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Resistance of transgenic tall fescue to two major fungal diseases

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Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is an open-pollinated, perennial, cool-season turf and forage grass species of great economic importance. The main problems of maintenance of tall fescue, and many other turfgrasses, are two severe fungal diseases: gray leaf spot caused by *Magnaporthe grisea*, and brown patch caused by *Rhizoctonia solani*. Three genes from various sources have been introduced into two elite cultivars, 'Coronado' and 'Matador', of tall fescue through *Agrobacterium*-mediated transformation, and conferred resistance to the diseases. Two genes, the alfalfa β-1,3-glucanase *AGLU1* gene and a truncated frog dermaseptin SI gene, conferred resistance to both diseases. The rice *Pi9* gene was specific against gray leaf spot. Of 15 T_0 transgenic plants examined, 6 showed high levels of resistance to *M. grisea*, and 3 had enhanced resistance to *R. solani*. The resistance in most of these cases was highly significant.

Keywords: alfalfa *AGLU1* gene; Dermaseptin SI gene; Fungal resistance; *Magnaporthe grisea*; *Rhizoctonia solani*; Rice *Pi9* gene

1. Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is an open-pollinated, perennial, cool-season turf and forage grass species of great economic importance in Europe, Asia, North Africa, and North America. As a turfgrass, it is widely used in home lawns, parks, golf courses, playgrounds, and athletic fields. Tall fescue can also serve as a utility turfgrass for soil solidification and as a feed for the livestock [1]. A major problem in growing tall fescue is its susceptibility to fungal diseases.

Gray leaf spot disease, caused by ascomycete fungus *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa, has recently become a severe disease of tall fescue in the Southeastern U.S., and of perennial ryegrass in many areas of the country [2]. Initial symptoms of the disease are round or oval leaf spots that are tan with a dark brown border. Under optimal environmental conditions and on a susceptible host, the leaf spot may expand and increase in number rapidly, leading to widespread blighting of the turfgrass foliage. Partial resistance to the disease was recently observed in perennial ryegrass and its inheritance studied [3,4]. *M. grisea* is also the causal pathogen of rice blast, one of the most important fungal diseases in rice. Extensive research on the biology of the fungal pathogen, its pathogenesis, and the resistance genes from rice has been conducted [5]. Recently, the sequencing of *M. grisea* genome was completed [6], which will facilitate further research on the molecular processes of pathogen infection and shed lights on new mechanisms for enhancing resistance in host crops.

Brown patch is caused by multiple strains (anastomosis groups) of the soilborne basidiomycete fungus, *Rhizoctonia solani*. It is the most severe and frequently occurring disease on tall fescue lawns during warm and humid conditions in the summer. It is also one of the most serious diseases of other turfgrass species [7]. Brown patch symptoms are first observed as small, circular or irregularly shaped lesions with a dark brown border. With growth of the hyphae, the pathogen spreads to other plants, forming brown-colored patches, which often coalesce to blight large sections of the turf, ranging from 6 inches to several feet in diameter [8].

Earlier reports on engineered fungal resistance in transgenic plants often used plant β-1,3-glucanase and/or chitinase genes [9–12], due to their ability to degrade β-1,3-glucan and chitin,

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two major components of fungal cell walls. Chitinase may also inhibit fungal growth by binding to chitin of the fungal cell wall [13]. Expression of ribosome-inactivating proteins (RIPs [14]) or metabolic engineering to produce a phytoalexin [15] for fungal resistance has also been reported to increase fungal resistance in plants. Some small antimicrobial peptides [16,17], including plant defensins [18,19], showed enhanced resistance to certain fungal pathogens. Moreover, disease resistance (R) genes, when introduced or over-expressed in plants, can trigger plant defense systems against fungal pathogens [20].

Conventional breeding and genetics studies on turfgrass resistance to fungal diseases have been reported [3,4,21], and brown patch disease resistant cultivars through conventional breeding efforts have been released [22–25]. However, it is unclear whether the resistance is against specific races or a broad spectrum of races. Moreover, it was observed in conventional breeding that brown patch resistant cultivars tend to be susceptible to gray leaf spot, and vice versa (Rose-Fricker, personal communication), making breeding resistance to both pathogens very difficult. Engineered resistance to fungal pathogens in turfgrass species has been reported. Over-expression of a receptor protein kinase PR5K [26] or the rice gene TLPD34, encoding a thaumatin-like protein [27], in transgenic creeping bentgrass plants enhanced resistance to dollar spot disease caused by *Sclerotinia homoeocarpa* (F.T. Bennett). We recently demonstrated that the bacteriophage T4 lysozyme gene conferred resistance to both gray leaf spot and brown patch diseases in transgenic tall fescue plants [28]. In this study, we tested the alfalfa β-1,3-glucanase *AGLU1* gene [29], a truncated dermaseptin SI gene from the South American arboreal frog *Phyllomedusa sauvagei* [30,31], and the rice *Pi9* gene, which is an R gene resistant to the rice blast disease [18], respectively. We observed remarkably enhanced resistance to the turfgrass isolates of the gray leaf spot pathogen, and improved resistance to the brown patch disease.

