and right are markers that flank the EST or BRGA.

^yMarkers found on this linkage group were not mapped by Bethel et al. (2006).

^xThese markers group together on a linkage group not previously identified by Bethel et al. (2006).

^wThese 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, tetraploid, 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 ‘Tifton 10’, as six homologous chromosomes are present, the most BRGA groups were amplified from triploid ‘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 PLP/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. augustinegrass, 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 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 ES299276, 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.

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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	<i>Oryza sativa</i>	9e ⁻⁵⁶
ES292254	NBS-LRR-like protein	AF456245	<i>O. sativa</i>	7e ⁻¹⁵
ES292682	NBS-LRR disease-resistance protein	ABB88855	<i>O. sativa</i>	6e ⁻⁰⁸
ES293253	Putative NBS-LRR-resistance protein	AAM47598	<i>Capsicum annuum</i>	2e ⁻⁰⁷
ES295852	NB-ARC domain containing	ACX68754	<i>O. sativa</i>	9e ⁻³⁴
ES295859	NB-ARC domain containing	ACX68900	<i>O. sativa</i>	2e ⁻⁸⁵
ES296552	Putative NB-ARC domain	XP_002450600	<i>Sorghum bicolor</i>	2e ⁻⁸⁸
ES296578	NB-ARC domain containing	ACX69212	<i>O. sativa</i>	9e ⁻⁰⁹
ES297666	Putative NBS-LRR protein	CAD45027	<i>Hordeum vulgare</i>	7e ⁻²⁰
ES298588	Putative protein kinase Xa21 D	BAD19603	<i>O. sativa</i>	4e ⁻⁴⁹
ES299276 ^z	P-loop NTPase	XM_002457736	<i>S. bicolor</i>	1e ⁻²¹
ES299682	Serine-threonine protein kinase	XP_002512112	<i>Ricinus communis</i>	2e ⁻⁸⁶
ES299920	Putative disease response protein 206	NP_001151705	<i>O. sativa</i>	1e ⁻⁵⁹
ES302322	Plant-hopper-induced resistance protein	AAQ54305	<i>O. sativa</i>	5e ⁻⁶⁶
ES303684	Putative NBS-LRR	CAJ26375	<i>Brachypodium sylvaticum</i>	1e ⁻²⁸
ES303694	Putative disease-response protein 206	NP_001149580	<i>Zea mays</i>	7e ⁻²⁴
ES304077	NB-ARC domain containing	ABA91338	<i>O. sativa</i>	4e ⁻¹¹
ES304457	NB-ARC domain containing	ABG22544	<i>O. sativa</i>	5e ⁻¹³
ES304944	NB-ARC domain containing	ACX68999	<i>O. sativa</i>	1e ⁻¹⁶
ES305038	LRR19	AAK20736	<i>Triticum aestivum</i>	3e ⁻³⁵
ES306059	Putative blight-associated protein p12	BAD09315	<i>O. sativa</i>	1e ⁻³²
ES306149	Putative disease-resistance protein RPM1	ABA97409	<i>O. sativa</i>	2e ⁻⁰⁵
ES307083	Putative disease-resistance protein	NP_001064537	<i>O. sativa</i>	4e ⁻⁴⁶
ES307377	NB-ARC domain containing	ACX69175	<i>O. sativa</i>	4e ⁻⁴⁵

