African Journal of Biotechnology Vol. 10(72), pp. 16128-16137, 16 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1456
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Maize defensin *ZmDEF1* is involved in plant response to fungal phytopathogens

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Accepted 21 September, 2011

A seed-specific cDNA *Zea mays* defensin 1 (*ZmDEF1*), which encodes a novel plant defensin, was isolated from maize (*Zea mays* L. cv. NongDa108). ZmDEF1 contains a predicted signal peptide of 31 amino acids at the N-terminus domain and a mature peptide of 49 amino acids with a calculated molecular mass of 5.4 kDa. Expression data demonstrated that this gene is expressed specially in immature and mature seeds. In contrast to defensins from other plant species, the expression of *ZmDEF1* cannot be detected in seedlings, even under induction of methyl jasmonate (MeJA) and abscisic acid (ABA). The recombinant ZmDEF1 displays an inhibitive activity against the fungal pathogen, *Phytophthora parasitica* var. nicotianae. Ectopic expression of the *ZmDEF1* gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter conferred enhanced tolerance against *P. parasitica* in transgenic tobacco plants.

Key words: Antifungal peptides, *Phytophthora parasitica*, plant defensin, seed specific, transgenic tobacco.

INTRODUCTION

Plant defensins are a family of cysteine-rich peptides with a specific three-dimensional structure stabilized by four intramolecular disulfide bonds (García-Olmedo et al*.,* 1998; Thomma et al*.,* 2002). These small proteins are comprised 45 to 54 amino acids and found in many monocotyledonous and dicotyledonous plants (Meyer et al*.,* 1996), including wheat (Koike et al*.,* 2002), barley (Mendez et al*.,* 1990), spinach (Segura et al*.,* 1998), pea (Almeida et al*.,* 2000), radish (Terras et al*.,* 1992) and sunflower (Urdangarin et al*.,* 2000). Plant defensins were isolated first from seeds (Thomma et al*.,* 2002), but have also been identified in other tissues including leaves (Kragh et al*.,* 1995), pods (Chiang and Hadwiger, 1991), tubers (Moreno et al*.,* 1994), fruit (Aluru et al*.,* 1999), roots (Sharma and Lönneborg, 1996), bark (Wisniewski et al*.,* 2003) and floral tissues (Gu et al*.,* 1992). Plant defensins can be divided into two major classes according to the structure of the precursor proteins predicted from cDNA clones (Lay et al*.,* 2003). The precursor proteins of Class I are composed of an endoplasmic reticulum (ER) signal sequence and a mature defensin domain. The Class II defensins are having larger precursors with C-terminal prodomains of about 33 amino acids. To date, these Class II defensins have only been found in solanaceous species (Milligan et al*.,* 1995; Brandstadter et al*.,* 1996).

Most plant defensins exhibit antifungal activity against various plant pathogens, especially a broad range of fungi (Broekaert et al*.,* 1997; Osborn et al*.,* 1995). It is generally accepted that plant defensins act at the level of the plasma membrane, similar to many other antimicrobial peptides. Radish seed antimicrobial protein 2 (Rs-AMP2) was shown to bind to the sphingolipid glucosylceramides of fungal membranes, causing inhibition of fungal growth by membrane permeabilization (Thevissen et al*.,* 1996; Mello et al*.,* 2011). However, the precise mechanism of protection for most defensins remains unclear. To date, plant defensins have been transformed into several plants to enhance resistance to agriculture pathogens. Constitutive expression of *Medicago sativa* defensin (alfAFP) in potato clearly enhanced resistance of potato plants to the fungus *Verticillium dahliae* (Gao et al*.,* 2000). Transgenic tobacco constitutively expressing

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Abbreviations: Me**JA**, Methyl jasmonate; **ABA,** abscisic acid.

the radish defensin Rs-AFP2 showed a sevenfold reduction in lesions of the untransformed plants upon infection with the fungal leaf pathogen *Alternaria longipes* (Terras et al., 1995).

We report here on the isolation and characterization of *ZmDEF1*, which encodes a defensin from *Zea mays*. The recombinant ZmDEF1 protein, expressed in the yeast, showed *in vitro* antifungal activity, and ectopic expression of the *ZmDEF1* gene in tobacco conferred enhanced tolerance against *Phytophthora parasitica.* These results indicate that ZmDEF1 is possibly involved in fungi resistance.