2. Materials and methods

2.1. Gene construction and Agrobacterium strains

Three binary vectors, pSD7, pSD12, and pNBS2, were used in the transformation experiments (Fig. 1). All three vectors were derivatives of the binary vector pCAMBIA1300, which has a hygromycin B resistant *hph* gene under control of the CaMV 35S promoter and the 35S terminator (http://www.cambia.org.au). The pSD7 vector has a construct of the alfalfa (*Medicago sativa*) β-1,3-glucanase *AGLU1* cDNA [29] under control of the maize *Ubi1* gene promoter (Fig. 1A [32]). Plasmid pSD12 contains a truncated dermaseptin SI gene of 18 AA from the South American arboreal frog *P. sauvagei* [30,31]. The 54 nt coding sequence was fused in-frame at its 5′-terminus with the ubiquitin monomer coding sequence of the rice *rubi3* gene, and the fusion gene was under control of the *rubi3* gene promoter with its 5′ UTR intron (Fig. 1B, [33]). Forward primer 5′-GTATTGTATCTGGCTCTTTGCC-3′, and reverse primer 5′-TCGAGCTCTTAGCCAGCGTGCAGAGCCATGGTGCCGA-

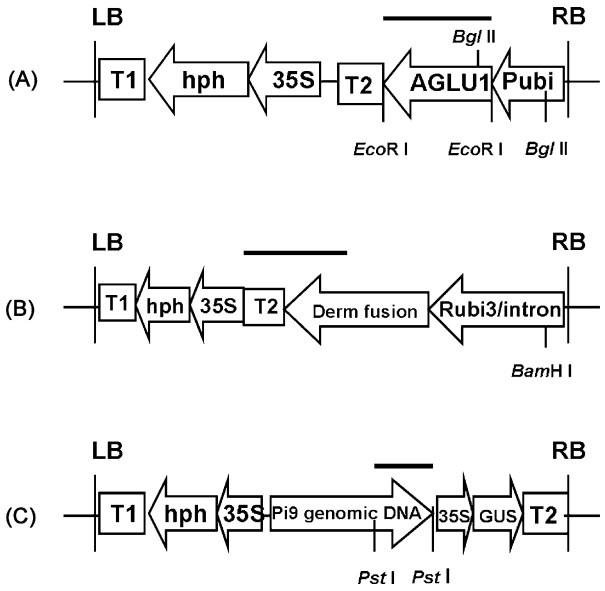

Fig. 1. Diagrams of the T-DNA regions of the binary vectors used in the transformation experiments. All the vectors are the derivatives of pCAMBIA1300. RB: right border; LB: left border; 35S: CaMV 35S promoter; *hph*: *E. coli* hygromycin phosphotransferase gene coding region; T1: CaMV 35S terminator; T2: *Agrobacterium tumefaciens nos* gene terminator. The probes used for Southern and Northern analyses are shown by bars. (A) pSD7. AGLU1: alfalfa *AGLU1* glucanase gene coding region; Pubi: maize *Ubi1* gene promoter. The BglII and EcoRI restriction sites are indicated. (B) pSD12. Rubi3/intron: rice *rubi3* gene promoter with its 5′ UTR intron; Derm fusion: in frame fusion of *rubi3* ubiquitin monomer coding sequence with the truncated 18-AA dermaseptin SI gene. The BamHI site is indicated. (C) pNBS2 containing the rice Pi9 genomic sequence and a *GUS* reporter gene construct. The PstI sites are indicated. GUS: *GUS* coding region with an intron insertion [18].

GCTTCTTCAGCATGGTCTTCCAGAGGGCGCCTCCACG-AAGGCGG-3′ (the anti-sense sequence of the truncated dermaseptin SI coding sequence is underlined) were designed and used for PCR to obtain the fusion gene from the plasmid pRESQ38 [33]. The pNBS2 vector has a 13.5 kb fragment of rice genomic DNA containing the *Pi9* gene (Fig. 1C [18]). Plasmids pSD7, pSD12, and pNBS2 were mobilized into *Agrobacterium tumefaciens* strain EHA105 (pTOK47) using the freeze–thaw method [34], respectively, as previously described [35].

