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Molecules and Cells

Expression of BrD1, a Plant Defensin from *Brassica rapa*, Confers Resistance against Brown Planthopper (*Nilaparvata lugens*) in Transgenic Rices

Man-Soo Choi, Yul-Ho Kim*, Hyang-Mi Park, Bo-Yoon Seo, Jin-Kyo Jung, Sun-Tae Kim[1], Min-Chul Kim[2], Dong-Bum Shin, Hong-Tai Yun, Im-Soo Choi, Chung-Kon Kim, and Jang-Yong Lee[3]

Plant defensins are small (5-10 kDa) basic peptides thought to be an important component of the defense pathway against fungal and/or bacterial pathogens. To understand the role of plant defensins in protecting plants against the brown planthopper, a type of insect herbivore, we isolated the *Brassica rapa Defensin 1* (*BrD1*) gene and introduced it into rice (*Oryza sativa* L.) to produce stable transgenic plants. The BrD1 protein is homologous to other plant defensins and contains both an N-terminal endoplasmic reticulum signal sequence and a defensin domain, which are highly conserved in all plant defensins. Based on a phylogenetic analysis of the defensin domain of various plant defensins, we established that BrD1 belongs to a distinct subgroup of plant defensins. Relative to the wild type, transgenic rices expressing *BrD1* exhibit strong resistance to brown planthopper nymphs and female adults. These results suggest that BrD1 exhibits insecticidal activity, and might be useful for developing cereal crop plants resistant to sap-sucking insects, such as the brown planthopper.

INTRODUCTION

Rice is an important cereal and a source of calories for more than one third of world population. Rice productivity is affected by several biotic and abiotic stresses. The major pests affecting rice production include bacterial blight, blast, and brown planthopper. The brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the major insect pests of cultivated rice in Asia. BPHs exerts its damaging effects by directly sucking phloem sap from rices (Cha et al., 2008) and transmitting viral diseases such as grassy stunt virus and ragged stunt virus (Falk et al., 1998). Furthermore, several genes conferring resistance to BPH have been identified in different rice cultivars and introduced into other elite rice varieties using conventional breeding techniques to develop BPH-resistant lines (Kim and Shon, 2005). However, with the emergence of new BPH biotypes (biotypes 2 and 3), the major genes conferring resistance to BPH lost their protective function (Gallagher et al., 1994). Therefore, there is a need to identify a new gene that confers resistance to BPH.

All living organisms, ranging from microorganisms to plants and mammals, share some common elements in their mechanism of defense against pathogen attacks. In the innate immune response, defensins are conserved between insects, mammals, and plants. In the beginning of the 1990s, the first plant defensins were isolated from wheat and barley grains (Mendez et al., 1990). These proteins are encoded by small multi-gene families and are expressed in various plant tissues (Epple et al., 1997; Terras et al., 1995; Thomma et al., 2002). Thirteen genes in the *A. thaliana* genome were found to encode putative plant defensins (Thomma et al., 2002). Furthermore, two putative proteins containing plant defensin domains were identified that contain the conserved cysteine residues, but do not share significant homology with other defensins (Thomma et al., 2002).

Although plant defensins all have similar three-dimensional structures, the plant defensin family is quite diverse in amino acid composition and biological activity (Lay and Anderson, 2005; Thomma et al., 2002). Some defensins fail to show any antifungal and, occasionally, antibacterial activity *in vitro* (Roy-Barman et al., 2006). In addition, plant defensins were shown to interact directly with plasma membrane components, such as sphingolipids from mutants of *Saccharomyces cerevisiae* (Thevissen et al., 2003), and glucosylceramides from yeast species *Candida albicans* and *Pichia pastoris* (Thevissen et al., 2004). The antifungal activity of these plant defensins is directly associated with the deleterious alteration of the pathogen membrane system. However, little is known about the biological role of plant defensins in insect resistance.

To expand our knowledge of the biological function of plant

National Institute of Crop Science, Rural Development Administration, Suwon 441-857, Korea, [1]Department of Plant Bioscience, Pusan National University, Miryang, Korea, [2]Division of Applied Life Science (Brain Korea 21 Program), Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Korea, [3]National Academy of Agricultural Science, Rural Development Administration, Suwon 441-857, Korea
*Correspondence: kimyuh77@korea.kr

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defensins in the defense mechanism against phloem-feeding insects, we isolated a cDNA encoding a novel plant defensin protein, *Brassica rapa* Defensin1 (designated BrD1), from Chinese cabbage, and generated transgenic lines of rice (*Oryza sativa* L.) stably expressing this gene. Compared to wild-type plants, transgenic rices over-expressing *BrD1* resisted BPH feeding and reduced the survival rate of the insect. This is the first report of a plant defensin exhibiting insecticidal activity against a sap-sucking insect, and provides a tool that may be useful in the development of BPH-resistant rices.