^zTblastx 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) ^z	Amplicon size (bp) (nondigested, digested) (-Dominant parent)	SEG ^y
BRGA38-F	M13 ^x -AATCTAGCGTTCCCTGACGA	50	151 ^w ,50-T574	No
BRGA38-R	AGGTCCATGCTTCAGACGAC			
BRGA81-F	M13-GCATTGGCAGAGCATGTAAA	56.7	156-T574	No
BRGA81-R	TCGCGTGGTTATTGTGATGT			
BRGA131-F	TTTCGAAGCTGCTGTTGTTG	63.4	375-T89	No
BRGA131-R	GCGACCAACTCTGTTTGCTC			
BRGA163-F	TGGCTGTGTGTGACAGATGA	45	402-T89	No
BRGA163-R	ACACTTCCCAATTTCCACCA			
BRGA167-F	CGCTTGGGCAGGTAGTGTAT	45	500 ^w ,400-T574	No
BRGA167-R	TCTCAACAAATGCTGCTTGC			
BRGA179-F	GCATGGCTTTGTGTTTCTCA	45	328-T89	Yes
BRGA179-R	ACAGCTCCCATCCTACATCG			
BRGA201-F	M13-GGACCATATGCATCGTGTG	63.4	124-T89	Yes
BRGA201-R	GGCCTGAAGATGTTGCACTT			
BRGA208-F	CATGAGCAGAGGATACAGGAGA	45	367 ^w ,300-T89	Yes
BRGA208-R	TCAACAGGTCTAGGCCATCC			
BRGA229-F	M13-GCTGCAGCCACAGGAATAAT	45	108-T574,126-T89	Yes ^v
BRGA229-R	TCCTATGACCACCGACATCA			
ES292237-F	M13- AGCTCAGGGAAGTGGTCTCA	64.6	160-T89	No
ES292237-R	GGGTCAATGGTTTCAGCACT			
ES292682-F	M13-GGTGGCTAGGCTTGGTATGA	50	122,140-T89	Yes ^v
ES292682-R	CACCCAGGTCATGGTAGCTT			

continued next page

Supplemental Table 2. Continued.

Primer	Sequence	Ta (°C) ^z	Amplicon size (bp) (nondigested, digested) (-Dominant parent)	SEG ^y
ES293253-F	M13-AGAGCTGTGGAGGCACACTT	45	150 ^u , 148-T89	No
ES293253-R	TCCTCAAGCCTAGGCGTACT			
ES295859-F	M13-ACCAAAGTGGTTCCCATTC	45	94-T89	Yes
ES295859-R	GTCTTCCGTGGCACTTAGTTTCAG			
ES296552-F	M13-GTGGTAAATCTTGCCCAATCAAC	63.2	96-T89	No
ES296552-R	GCAGAAGAGGGTAAATGCATCAC			
ES296578-F	AGTACCCAGCCCAGTCACAG	45	576-T89	Yes
ES296578-R	CACGTGTCAACAGGTGATCC			
ES297666-F	M13- GCAGGTCCATCGATGAAAT	45	126-T89	Yes
ES297666-R	GCCGGCAAAAATATCAAGAA			
ES298588-F	M13-CCCCCGAAATAGGTTTCTTC	50	134-T89	Yes
ES298588-R	AAGCAGGATTTGCTGCACTT			
ES299276-F	M13- CGAGCTTTCGTCTTCGTAGG	50	146-T89	Yes
ES299276-R	TGTTTCGATGCTGGAGATGAG			
ES299682-F	M13-CAAGATGTGCTTCCACCAGA	50	158 ^w , 156-T574	Yes ^t
ES299682-R	GCCAGTAACCGATTTTCAT		156 ^w , 154-T89	
ES299920-F	M13-ACCTACATGCTGGGGTCCAT	40	260-T574	No
ES299920-R	CTCTAACCTCCTCCGACGTG			
ES302322-F	M13-CTCCGCATCTACCTCGAGTC	45	148-T89	Yes
ES302322-R	GAGAGCCACATGCTGTTTCA			
ES303684-F	GGGGTCATTTGCGTGACTT	45	523-T89	Yes
ES303684-R	CCCATCTCTTCAAAGCTTGC			
ES303694-F	M13-AGTCCTCCTGCCTTCTCAT	61.8	122-T89	Yes
ES303694-R	GTAGTGCTCGTGTCGTCAGC			
ES304077-F	M13-GTTGGGCAAGACCACTTGTT	50	150-T89	Yes
ES304077-R	CCAATCGTTTGGGTCTGAAC			
ES304457-F	M13-TCCCTTCATCCTACCAAAG	50	268,270-T89	Yes ^v
ES304457-R	CAGAAGCTCTATCGCCTCGT			
ES305038-F	M13-GGTTTGGCGTCACTTAGCTC	50	130-T89	No
ES305038-R	GCCTCCACCATACTCTCAA			
ES306059-F	M13-GTTCACTGTGATGGCACTGG	50	167-T89	No
ES306059-R	CGTGGTGCTTTTGTCACTGT			
ES306149-F	M13-AGGAAAAATGGGTTCGAAGG	50	138-T574	Yes
ES306149-R	TAATATGGCAGGCGATTTC			
ES307083-F	M13-GGGTACACGCTCAGCAAGAT	66.8	122-T89	No
ES307083-R	GCAAGAGTTGAGACGGGTTTC			
ES307377-F	M13-AACGGTGTCGGAAGACAAC	61.8	129-T89	Yes
ES307377-R	TCCATTTGTCGTGATCGTGT			