2.2. Tall fescue transformation

Mature seeds from tall fescue cultivars ‘Coronado’ [36] and ‘Matador’ [24] were used to induce embryogenic calli as explants for *A. tumefaciens*-mediated transformation as described previously [28]. In brief, the infected calli were cultured under 250 mg l^{-1} hygromycin B (hyg B, A.G. Scientific, San Diego, CA, USA) selection at 25 °C in the dark for 8 weeks. Hyg B resistant calli were transferred to the selective regeneration medium (with 50 mg l^{-1} hyg B) for shoot induction. Root growth was induced from regenerated shoots on a rooting medium containing 50 mg l^{-1} hyg B. About 4 weeks later, the plantlets were transplanted into potting soil (Metro-Mix 200, Scotts, Marysville, OH) and subsequently grown in a greenhouse at 25 °C. All chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise specified.

2.3. Southern blot analysis

Twenty-five micrograms of genomic DNA isolated from each sample was digested overnight, separated by electrophoresis in a 0.8% agarose gel and blotted onto Hybond-N^+ membranes (Amersham, Little Chalfont, Bucks, UK) [28]. The restriction enzymes BglII, BamHI, or PstI (Promega, Madison, WI, USA) were used to digest genomic DNA of transgenic plants from pSD7, pSD12, and pNBS2, respectively (Fig. 1). The full-length coding region of *AGLU1* (1.3 kb EcoRI fragment of the pSD7) was used as a probe to detect this transgene. A 0.3 kb PCR fragment of pSD12 containing the dermaseptin SI coding sequence and the *nos* terminator sequence, or a 3 kb PstI fragment of pNBS2 containing a segment of the *Pi9* genomic DNA [18] was employed to probe these two transgenes, respectively. All probes were labeled with [α-^{32}p]-dCTP (Amersham) as previously described [28]. The membranes were exposed to Kodak BioMax MS film for autoradiography (Kodak Eastman, Rochester, NY, USA).

2.4. Northern blot analysis and RT-PCR assays

Twenty micrograms of total RNA extracted from plant leaves using Trizol® Reagent (Invitrogen, Carlsbad, CA, USA) was subjected to electrophoresis in 1% agarose gels prepared with 1× MOPS [37]. After electrophoresis, RNA was transferred to a Hybond N^+ hybridization membrane according to the manufacturer's instructions. The same probes described for Southern analysis were used for Northern hybridizations. The blotted membranes were probed in Denhart's hybridization solution and washed according to the manufacture's instructions. Kodak BioMax MS film was used for autoradiography.

RT-PCR analysis was performed to confirm or detect the mRNA expression of *AGLU1* or *Pi9* gene. Total RNA was extracted from plant leaves using RNeasy® Plant Mini Kit (Qiagen, Valencia, CA, USA). During the extraction process, RQ1 RNase-free DNase (Promega, Madison, WI, USA) was used to remove genomic DNA. One microgram RNA from each plant was reverse transcribed with the reverse primer Glu2 (5′-CCAACCACTCTCAGATAC-3′) or NBS2-H (5′-AACATGAGTAGAAACAAATTAGTTTG-3′, [18]) using AMV reverse transcriptase (Promega) at 42 °C for 60 min. PCR was carried out with 2 μl of RT reaction product, and Glu1(5′-CCTTCTTTCTTTGCTCCA-3′) and Glu2 primers for the *AGLU1* gene fragment amplification, or NBS2-G (5′-TGCCCAACCTTTACCCACTGTA-3′, [18]) and NBS2-H for the rice *Pi9* gene fragment amplification. The standard 35-cycle PCRs were performed at 50 °C using the AccuPower® PCR Premix (Bioneer, Daejeon, Korea). PCR products were analyzed on a 0.9% agarose gel.

2.5. Plant inoculation with M. grisea

Two isolates of *M. grisea* obtained from tall fescue, 1213-59 and 533-78 [2], were plated on potato dextrose agar medium (Becton, Dickinson and Company, Sparks, MD, USA) amended with 50 mg l^{-1} each of tetracycline, streptomycin, and chloramphenicol, and grown for seven days at 25 °C in the dark. To produce conidia, each isolate was transferred to 1.5% Difco™ water agar medium overlaid with 12 sterilized alfalfa stem sections (6 cm long) at 25 °C under continuous fluorescent lighting. Two weeks later, conidia from the two isolates were harvested and mixed, and the final concentration was adjusted to 2×10^5 conidia ml^{-1} using a hemacytometer under a light microscope [2].

Inoculations with *M. grisea* were performed in a growth chamber with temperature and humidity control, at the NCSU Phytotron. The fungicide flutolanil (Prostar®, Bayer Environmental Science, Research Triangle Park, NC, USA), which has no activity against *M. grisea* was applied to the test plants 4 days before inoculation to prevent contamination from *R. solani* (L.P. Tredway, personal observation). Each plant was sprayed with 7.5 ml of conidia suspension using an airbrush sprayer (Badger Air-Brush Co., Franklin Park, IL, USA). The plants were kept at 24 °C, 100% relative humidity (RH), in the dark for the first 24 h, and subsequently incubated in a lighted growth chamber with a 12/12 h (day/night) photoperiod. Temperature and humidity conditions were 30 °C with 70% RH during daytime, and 24 °C with 100% RH at night. The photosynthetic photon flux density (PPFD) was 600 μmol m^{-2} s^{-1} (400–700 nm) as measured at plant level from a combination of cool white fluorescent and incandescent lamps at an input wattage ratio of 10:3. Disease development was evaluated 10–14 days after inoculation. Disease incidence was calculated as the percentage of symptomatic leaves out of the total number of leaves in each plant [2]. Lesion length was measured from ten representative leaves from each plant. The experiment was repeated twice.