MATERIALS AND METHODS

Plant materials and fungal strains

Z. mays L. cv. NongDa108 was provided by Professor Qi-Feng Xu, China Agriculture University. *Nicotiana tabacum* var. Xanthi nc. was used for transformation. Fungal strain *P. parasitica* var. nicotianae was provided by Professor Yi Shi, Tobacco Research Institute of Chinese Academy Agricultural Sciences.

Cloning of the *ZmDEF1* gene

RNA was isolated from *Z. mays* seeds by TRIzol method according the instructions (Invitrogen, USA) and treated with DNase I enzyme (TaKaRa, Japan). A 50 µL reverse transcription reaction was prepared consisting of 2 µg total RNA, 1 × reverse transcription buffer, 1 mM oligo dT_{18} primer, 1 mM dNTP, 2 U RNasin and 2 U M-MLV. The reaction was incubated for 60 min at 42°C prior to PCR reactions. A sense primer, Def1 [5′-GATGGCKCYGTCTCGWCG-3′], and an antisense primer, Def2 [5′-ACTAGCAKAYCTTCTTGCAGA-3′] were designed based on nucleotide sequence of the *Triticum aestivum* defensin (GenBank accession number AB089942). Def1 and Def2 were used in PCR amplification with 1 µL cDNA reaction mixture earlier obtained as template. Amplification conditions were: 95°C pre-denaturation for 5 min, 30 cycles: 95°C for 1 min, 51°C for 1 min and 72°C for 1 min, then 10 min at 72°C. The PCR products were recovered from 0.8% agarose gel. The purified fragment was cloned into pMD 18-T vector (TaKaRa, Japan), named p18T-ZmDEF and the nucleotide sequence of the cDNA insert was determined by sequencing (Sangon, China).

Treatments of seedlings with MeJA and ABA

Maize seeds were preconditioned in sterile distilled water for two days in darkness at 28°C and then grown in an artificial climate box under a white fluorescent lamp on a 16 h-light/8 h-dark cycle at 27°C. Maize seedlings with three leaves were sprayed with 100µM MeJA (Sigma, USA) or H_2O as control. For the ABA treatments, the seedlings were placed in flasks filled with distilled water (control) or 100 µM ABA (Sigma, USA) for 6 h. ABA and MeJA were added as stock (100 mM to give a final concentration of 100 µM) in ethanol, and an equal amount of ethanol was added to a control sample. After treatments, the second fully expanded leaves were picked for total RNA extraction and RT-PCR.

Expression of ZmDEF1 in *Pichia pastoris*

The coding sequence of the *ZmDEF1* mature peptide was obtained by PCR amplification with a forward primer D5 incorporating an *Eco*RI site (5′-CGGAATTCATGAGGCACTGCCTGTCGCAGAG-3′) and a reverse primer D3 with a *Not*I site (5′-TAGCGGCCGCATCTCAGTGGTGG-3′). The amplified product was digested with *Eco*RI/*Not*I and ligated into *Eco*RI and *Not*I sites of the vector pPIC9K (Invitrogen USA). This expression plasmid was named pPIC9K-ZmD. The identity of the insert was verified by sequencing. The vector pPIC9K-ZmD was linearized with *Sac*I and introduced into the *P. pastoris* strain GS115 according to the manufacturer's instructions (Invitrogen, USA). *P. pastoris* cells containing ZmEDF1 were grown at 30°C for 16 h in 100 ml BMGY medium (2% Peptone, 1% yeast extract, 1.34% yeast nitrogen base without amino Acids (YNB), 0.5% Biotin, 1% glycerol and 100 mM potassium phosphate). The supernatant was removed by centrifugation at 10,000 × g for 5 min, and the pellet was re-suspended in 1000ml BMMY (2% Peptone, 1% yeast extract, 1.34% YNB, 0.5% Biotin, 0.5% Methanol and 100 mM potassium phosphate) medium, followed by shaking at 30°C for 120 h. Methanol was added to a final concentration of 0.5% and aliquots were collected for ZmDEF1 content analysis every 12 h.