MATERIALS AND METHODS

Isolation of *BrD1* cDNA

For full-length cDNA cloning, *BrD1*-specific primers were designed based on the *CPL29* sequence (GenBank Accession Number: AF528180), which encodes the *Brassica rapa* defensin gene (Park et al., 2005). The forward primer was 5′-CACCAACAATGGTGAAAGCGCAGAAG-3′, and the reverse primer was 5′-GCACAACTTCTGCGCTTTCACCATTG-3′. To isolate full-length *BrD1* cDNA, total RNA was extracted from the leaves of two-week-old *Brassica rapa* (Korea variety, Jangmi) seedlings, used for cDNA synthesis, and then PCR amplified. The PCR reaction mix consisted of 1 μl cDNA template, 2 μl 10 × PCR buffer, 1 μl of the gene-specific primer (10 μM), 2 μl dNTPs (2.5 mM), 0.25 μl Pfu Taq-Pol (5 U/μl) (BIOTOOLS, Spain), and 15.75 μl ddH_2O. Thirty cycles were performed of pre-denaturation for 3 min at 94°C, denaturation for 30 s at 94°C, annealing for 30 s at 58°C, and extension for 30 s at 72°C. After the last cycle, the amplification was extended for 7 min at 72°C. The PCR products were purified using a DNA Gel Extraction Kit (GeneAll, Korea), and cloned into the pGEM-T Easy Vector (Promega, USA).

Genomic DNA gel-blot analysis

The extraction of genomic DNA was carried out according to the cetyltrimethylammonium bromide (CTAB) method (Clarke, 2009), and remaining RNAs were removed by adding 10 μg RNase A (Sigma-Aldrich Chemie GmbH). Approximately 20 μg of genomic DNA extracted from *Brassica rapa* or rice (cv. Ilmibyeo) were digested with various restriction enzymes, including *Bam*HI, *Eco*RI, *Pst*I, and *Xba*I. The digested DNA fragments were fractionated on a 0.8% agarose gel in 0.5× TBE buffer. The supercoiled DNA ladder (Promega, USA) was used as a size marker. After electrophoresis, Southern blot analysis was conducted with a full-length *BrD1* cDNA probe labeled with DIG-High Prime DNA Labeling and signals were detected using the Detection Starter Kit II (Roche Diagnostics, Switzerland).

Quantitative real-time PCR analysis

The expression of *BrD1* gene in transgenic plants was analyzed by real-time quantitative reverse transcriptase PCR using the fluorescent intercalating dye SYBR Green in a detection system. A rice 18S ribosomal RNA was used as a standard control in the RT-PCR reactions. For real-time quantitative RT-PCR, total RNAs were isolated using the RNeasy Plant Mini Kit, according to the manufacturer's instructions (QIAGEN, Germany). The first strands of cDNA were synthesized using the AffinityScript™ Multiple Temperature Reverse Transcriptase Kit (Stratagene, USA). Quantitative RT-PCR analysis was performed with a pair of gene-specific primers. The forward primer was 5′-TTCGCTGCTCTCGTTCTCTTTGCTGC-3′, and the reverse primer was 5′-TCCCTCAAGGTTGATGCACTGGTTCT-3′. Amplification conditions were 25 cycles of 94°C for 3 min, 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by one cycle of 72°C for 7 min. The data of the real-time RT-PCR are mean values and standard error (bar) of three independent experiments.

Sequence alignment and phylogenetic analysis

Multiple amino acid sequence alignment was performed using the program ClustalW with BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html). A phylogenetic tree was constructed using the neighbor-joining method, and support values for nodes on the tree were estimated with 1000 bootstrap replicates. Programs used here were provided by the DDBJ (http://www.ddbj.nig.ac.jp/search/clustalw-e.html).