2.6. Plant inoculation with R. solani

Transgenic plants were challenged with *R. solani* Kuhn subgroup AG1-1A, the most common cause of brown patch in tall fescue [38]. Cultures were grown on potato dextrose agar at 25 °C for 3 days prior to inoculation. The inoculation was carried out in a growth chamber. An agar plug (3 mm × 3 mm) infested with mycelium was placed on the midrib of each leaf for inoculation. Four young leaves from each plant were randomly chosen for inoculation. After inoculation, plants were grown in a lighted growth chamber under a 14/10 h (day/night) photoperiod with the same light conditions as described above. The temperature and RH were 30 °C and 70% during daytime, and 24 °C and 95% at night. After 14 days, disease development was evaluated by measuring lesion size from the point of inoculation to the farthest point of lesion expansion. The experiment was repeated three times.

2.7. Statistical analysis

Both inoculation tests used randomized complete block design. Brown patch resistance was analyzed by ANOVA with the lesion size data log transformed. ANOVA was also

Table 1
The original and modified nucleotide sequences, and the amino acid sequence, of the truncated dermaseptin SI gene used in the experiments

Original	GCT CTG TGG AAA ACT ATG TTA AAA AAA TTA GGA ACA ATG GCT TTA CAT GCA GGA
Modified	GCC CTC TGG AAG ACC ATG CTG AAG AAG CTC GGC ACC ATG GCT CTG CAC GCT GGC
AA	A L W K T M L K K L G T M A L H A G

Note: Altered nucleotides are underlined.

employed for gray leaf spot tests using lesion length and disease incidence data with SAS v.9.2 (SAS Institute, Cary, NC, USA). CONTRAST statements were used to compare each transgenic plant to a non-transformed control plant or the mean of the control plants.

3. Results

A total of 15 T_0 transgenic plants were obtained through *Agrobacterium*-mediated transformation from the three gene constructs. Among them, 12 contained the *AGLU1* gene (Glu plants), two expressed the dermaseptin SI fusion gene (Derm plants), and one had the rice *Pi9* gene, including its own promoter, exons and introns, and terminator (Pi9 plant, [18]). All the independently transformed plant lines looked normal and grew vigorously. The truncated dermaseptin SI gene used in the experiments encodes a peptide of its N-terminal 18-AA residues (Table 1), which was shown to have active antimicrobial activity [30]. To increase the expression of the small peptide, the rice *rubi3* promoter and a fusion with the *rubi3* ubiquitin monomer coding sequence were used for the construct. It was demonstrated that the N-terminal fusion of the rice *rubi3* ubiquitin monomer coding sequence in frame with a transgene coding sequence enhances transgene expression by about four fold and the original protein encoded by the transgene is released in such a construct after excision of the ubiquitin monomer by an ubiquitin cleavage mechanism inside the cells [33]. Moreover, plant preferred codons [39] were used in the synthetic gene (Table 1) to facilitate efficient translation.

3.1. Southern analysis of the transgenic plants

Southern analysis was performed on the putative transgenic plants and the transgenic nature of these plants was confirmed. Of these plants, 4 Glu transgenic plants, 2 Derm plants, and 1 Pi9 plant, which exhibited disease resistance (below), are shown in Fig. 2. The various hybridization patterns confirm that the plants were from independent transformation events.

3.2. Northern and RT-PCR analyses of the transgenic plants

Northern blot analyses were performed to detect transgene expression at RNA level among the resistant transgenic plants (Fig. 3A and B). All the resistant transgenic plants with *AGLU1* (Glu17a was not tested) or dermaseptin SI fusion gene showed detectable transcript accumulation. However, unexpected multiple RNA species, in addition to the expected 1.5 kb mRNA, hybridized with the *AGLU1* probe in the Glu plants. Northern analysis failed to detect the *Pi9* transcript in the Pi9-1 plant (data not shown) most likely due to its low natural expression [18].