^zTa was the annealing temperature used.^ySEG was whether the polymorphic marker was segregating in the F₁ population.^xForward primers were labeled with a 5' M13 Tag (TGTAACACGACGGCCAGT) 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.^wAmplicon was digested with *Hpa*II to generate a polymorphism between the mapping parents T89 and T574.^vBoth amplicons from T89 were segregating.^uAmplicon was digested with *Eco*RV to generate a polymorphism between the mapping parents T89 and T574.^tOnly 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) ^z
BRGA131F	TTTCGAAGCTGCTGTTGTTG	375
BRGA131R	GCGACCAACTCTGTTTGCTC	
BRGA136F	CTCGCAGACCTTCACAATCA	431
BRGA136R	GGCATTTCCTTGAGCATACTGG	
BRGA162F	ACTTCAGAGCCACCGATGTT	393
BRGA162R	TGACGACACACTTACCGATCA	
BRGA163F	TGGCTGTGTGTGACAGATGA	402
BRGA163R	ACACTTCCCAATTTCCACCA	
BRGA167F	CGCTTGGGCAGGTAGTGTAT	411
BRGA167R	TCTCAACAAATGCTGCTTGC	
BRGA173F	GCCGGAAAAGGTTCTCTACTT	257
BRGA173R	TGTTTGCTCTTGCACTCCTC	
BRGA179F	GCATGGCTTTGTGTTTCTCA	328
BRGA179R	ACAGCTCCCATCCTACATCG	
BRGA208F	CATGAGCAGAGGATACAGGAGA	367
BRGA208R	TCAACAGGTCTAGGCCATCC	
ES295859F	TCCAGGCATTACCAACCTTC	660
ES295859R	AGAGCCCCTTCATGAAACCT	
ES296552F	AGTACCCAGCCCAGTCACAG	576
ES296552R	CACGTGTCAACAGGTGATCC	
ES296578F	GCTCCATCTTGGACTTGGAC	587
ES296578R	CTTTGTGTGCGGTGTCGCTAA	
ES303684F	GGGGTCATTTGCGTGTACTT	523
ES303684R	CCCATCTCTTCAAAGCTTGC	
ES304944F	ATCGCAAACACCGAGCTATT	510
ES304944R	TCTTGTCTCTCGAACATGCTG	

^zThe annealing temperature used to amplify these fragments among the turf genera was 45 °C.