Vector construction and plant transformation

The vector used for rice transformation was constructed by inserting the *BrD1* gene between the rice cytochrom C promoter and the *PinII* terminator in the modified binary vector (Jang et al., 2002) (Fig. 3A). *A. tumefaciens* strain LBA4404 carrying pMJ101/*BrD1* was used for transformation of the rice variety Ilmibyeo, as described by Sohn et al. (2006). Eleven independent transgenic lines were primarily selected using 6 mg/L phosphinothricin (ppt), and then further selected with a 0.3% basta herbicide spray.

Insect bioassays with transgenic plants

A two-week-old seedling of each rice variety was carefully uprooted from the soil and washed with tap water to remove the remaining soil from the roots. The root of each rice seedling was curled around cotton and put into a glass tube. Five fifth-instar nymphs or female adults collected from a cultured BPH population were inserted into each of the glass tubes, and were observed over a period of 10 days during the feeding experiments. All experiments were replicated five to six times, with similar results. BPH survival and plant damage were monitored every day until the susceptible plant withered. The survival rate was calculated on the second day to avoid counting artificially damaged insects. A survivorship test was conducted in environmental conditions under 25 ± 2°C, 60 ± 5% Relative Humidity, and a 15 h light/9 h dark photoperiod.

RESULTS AND DISCUSSION

Isolation of a cDNA clone encoding the *Brassica rapa* defensin, BrD1

To isolate a cDNA clone corresponding to the *Brassica rapa* defensin gene, we conducted RT-PCR with primers designed using the sequence of the previously reported defensin gene, *CPL29* (Genbank Accession Number: AF528180; Park et al., 2005), whose expression was induced by ethylene and wounding, but not by salicylic acid or methyl jasmonate, in Chinese cabbage. Sequence analysis of the PCR product indicated that the cloned cDNA fragment contains an entire open reading frame (ORF) of 243 bp in length, and 21 bp and 99 bp of the 5′- and 3′-untranslated regions, respectively (Fig. 1A). Analysis of the deduced amino acid sequences indicated that this cDNA encodes a protein composed of 80 amino acids, with an estimated molecular mass of 8.8 kDa. A BLASTP search in the Swiss-Prot database using the conceptual translation product of this cDNA clone suggested that it belongs to a member of the plant defensin family, as it possesses a putative endoplasmic reticulum signal peptide at its N-terminus and a defensin domain with amino acids that are highly conserved in the defensin domains of plant defensin family members (Fig. 1A). Thus, we named this cDNA clone *Brassica rapa Defensin1, BrD1*.