To further confirm expression of the *AGLU1* transgene, or to detect the *Pi9* gene expression in these transgenic plants, RT-PCR analysis was carried out for these plants (Fig. 3C–F). DNA

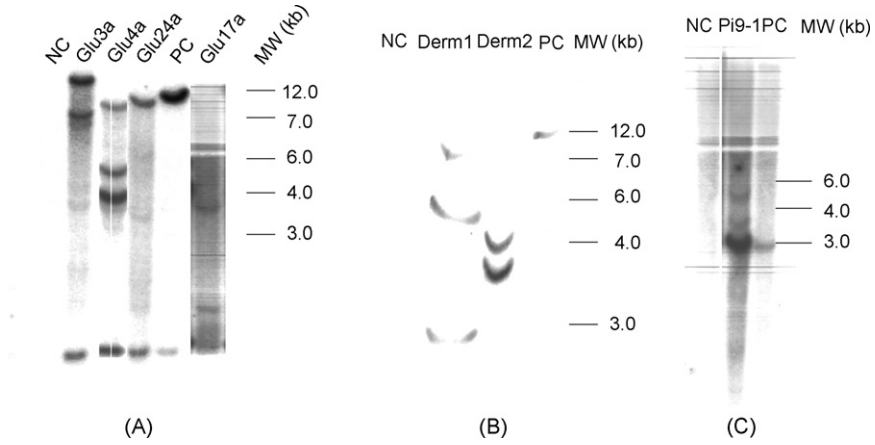

Fig. 2. Southern analysis of the transgenic plants. NC: negative control, DNA from a non-transformed tall fescue plant; PC: positive control and reconstruction, respective plasmid DNA equivalent to one copy of the transgene in a 2C tall fescue genome, mixed with 25 μg non-transgenic plant DNA; MW: DNA molecular weight marker. (A) Blot of Glu transgenic plants using *AGLU1* coding sequence as the probe. (B) Blot of Derm transgenic plants containing the dermaseptin SI fusion gene, using dermaseptin SI coding sequence and the *nos* terminator sequence as the probe. (C) Blot of the Pi9-1 transgenic plant.