	1	10	20	30	40	50	60
	<u>P-loop</u>			<u>RNBS-A-nonTIR</u>			
BRGA172	GGVGKTT	LAKMVYNDPRVQKH	FELKMWYCVSENF	EVTTIMRSVIE	-LG-----	TNERC	
BRGA161	GGVGKTT	LAKMVYNDPRVQKH	FELKMWYCVSENF	EVTTIMRSVIE	-LG-----	TNERC	
BRGA168	GGVGKTT	LAEMVYNDRCRVQKH	FELKMWYCVSENF	EATTIMRSVIE	-LG-----	TNERC	
BRGA131	GGVGKTT	LAKMVYNDSRVQNH	FELKLWYCVSKNF	EAAVVVRSVIE	-L-----	ATNGRC	
BRGA162	GGVGKTT	LAEMVYNDRCRVQKH	FELKMWHCVSKNF	RATDVVRSIIELA	-----	TVEPC	
BRGA163	GGVGKTT	LAKVVYNDRCRVQKH	FELIMWLCVTDDF	SVSGLVRSIVELA	-----	TKKPC	
BRGA167	GGVGKTT	LGQVVYTDSDRVVQY	FDLRAWVCVSEYF	DVKNITRKIIITSL	-----	TRSCC	
BRGA132	GGVGKTT	LGQVVYADSRVVQY	FDLRAWVCVSEYF	DVKNITRKIIITSL	-----	TRSCC	
BRGA169	GGVGKTT	LGQVVYADSRVVQY	FDLRAWVCVSEYF	DVKNITRKIIITSL	-----	TRSCC	
BRGA179	GGVGKTT	LAQKVYNDNKIKGK	FSRKAWLCVSQEV	SVVSLKELLRNIG	-----	ADY	
BRGA208	GGVGKTT	LAQKIYHEQRIQEK	FQIPIWLCSISQSY	TEIDLLKQAIMAG	-----	GSY	
BRGA211	GGVGKTT	LAQKIYHEQRIQEK	FQIPIWLCSISQSY	TEIDLLKQAIMAG	-----	GSY	
BRGA202	GGVGKTT	LAQKIYHEQRIQEK	FQIPIWLCSISQSY	TEIDLLKQAIMAG	-----	GSC	
BRGA216	GGVGKTT	LAQKIYHEQRIQEK	FQIPIWLCSISQSY	TEIDLLKQAIMAG	-----	GSY	
BRGA136	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA66	GGVGKTT	FVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGNSRASPSNTKT			
BRGA147	GGVGKTT	FVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGNSRASSSNTKT			
BRGA153	GGVGKTT	FVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGNSRASSSNTKT			
BRGA67	GGVGKTT	FVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGNSRASSSNTKT			
BRGA182	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCPNAKT			
BRGA186	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA184	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA185	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA221	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA149	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA34	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TISDLLRKILKELGCNSASCSNAKT			
BRGA33	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA31	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA79	GGVGKTT	LVTRLYKEV	-ATSHFECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA181	GGVGKTT	LVTRLYKEV	-ATSHLECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			
BRGA238	GGRGKTT	LVTRLYKEV	-ATSHLECAAWVAVSQTF	TINDLLRKILKELGCNSRASCSNAKT			

	<u>Kinase-2</u>									
	<u>RNBS-B</u>									
BRGA172	DMRDNIEMLKGR	LQEVIGRKRFL	LILDDVW	NEDQRKWEDE	LRPLLCS	IGGC	GSAIVMTS			
BRGA161	DMRDNVEMLKGR	LQEVIGRKRFL	LILDDVW	NEDQRKWEDE	LRPLLCS	IGGC	GSAIVMTS			
BRGA168	DMRDNIEMLKGR	LQEVIGRKRFL	LILDDVW	NEDQRKWEDE	LRPLLCS	IGGC	GSAIVMTS			
BRGA131	DLPDNIEMLLKGR	LQEVIGRKRFL	LILDDVW	NEKHVKWEED	LRPLLCS	IGGC	GSTIVVTS			
BRGA162	DLPDNIEMLLKGR	LQEVIGRKRFL	LILDDVW	NEDQRKWEDE	LRPLLCS	IGGC	GSMIVVTS			
BRGA163	LLTDRIELLSHY	LLEVVQKRYL	LVLLDDVW	NEEQKWEDLRP	-LLCSAGAP		GRVVIVTT			
BRGA167	RHIQD	-GGLQGALVDH	VKEKSV	FLVLDDVW	NERRDYWELL	CMPLASKQ	---	CSIIVTT		
BRGA132	RHIQD	-GGLQGALVDH	VKEKSV	FLVLDDVW	NERRDYWELL	CMPLASKQ	---	CSIIVTT		
BRGA169	RHIQD	-GGLQGALVDH	VKEKSV	FLVLDDVW	NERRDYWELL	CMPLASKQ	---	CSIIVTT		
BRGA179	QQDETAGE	LTKKLATAVEN	NMSFF	FLVLDDIWE	HE--VWNTILR	---	TPLNAA	TSVILVTT		
BRGA208	DQLEAKSELLRL	MDTVTGKSI	FLVLDDVW	KSD--VWNKLLQ	--MPFETGV	NLTLPVTT				
BRGA211	DQLEAKSELLRL	MDTVTGKSI	FLVLDDVW	KSD--VWNKLLQ	--MPFETGV	NLTLPVTT				
BRGA202	DQLEAKSELLRL	MDTVSGKTI	FLVLDDVW	KSD--VWNELLQ	--TPFETGV	NLTLPVTT				
BRGA216	DQLEAKSELLRL	MDTVSGKSI	FLVLDDVW	KSD--VWNELLQ	--TPFETGV	NLTLPVTT				
BRGA136	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA66	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA147	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA153	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA67	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA182	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA186	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA184	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA185	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA221	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA149	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA34	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA33	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA31	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA79	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA181	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				
BRGA238	DTDDYRSLME	AVQGHLFQRRY	LVVLDDI	WDPH--LWGKLLN	--LAFPD	DATSRVVITT				