We compared the deduced amino acid sequence of *BrD1*

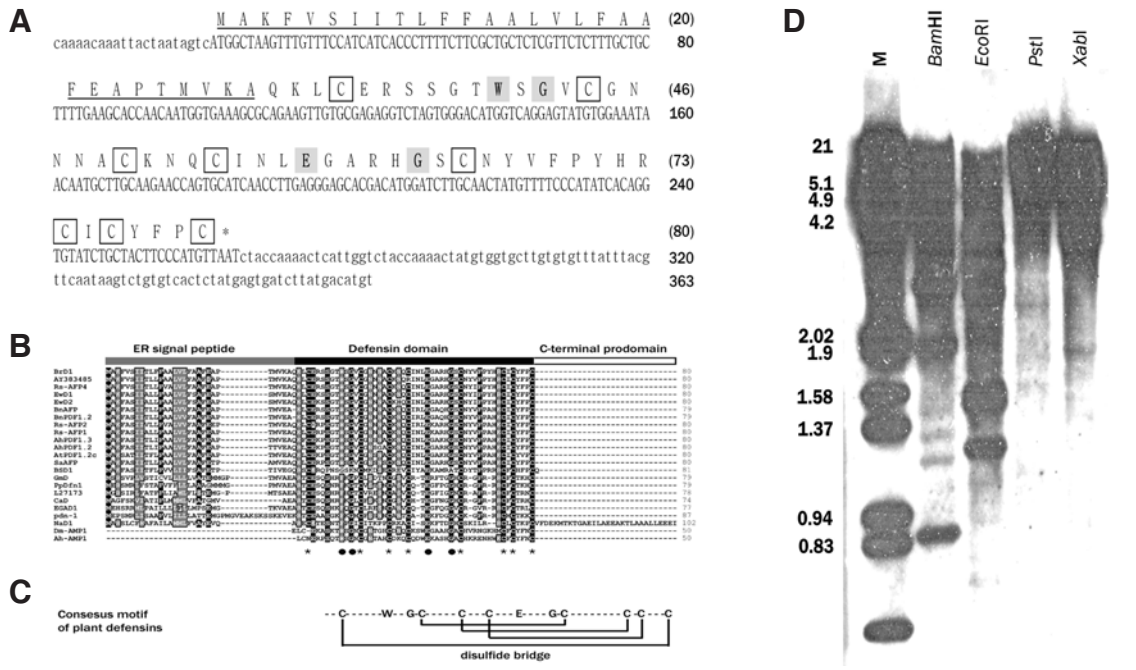

Fig. 1. Characterization of *BrD1*. (A) Nucleotide and deduced amino acid sequence of the *BrD1* cDNA clone. The putative signal peptide is underlined. Square boxes indicate the conserved cysteine residues, and other conserved residues are shaded. (B) Comparison of the deduced amino acid sequence of BrD1 with those of 22 other plant defensins. Spaces have been introduced to maximize the alignment. Residues conserved in some or most of the sequences are shaded in grey and black, respectively. The alignment was generated using ClustalW (http://www.ddbj.nig.ac.jp). (C) The consensus motif of the above proteins and the positions of putative disulfide bridges. (D) Southern blot analysis of the *BrD1* gene in the *Brassica rapa* genome. Twenty micrograms of genomic DNA was isolated from leaves of *Brassica rapa*, digested with the indicated restriction endonucleases (*Bam*HI, *Eco*RI, *Pst*I, or *Xba*I), blotted onto a nylon membrane, probed, and hybridized with a DIG-labeled *BrD1* probe. M, DNA size marker. Numbers on the left, size (kp) of DNA fragments in marker.

with those of other plant defensins, including AY383485, Rs-AFP, Rs-AFP1, Rs-AFP2, EwD1, EwD2, SaAFP, AhPDF1.2, AhPDF1.3, and AtPDF1.2c, and established that similarity in overall amino acid sequence ranged from 85% to 98% (Fig. 1B). However, the amino acid sequence of BrD1 shared relatively low identity with those of Dm-AMP1 (54%), Ah-AMP1 (50%), NaD1 (33%), and pdn-1 (33%). The amino acid sequence of the endoplasmic reticulum signal peptide of BrD1 also differed from those of other plant defensins (Fig. 1B). Particularly, the amino acid sequence of BrD1 differed from that of CPL29 by a single amino acid; the Phe^{11} residue of CPL29 was substituted with a Leu^{11} in BrD1 (Fig. 1B). Amino acid variation may just be the reason for a different variety of *Brassica rapa*.

The presence and positions of the eight cysteine residues that are known to be conserved in all plant defensins are also highly conserved in BrD1 (Fig. 1C). Furthermore, other well-conserved amino acids in all plant defensins, including two glycine residues at amino acid positions 13 and 34 a tryptophan residue at position 11, and a glutamate residue at position 29, are also found in BrD1 (Fig. 1C). The structural features of the BrD1 protein suggest that BrD1 may function as a plant defensin.

To determine the copy number of the *BrD1* gene in the genome of *Brassica rapa*, genomic DNA gel blot analysis was performed (Fig. 1D). Genomic DNA was digested with various restriction enzymes, such as *Bam*HI, *Eco*RI, *Pst*I, and *Xba*I, which do not exist within the *BrD1* gene. The blot was hybridized using DIG-labeled *BrD1* full-length cDNA as the probe. As shown in Fig. 1D, multiple fragments of digested *Brassica rapa* genomic DNA hybridized to the probe, indicating that *BrD1* is a member of a multigene family. Our result is consistent with previous reports that show that plant defensins are grouped into small multigene families in other plants (Thomma et al., 2002). The *Arabidopsis* genome contains 13 defensin genes that encode putative plant defensins, and an additional two genes that encode putative proteins containing plant defensin-like domains (Thomma et al., 2002). Southern blot analysis revealed that *Brassica rapa* contains a number of defensin genes with a high level of sequence similarity to each other.

Phylogenetic analysis of plant defensin family members

To establish the evolutionary history of plant defensin genes, an unrooted phylogenetic tree was generated using CLASAL W (Fig. 2). Phylogenetic analysis was carried out with 80 amino acid sequences of premature plant defensins. BrD1 was included in the same subgroup as CPL29 and AY383285, defensin proteins from Chinese cabbage and potato, respectively, but isolated from another subgroup that included Rs-AFP4 (Accession number: O24331), Dm-AMP1 (Accession number: AAB34972), Ah-AMP1 (Accession number: AAB34970), and NaD1 (Accession number: AAN70999), which have antimicrobial and antifungal activities. These results suggest that BrD1 has similar functions to CPL29 and AY383285, but different functions from other plant defensins.