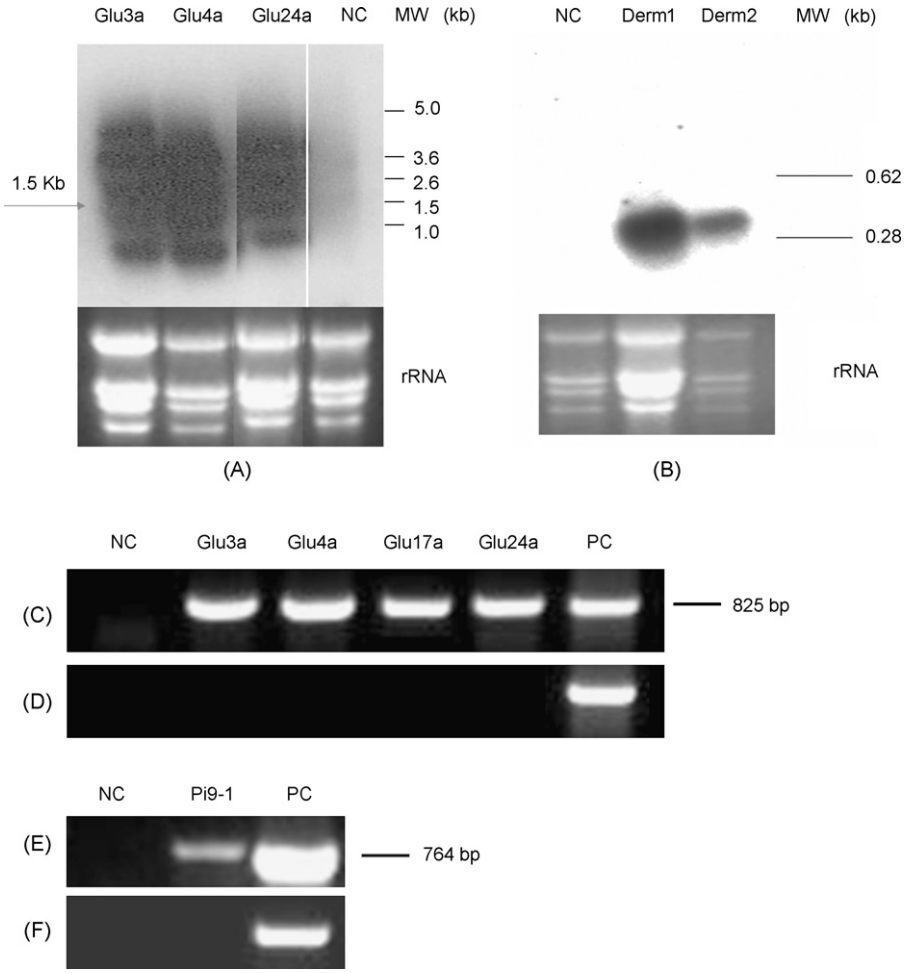

Fig. 3. Northern and RT-PCR analyses of the transgenic plants. NC: negative control, RNA from a non-transformed tall fescue plant. MW: RNA molecular weight marker. Photos of rRNAs from ethidium bromide (EtBr) stained gels are shown as loading controls. (A) Northern analysis of Glu transgenic plants using *AGLU1* coding sequence as a probe. The expected 1.5 kb *AGLU1* mRNA is indicated. (B) Northern analysis of the Derm transgenic plants expressing the dermaseptin SI fusion gene, using the dermaseptin SI coding and *nos* terminator sequences as probes. (C) RT-PCR analysis of four Glu plants. An 825 bp *AGLU1* PCR fragment was expected. PC, positive control, PCR using genomic DNA from transgenic plant Glu3a. (D) Standard PCR analysis using RNA samples in C to show no genomic DNA contamination in these RNA samples. (E) RT-PCR analysis of the Pi9 plant. The expected 764 bp Pi9 amplified fragment was indicated. PC, positive control, PCR using genomic DNA from transgenic plant Pi9-1. (F) Standard PCR analysis using RNA samples in E to demonstrate no genomic DNA contamination in these RNA samples.

fragments with expected size (825 or 764 bp) were obtained after PCR using reverse-transcribed cDNAs as templates in all the four Glu plants or in the Pi9 plant, respectively, indicating transgene expression in these plants (Fig. 3C and E). No amplification was observed when PCR reaction was performed on the same set of RNA samples without reverse transcription (Fig. 3D and F), suggesting no genomic DNA contamination in the RNA preparations.

3.3. Inoculation test for gray leaf spot disease resistance

Twelve Glu transgenic plants, 2 Derm transgenic plants and 1 Pi9 transgenic plant were challenged with a mixture of two *M. grisea* isolates obtained from tall fescue in a replicated experiment under controlled environment. Three Glu plants (Glu3a, 4a, and 17a), 2 Derm plants (Derm1 and Derm2), and the Pi9 plant (Pi9-1) exhibited high resistance with the lesion length reduced by 75–90%, and disease incidence decreased by 55–85% when compared to the non-transformed control plants (Fig. 4A–C). Statistical analysis indicated that the lesion length reduction in all the six transgenic plants was highly significant ($P < 0.01$, Fig. 5A and C), suggesting that the invasion of tall fescue plants by *M. grisea* was substantially suppressed in these plants. The six transgenic plants also exhibited lower disease incidence ($P < 0.01$), indicating that the infection process was also inhibited (Fig. 5B and D).

3.4. Inoculation test for brown patch disease resistance

To test the effects of *AGLU1*, or dermaseptin SI, transgene on brown patch resistance for tall fescue, all the Glu plants and the Derm1 plant (Derm2 plant was too small to be tested at the time) were inoculated with the fungal pathogen *R. solani*, subgroup AG1-1A, which was isolated from infected tall fescue plants. Measurement of lesion length two weeks after inoculation indicated that lesion development was suppressed by about 70% in two Glu transgenic plants and in Derm1 transgenic plant when compared to the non-transformed control

Fig. 4. Representative inoculation test results of the transgenic plants in comparison to non-transgenic control plants (CK). (A–C) Leaves of the transgenic plants Glu17a containing the *AGLU1* gene, Derm1 containing the dermaseptin SI fusion gene, and Pi9-1 containing the rice *pi9* gene, inoculated with *M. grisea*. Photos were taken 10–14 days after inoculation. (D and E) Leaves of transgenic plants Glu24a and Derm1 inoculated with *R. solani*. Photos were taken 14 days after inoculation.

plants (Fig. 4D and E). Based on lesion development, resistance was highly significant for transgenic plant Derm1 ($P = 0.0098$, Fig. 6C), and significant for transgenic plant Glu24a ($P = 0.0177$, Fig. 6B). A slight improvement in the resistance in transgenic plant Glu3a was also observed ($P = 0.0555$, Fig. 6A). Among the four brown patch resistant plants, two also showed resistance to gray leaf spot, indicating that these two plants (Glu3a and Derm1) were resistant to both the diseases. *Pi9* gene is an R gene specifically against *M. grisea* [18], and thus the Pi9 plant was not tested for resistance to *R. solani*.