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.

BRGA172 RLQQVASIMGTLPHHELKILSEESWKLFSMKAFANRGVQE-QTELVSTSKRIVNNCKGL
 BRGA161 RLQQVASIMGTLPHHELKILSEESWKLFSMKAFANRGVQE-QTELVSTSKRIVNNCKGL
 BRGA168 RLQQVASIMGTLPHHELKILSEESWKLFSMKAFANRGVQE-QTELVSTSKRIVNNCKGL
 BRGA131 RLQQVATIMGTVPHHNLKILTEESRQLFSTKAFANRVQEQ--TELVAIGKRIVNNCKGL
 BRGA162 QSRQVASIMGTLPLYELACLSEHESWELFSKKA FNRLQKQ---DELILIGKCVNMCKGL
 BRGA163 RSEQVASIMGTLPSHMSISYLNQDDSWELFRKKAFCREEDEQ--PKLVEIGKCIVKKCKGL
 BRGA167 RSKVVARLVQTMPFYNLKCLGPDSDWSL FKQAAFVEKGIAT-PVNL MNIGRSITEKCKGL
 BRGA132 RSKVVARLVQTMPFYNLKCLGPDSDWSL FKQAAFVEKGIAT-PVNFVNIGRSITEKCKGL
 BRGA169 RSKVVARLVQTMPFYNLKCLGPDSDWSL FKQAAFVEKGIAT-PVNFVSIIGRSITEKCKGL
 BRGA179 RKDTVARAIGVEDVHRVELMSNDVGWELLWKS--MNI SKETDVQNLRGIGMDIVRMCGGL
 BRGA208 RDL DVLGQM HAKYTHKV NQINITDGLDLLMERSFR--PDEQIDDGFRDVGRI VQKCDGL
 BRGA211 RDL DVLGQM HAKYTHKV NQINITDGLDLLIKRSFR--PDEQIDDGFRDVGRI VQKCDGL
 BRGA202 RDL DVLGQM HAKYTHKV NKMNITDGLDLLIRRSFR--PDEQINDGFRDVGRI VQKCDGL
 BRGA216 RDL DVLGQM HAKYTHKV NKNITDGLDLLIKRSFR--PDEQINDGFRNVGRI VQKCDGL
 BRGA136 RSNEVAKAATRERTMMLEPLQSSEAWTLFCNITFRDIPNRTCPTHLEELATSM LKKCHGL
 BRGA66 RSNEVAKAATRERTMMLEPLQSSEAWTLFCNITFRDIPNRTCPTHLEELATSM LKGCHGL
 BRGA147 RSNEVAKAATRERTMMLEPLQSSEAWTLFCNITFRDIPNRTCPTHLEELATSM LKRCHGL
 BRGA153 RSNEVAKAATRERTMMLEPLQSSEAWTLFCNITFRDIPNRTCPTHLEELATSM LKRCHGL
 BRGA67 RSNEVAKAATRERTMMLEPLQSSEAWTLFCNITFRDIPNRACPTHLEELATSM LKRCHGL
 BRGA182 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA186 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA184 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA185 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA221 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA149 RSNEVAKAATRERIMMLEPLQLSGAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA34 RSNEVAKAATRERIMMLES LQLSEAWTLFCNITLRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA33 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA31 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA79 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRT RPSHLEELATSM LKRCHGL
 BRGA181 RSNEVAKAATRERIMMLEPLQLSEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL
 BRGA238 RSNEVAKAATRERIMMLEPLQLCEAWTLFCNITFRDVPNRTCPSHLEELATSM LKRCHGL