Generation of a transgenic rice plant expressing *BrD1*

To characterize the biological function of plant defensin, we attempted to express the recombinant protein in bacteria and yeast. There are plant defensins that are successfully expressed in *S. cerevisiae* and in *Pichia pastoris* (Wisniewski et

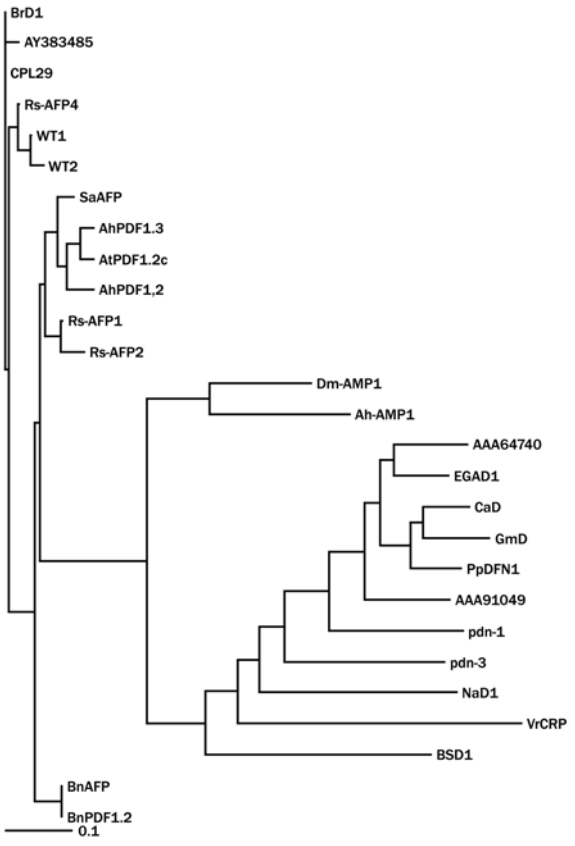

Fig. 2. Phylogenetic relationship between BrD1 and 22 plant defensins from other plants. The rooted tree was constructed using the TreeView X software after a multiple sequence comparison using the Clustal method of the Clustal W program.

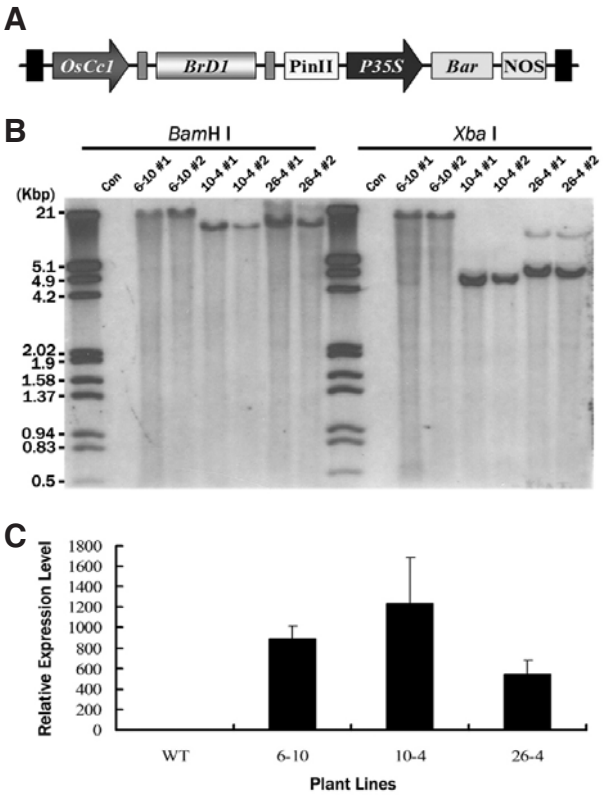

Fig. 3. Characterization of transgenic plants expressing *BrD1*. (A) Schematic diagram of the *BrD1* construct used for transformation of rice. OsCc1, rice cytochrome c promoter. (B) Southern blot analysis of wild-type and transgenic rices (6-10, 10-4, and 26-4) expressing *BrD1*. Twenty micrograms of genomic DNA isolated from wild-type and transgenic rices were digested with *Bam*HI or *Xba*I, and hybridized with a DIG-labeled *BrD1* probe. M, DNA size marker. Numbers on the left, size (kb) of DNA fragments in marker. (C) Expression level of *BrD1* in transgenic plants was quantified by real-time RT-PCR. Relative values of *BrD1* gene expressions in transgenic plant are shown as percentage of *BrD1* expression activity in wild-type plant. Error bars represent standard deviation.