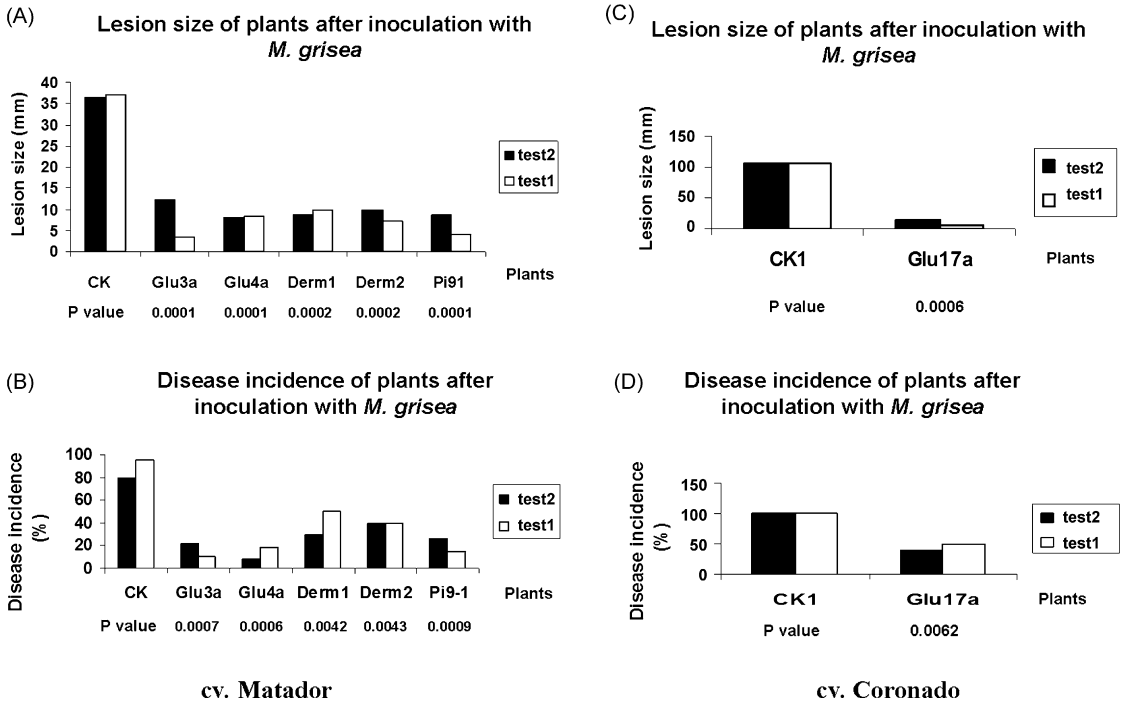

Fig. 5. Analysis of *M. grisea* inoculation test on transgenic plants with various cultivar background. (A and C) Lesion size of the transgenic plants resistant to *M. grisea* in comparison with non-transformed control plants. (B and D) Disease incidence analysis of the above plants. *P* value of each resistant transgenic plant is shown.

4. Discussion

We have successfully generated transgenic tall fescue plants with enhanced resistance to *M. grisea* and *R. solani*, the two most dreaded fungal pathogens of turfgrasses.

β-1,3-Glucan is a major component of fungal cell walls. Plants have evolved to have β-1,3-glucanases as one of the major PR (pathogenesis related) proteins in their defense systems [40]. It is believed that β-1,3-glucanases directly digest the β-1,3-glucans of fungal cell walls, resulting in cell lysis, and/or release of bioactive cell wall fragments as elicitors for inducing plant defense reactions [41]. It was reported that expression of the alfalfa *AGLU1* β-1,3-glucanase gene in transgenic tobacco conferred resistance to a fungal pathogen *C. nicotianae* [10]. Wang et al. [42] attempted to introduce the alfalfa *AGLU1* gene into creeping bentgrass but could not observe its expression, and concluded that the dicot gene may not be expressed in monocot plants. In our study, among 12 tall fescue plants containing the *AGLU1* gene, three were highly resistant to *M. grisea* and two were resistant to *R. solani*. Northern and RT-PCR analyses clearly detected *AGLU1* expression in these plants. Opposite to the previous report, our results revealed the *AGLU1* gene can be expressed in tall fescue, and could be successfully used for fungal disease resistance strategies in monocot plants. We observed irregular *AGLU1* expression pattern in Northern analysis. Similar results were observed with T4 lysozyme gene construct [28]. Although the reason for the unusual expression pattern was not clear, it can be traced back to an intermediate plasmid used for both gene constructs. The CAMBIA1300-based vector has a maize *Ubi1* promoter, a *nos* terminator, and a polylinker in between. Restriction digestion results matched the expected map of the plasmid. Although we are not sure what exactly happened for the transgene expression from this construct, possible reasons for the multiple mRNA species of the transgene include transcription readthrough, RNA processing events, or alternative transcription start sites. The intermediate plasmid was not used in construction of the dermaseptin SI transgene, and this transgene expression was normal and only the expected mRNA was detected.