	HD
BRGA172	E---
BRGA161	E---
BRGA168	E---
BRGA131	E---
BRGA162	E---
BRGA163	E---
BRGA167	E---
BRGA132	E---
BRGA169	E---
BRGA179	PLAL
BRGA208	PLA-
BRGA211	PLA-
BRGA202	PLA-
BRH216	PLA-
BRGA136	PLAL
BRGA66	PLAL
BRGA147	PLAL
BRGA153	PLAL
BRGA67	PLAL
BRGA182	PLAL
BRGA186	PLAL
BRGA184	PLAL
BRGA185	PLAL
BRGA221	PLA-
BRGA149	PLAL
BRGA34	PLAL
BRGA33	PLAL
BRGA31	PLAL
BRGA79	PLAL
BRGA181	PLAL
BRGA238	PLA-

Supplemental Fig. 1. (Continued).

TRGA	Primer	Genotype	analog (BLASTx)	Accession	Organism	e-value
TRGA1	ES295859	Empire	putative disease resistance protein	BAC15497	<i>Oryza sativa</i>	6e ⁻⁷⁶
TRGA2	ES296552	Empire	putative NBS-LRR protein	ACD70335	<i>Saccharum arunaceum</i>	7e ⁻⁶⁹
TRGA3	BRGA173	SI1	NBS-LRR like protein	ABW04965	<i>Eleusine coracana</i>	5e ⁻²⁴

TRGA1	GITNIPNVNLLAGKRCYHHNGLWPISEFWGLHVPKKLGNLDALRTLAQVAFTESTTQGI
ES295859	GITNIPNVNLLAGKRYHHNGLWPISEFWGLYVPKKLGNLDALRTLAQVAFTESTTQGI
TRGA1	SELGKLPRLNKLGVMMFVDDDSWASLISALENLSGNLCSLLWLRPDGVMNFACLDLSLR
ES295859	SELGKLSGLKLGVMFVDDDSWASLISALNLSGNLCSLLWLRPDGAMNYDCNLSLR
TRGA1	PMFMKSINFRGQLIKLPKWFPLLSNLTELTIRATELSATEDLRVLARLPSTLYLRLHHS
ES295859	PMFMKSINFRGQLIKLPKWFPLLSNLTELTIRATELSATEDLKVLARLPSTLYLRLHHS
TRGA1	AFVHTEFSVAASEFPCLKLLVIHLATHEPWFARFHEGA
ES295859	AFVQPEFAVAALDFPRLKLLVIHLALPQTWTFARFHEGA

	Kinase-2	RNBS-B
TRGA2	-YPAQSQLADLDAIDLKKEIKKRVDGKCLLVLDVVDKEAFHRIISDALQNQAIRVIT	
ES296552	<u>eYPAQSQLADLDAIDLKVKITEKLTA</u> GKCLLVLDVVSQEAFLQIRDTLQNQAIRVIT	
TRGA2	TRQEHVATLAQPTQLKLPLEHSDAFSLFCRKAFYNSMESKCPPELEKLANNIVGKCOG	
ES296552	TRQEHVVNLAQSTHQLRLKPLEHSDAFTLFCKKAFCNRMHRCPODLNLANNVVDKCRG	
	HD	
TRGA2	LPLATVISIAGMLSSLPOKEYVWNEMYQLRSELANDNHVSAILNLSYHDLSGELRNCFLY	
ES296552	LPLATVISIGMLSSLPPTEYVWSEMYQLRSELANDNHVSAILNLSYHDLSGELRNCFLY	
	RNBS-D-NonTIR	
TRGA2	CSLFPEDHLLTR	
ES296552	CSLFPEDHLLTR	

	Kinase-2	RNBS-B
TRGA3	RKRFLLMXDDVWNEDQNKWEEDLRPLLCSIGGLGSMIVVTSRSRQVAEIMGTLPPEHELV	
BRGA173	RKRFLILDDVWNEDQNKWEEDLRPLLCSIGGCGSAIVMTSLRQQVASIMGTLPPEHELV	
TRGA3	CLSEDDSWELFSKKXFS-RGVQEQT	
BRGA173	ILSEEEESWKLFSMKAFARGVQEQT	

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.