al., 2003). BrD1 is not expressed in bacteria or yeast such as most of plant defensin (data not shown). Antimicrobial proteins are frequently recalcitrant during expression in bacterial and yeast cells as a result of their toxicity to the host (Saitoh et al., 2001). Recently, a wasabi defensin (WT1) was produced in the leaves of *Nicitiana benthaminana* by infecting the plants with a potato virus X vector carrying the *WT1*. Therefore, plant transformation is efficiently a tool for expression of BrD1 protein.

To investigate whether *BrD1* confers resistance to sap-sucking insect pests, transgenic rices expressing *BrD1* under the control of the rice cytochrome C (*OsCc1*) promoter (Jang et al., 2002) were generated (Fig. 3A). Eleven independent transgenic lines were primarily selected using 6 mg/L phosphinothricin and then further selected with 0.3% basta herbicide spray. Among them, transgenic lines 6-10, 10-4, and 26-4, which had phenotypes that were indistinguishable from those of wild-type plants, were used for further experiments. To determine transgene integration and copy number of the integrated T-DNA in transgenic lines harboring *BrD1*, Southern blot analysis was initially carried out with genomic DNA isolated from leaves of both a wild-type plant and the three selected transgenic lines (6-10, 10-4, and 26-4 line). Genomic DNA was digested with *Bam*HI or *Xba*I and probed either with the *BrD1* coding sequence or the *bar* gene. Southern blot analysis revealed the presence of a single band in all transgenic plants, but no hybridized band was detected in the wild type. This result implies that T-DNA is successfully integrated as a single copy into the genome of transgenic rices (Fig. 3B). To investigate whether *BrD1* was successfully expressed in transgenic lines, real-time RT-PCR (Fig. 3C) was performed in a wild-type plant and three independent T3 transgenic rices (lines 6-10, 10-4, and 26-4 lines, respectively) using gene-specific primers. *BrD1* was highly expressed in all transgenic plants, but not in the wild-type plant. Two transgenic lines (6-10 and 10-4) showed particularly high levels of *BrD1* expression, and line 26-4 had a relatively low level of expression.

Insect bioassay of T2 transgenic plants expressing *BrD1* using BPH nymphs

Plant defensins have been extensively reported for the biological property to inhibit bacteria and fungi (Terras, 1995). It should be mentioned here if BrD1 have or not an antimicrobial activity as other plant defensins. However, transgenic plants expressing BrD1 are not appeared antifungal activity against rice blast (data not shown). These results suggest that BrD1 have a different function with other plant defensin as phylogenetic analysis.