The original dermaseptin SI gene encodes a 34-AA peptide. The peptide is cytolytic to bacteria, yeast, filamentous fungi, and protozoa, but has not shown cytolytic activity to mammalian cells [30]. Immunofluorescent, electron microscopic, and electrophysiological studies indicated that the interactions between the peptide and the lipid bilayer of cells caused changes in membrane functions, and resulted in the imbalance of the osmotic pressure and cell death [43]. Research also demonstrated that the 18-AA peptide from the N-terminus of the dermaseptin SI is sufficient for, and actually has improved, antimicrobial activity [30]. In an attempt to increase the expression of such a small peptide, plant preferred codons [39] were used when designing the synthetic gene (Table 1), and the rice *rubi3* promoter and an in-frame fusion with the *rubi3* ubiquitin monomer coding sequence were used for the construct. It was demonstrated that the N-terminal fusion of the rice *rubi3* ubiquitin monomer coding sequence in frame with a transgene coding sequence enhances transgene expression by about four fold and the original protein encoded by the transgene is released in such a construct after excision of the ubiquitin monomer by an ubiquitin cleavage mechanism inside the cells [33,44]. In our experiment, we obtained two

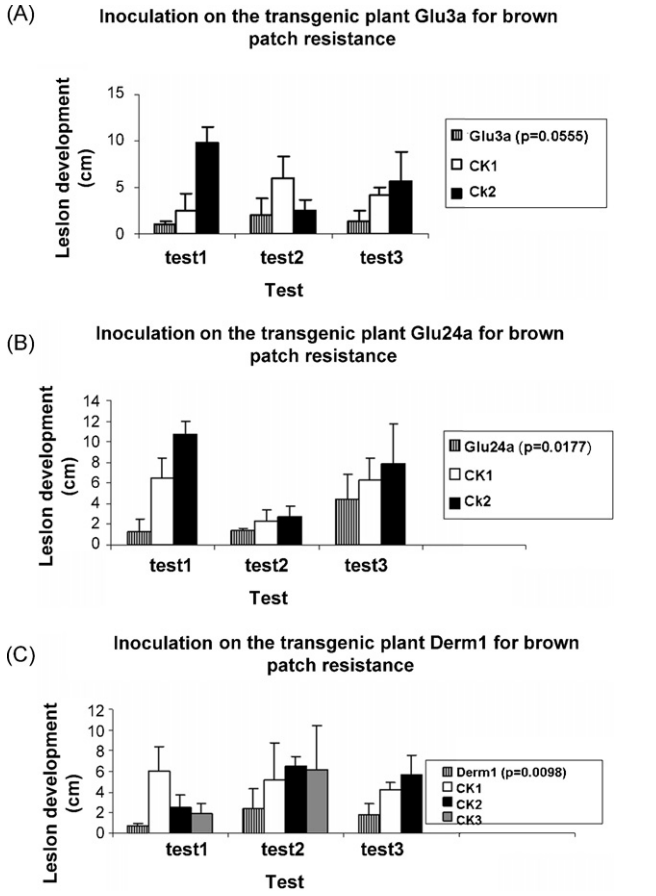

Fig. 6. Lesion development of three resistant transgenic plants (Glu3a and Derm1, cv. Matador; Glu24a, cv. Coronado) after inoculation with *R. solani* in comparison with non-transgenic control plants of corresponding cultivars (CK). *P* value of each transgenic plant is shown.

independent transgenic tall fescue plants from this gene construct. Both expressed the fusion gene well (Fig. 3B), and had high resistance to *M. grisea*. Derm1 was also challenged and exhibited good resistance to *R. solani*. This suggests a high potency of the transgene construct against both fungal pathogens. Moreover, the transgenic plants looked normal, indicating that the peptide apparently was not harmful to plant cells. Recently, resistance using a derivative of a dermaseptin B gene to fungal pathogens from seven genera in transgenic potato was reported [17]. Dermaseptin B is a 31-AA peptide and shares substantial homology with the truncated dermaseptin SI (12 identical AA out of 18, data not shown). To our knowledge, this is the first time dermaseptin SI is used to engineer fungal resistance in plants. The dermaseptins may have good potential for conferring resistance to many fungal pathogens, and wide applications in engineering fungal resistance in plants.

The isolated rice blast R gene, *Pi9*, belongs to a NBS-LRR R gene class. In general, an R gene has strong resistance to only one or a few closely related races of a pathogen species. The *Pi9* gene is unusual in that it exhibits high resistance to all the rice blast-causing races of *M. grisea* tested so far [18]. We demonstrated that it also confers resistance to two turfgrass isolates of this pathogen. Although we only obtained one transgenic tall fescue plant with the *Pi9* construct, it did have a high level of resistance to the turfgrass isolates of *M. grisea*, and may prove useful for resistance to other turfgrass isolates of the pathogen.

Brown patch and gray leaf spot are two most severe diseases of tall fescue and many other turfgrasses. The observed inverse relationship in resistance to the two fungal pathogens may obstruct conventional breeding efforts to obtain resistance to both pathogens. We have demonstrated that it can be achieved by using transgenic approach with transgenes from various sources ([28], this report). Field tests and inoculation with other fungal pathogens will be needed to further test the ability of these genes in conferring fungal resistance in natural environment. Our observation indicates that transgenic approach may be an efficient way to address the fungal disease problems of turfgrasses, and to reduce fungicide uses on turf lawns and golf courses so to improve our living environment.

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