Plant-derived genes conferring resistance to hemipteran

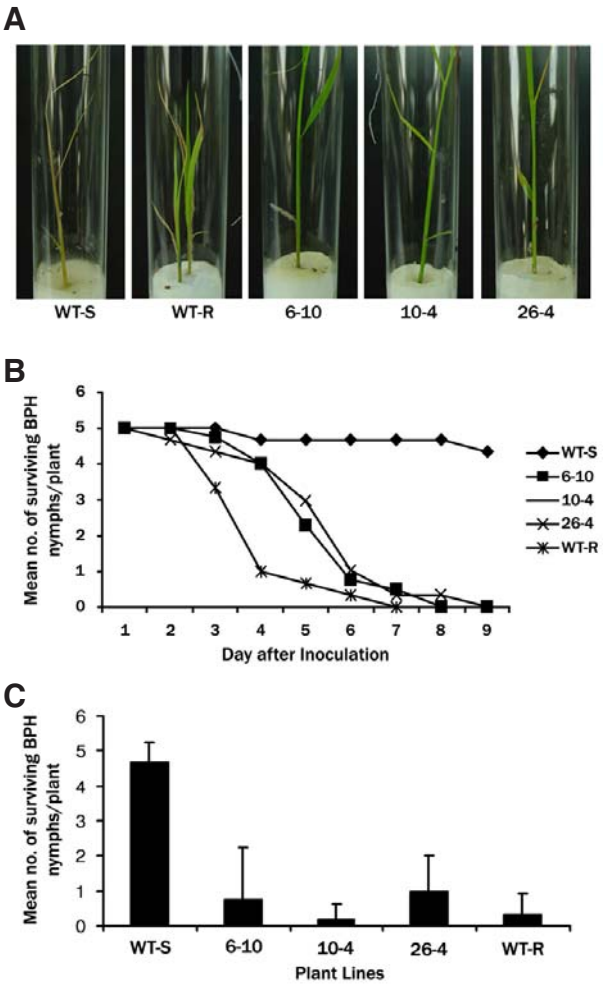

Fig. 4. Resistance of T2 transgenic rices expressing *BrD1* to BPH nymphs. (A) Disease symptoms of rice seedlings 10 days after inoculation with BPH nymphs. (B) Survival of BPH nymphs on transgenic rice lines expressing *BrD1*. Five fifith-instar nymphs were inoculated in each tube at 0 day. Untransformed control plants and transgenic plants were used for bioassay experiments. WT-S is the negative control that is susceptible to BPH (Ilmibyeo), and WT-R is the positive control that is resistant to BPH (Cheongcheongbyeo). (C) The number of survival in WT-R, WT-S, and transgenic plant at 5th days after inoculation with BPH nymphs.

groups of insect pests have been introduced into plants. Among genes derived from plants, inhibitors of digestive enzymes (proteases and amylase) and lectins were investigated for insecticidal activity (Schuler et al., 1999). Galanthus nivalis agglutinin (GNA) showed highly antinutritional and/or toxic effects against various phloem-feeding insect pests, based on the results of an artificial diet feeding system (Powell et al, 2001) and transgenic plants (Foissac et al., 2000; Loc et al., 2002; Ramesh et al., 2004). *Allium sativum* leaf agglutinin (ASAL), which has a high degree of sequence similarity with GNA (Bandyopadhyay et al., 2001; Dutta et al., 2005a; 2005b; Majumder et al., 2004), was isolated from garlic leaves. Subsequently, ASAL was found to be a potent agent for controlling sucking pests (Bandyopadhyay et al., 2001; Dutta et al., 2005a; 2005b), and it reduced the survival of the important hemipteran rice pest, green leafhopper (GLH). Recently, transgenic plants expressing protease/amylase inhibitor were found to be somewhat resistant against various plant-eating insects (Alfonso-Rubí et al., 2003; Lee et al., 1999).

To test the insecticidal activity of BrD1 against the BPH sap-sucking insect pest, we examined the survival rates of BPH nymphs on T2 transgenic rices (lines 6-10, 10-4, and 26-4) expressing *BrD1*. Resistant (WT-R; cv. Cheongcheongbyeo) and susceptible (WT-S; cv. Ilmibyeo) rice cultivars were used as positive and negative controls, respectively. We investigated the survival of BPH at one-day intervals for up to 10 days after inoculation. After being infested with fifth-instar nymphs during the bioassay, the transgenic plants and the WT-R plants were significantly more resistant to BPHs than were the WT-S plants (Fig. 4). WT-S plants exhibited slight wilting within five days of inoculation, and died at seven days post inoculation, but BPH nymphs remained relatively healthy for 10 d after inoculation (Fig. 4A). Some BPHs began to die at the first day after inoculation of the WT-R and transgenic plants, and most BPHs were dead by the sixth day after inoculation, while most BPHs survived until the ninth day after inoculation of the WT-S plants (Fig. 4B). The survival of BPH nymphs in transgenic plants (lines 6-10, 10-4, and 26-4) was reduced by ~83.9%, ~95.7% and ~78.6%, respectively, compared to the survival of nymphs in WT-S plants after the first five days of the assay period. Strikingly, the 10-4 transgenic line exhibited greater resistance to BPH nymphs, and had higher insecticidal activity (~95.7%) against BPH nymphs than the WT-R (~92.9%) of inoculated nymphs died within the assay period (Fig. 4C). The insecticidal activity against BPH nymphs correlated with the expression level of *BrD1* in the transgenic plants (Fig. 3C).

Enhanced tolerance to BPH female adults of T3 transgenic rice expressing *BrD1*

To evaluate the degree of resistance to BPH female adults, a bioassay was carried out using WT-S, WT-R, and two T3 transgenic lines, 10-4 and 26-4, which appeared to have the strongest and weakest resistance for BPH nymphs, respectively. Survival of BPH female adults was investigated in wild-type and transgenic plants that were inoculated with five BPH female adults, and then tracked through five days (Fig. 5). As observed in the bioassay with nymphs, all of the transgenic and WT-R rices appeared to have significant resistance, compared to the WT-S plants, for BPH female adults (Fig. 5A). As shown in Fig. 5B, all of the transgenic rices were more resistant to BPH four days after inoculation than were WT-S plants. The relative survival rate of BPH adults in transgenic plant lines 10-4 and 26-4 was reduced by ~45.2% and ~21.4%, respectively, compared to that in WT-S plants.

Furthermore, we carried out using the modified-seedbox test for resistance to BPH nymphs (Tan et al., 2004). Forty seeds of WT-S, WT-R, and T4 transgenic rice plant were grown in plastic trays (25 cm × 45 cm in length) for 3-week-old and 600 BPH nymphs were maintained in plastic trays at a density of approximately 5 insects per seedling. WT-R and T4 transgenic rices were evaluated to resistance phenotype to BPH when the seedling plants of WT-S were completely killed at 15 day after infestation (data not shown). T4 transgenic rices expressing BrD1 were effectively resistance to BPH in a massive survival test. Those results suggest that the constitutive expression of *BrD1* in transgenic rices confer resistance not only against BPH nymphs, but also against BPH adults.

Even though we could not observe the expression of the BrD1 protein in transgenic rice, our results indicated that the T-DNA was successfully integrated into the rice genome as a single copy (Fig. 3B), and that the introduced *BrD1* gene was

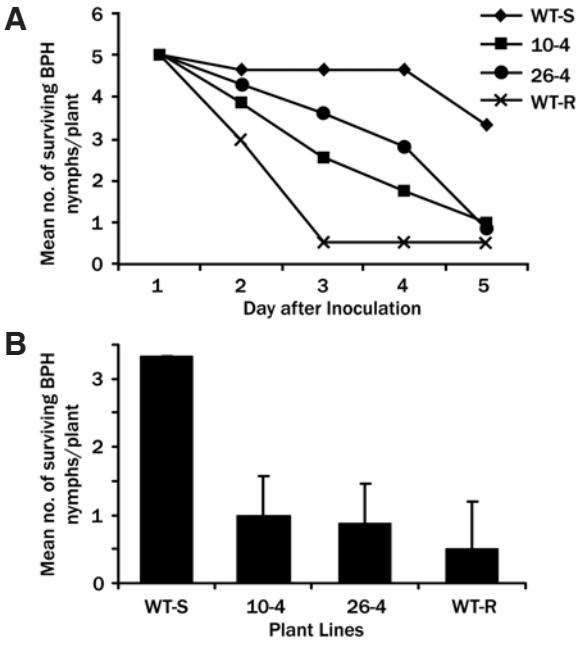

Fig. 5. Effects of T3 transgenic rices expressing *BrD1* on the survival of BPH female adults. (A) Survival of BPHs on transgenic rices. Five BPH adults were inoculated onto each plant at 0 day. A bioassay was performed as described in Fig. 4. (B) The number of survival in WT-R, WT-S, and transgenic plants at 4th days after inoculation with BPH female adults.

stably expressed (Fig. 3C) and inherited in the second and third generations (Figs. 4 and 5). In the insect bioassay, we established that transgenic rices have insecticidal activity that T2 plants were resistant only to the nymphs and T3 only to the female adults (Figs. 4 and 5). These results suggest that the stable expression of *BrD1* in transgenic rices imparts insecticidal activity for BPH nymphs and female adults.

To analyze a possible mechanism of BrD1 in rice transgenic plants, the electrical penetration graph (EPG) method was used in studies of BPH feeding behavior (data not shown). There was no difference between Ilmibyeo (WT-S) and transgenic rices in patterns of EPG. This result suggests that BrD1 could have an insecticidal effect on a digestive apparatus of BPH instead of feeding behavior.

In conclusion, although several defensins isolated from cereal crops appeared to exhibit insecticidal activity *in vitro*, they have not been reported to possess insecticidal activity *in planta*. BrD1, a defensin isolated from *Brassica rapa*, showed significant amino acid similarity with other plant defensins and exhibited insecticidal activity against BPH *in planta.* Also, these results might be useful for developing cereal crop plants resistant to sap-sucking insects, such as the brown planthopper. To obtain a detailed understanding of the insecticidal activity of transgenic rices expressing *BrD1,* it would be worth studying the mode of action of plant defensin BrD1 against sap-sucking insects.

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