Tricine-SDS-PAGE and Western blot

Crude protein was precipitated by adding acetone to a final concentration of 20%, then homogenized in 3% SDS, 1.5% mercaptoethanol, 30% glycerol, 0.01% coomassie blue G-250, 30 mM Tris-HCl (pH 7.0), and heated at 100°C for 5 min. Twenty microliters of total protein were loaded into each lane and separated by tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis. The electrophoresis was carried out according to the method of Schägger and Von Jagow (1987) and using a Bio-Rad Mini Electrophoresis system per the manufacturer's instructions. After electrophoresis, the separated proteins were transferred onto nitrocellulose membranes using an Electro Trans-blot apparatus (Bio-Rad, USA). The nitrocellulose membranes were blocked for 30 min in TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) with 5% BSA. The blots were incubated for 1 h with mouse anti-His Tag antibody in TBS containing 5% BSA, and then subsequently with alkaline-phosphatase-conjugated goat anti-mouse IgG antibody (Promega, USA) for 1 h. The color reaction was performed on the blots using nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in a buffer containing 0.1 M $NaHCO_3$ and 1.0 mM $MgCl_2$, pH 9.8.

Purification of ZmDEF1

A three day growth culture was harvested by centrifugation at 14,000 × g for 30 min, the supernatant was collected and protein content precipitated by adding ammonium sulfate at a rate of 1 g/min to 90% of saturation. The individual precipitated fractions were collected by centrifugation at 14,000 × g for 30 min and dissolved in 10 ml of PBS. The majority of ammonium sulfate was removed by dialyzation for 24 h. Purification of the fusion protein, tagged with hexa-His at the C-terminus, was carried out following the Ni-NTA purification system for proteins tagged with histidines (Invitrogen, USA). The fusion proteins were eluted by imidazole-containing buffer and dialyzed against 100 mM phosphate buffer (pH 7.5), and then stored at -20°C until used for antifungal activity assay.

In vitro antifungal activity assays

P. parasitica var. nicotianae was cultured on potato dextrose agar (PDA) medium plates at 25°C for 8 days. The pathogen was then inoculated into millet medium and placed at 28°C for 20 days and the resulting mycelium was rinsed with sterile water and then

Figure 1. Nucleotide and deduced amino acid sequences of *ZmDEF1* cDNA. The deduced amino acid sequence is shown below the cDNA sequence. The putative signal peptide is underlined. The putative translation initiation and stop codons are in bold.

filtered with pledget. The spore suspensions were added into unset PDA medium and then a 5 mm disc was removed from the plate. Solution of the fusion ZmDEF1 was added into the 5 mm hole, with PBS used as a negative control. The plates were placed in the dark at 25°C for three days.

Generation of tobacco lines expressing *ZmDEF1*

Plasmid p18T-ZmDEF was digested with *Hinc*II and *Sac*I and the insert was cloned into the pROK219. The CaMV 35S::*ZmDEF1* expression cassette was digested with *Hin*dIII and *Eco*RI and cloned into the pBI121 binary vector and sequenced. The LBA4404 strain of *Agrobacterium tumefaciens* was used to transform tobacco using the leaf disk transformation method (Horsch et al*.,* 1985). After regeneration on kanamycin selective medium, transformed tobacco lines were checked for the presence of the transgene by PCR.

Northern analysis

Total RNA was isolated from shoot tissue of tobacco using TRIzol reagent (Invitrogen, USA). 20 µg of total RNA was denatured and loaded into a 1.6% formaldehyde-agarose gel, then subsequently transferred onto a nylon membrane. Equal loading of the samples was confirmed by ethidium bromide staining. The membrane was hybridized to a ^{32}P-labeled probe in a hybridization solution containing 0.5 M Na_2HPO_4 pH 7.2, 1 mM EDTA, 1% BSA and 7% SDS at 65°C overnight. The template for the probe was a full-length 245 bp *ZmDEF1* cDNA. After hybridization, the filterate was washed twice with 2 × SSC (1 × SSC is 0.15 M NaCl, 15 mM sodium citrate) containing 0.1% SDS at 65°C for 20 min each, once with 0.2 × SSC containing 0.1% SDS at 65°C for 10 min, and then exposed to X-ray film at -70°C for 24 h.

Detached leaf bioassay

For the leaf bioassay, detached leaves from mature transgenic and control plants (transformed with an empty vector) were placed in Petri dishes containing wet filter papers. The leaves were wounded by gently pricking the abaxial side of the leaves several times with a sterile needle. Then 20 µL of fungal suspension was introduced to the wounded area and incubated at 28°C for 10 days.

Infection of tobacco plants with blast fungus

The seeds of T1 transgenic tobacco were germinated on MS medium containing 100 µg/ml kanamycin. The resistant plants were planted in pots containing plant growth medium and grown in the greenhouse at 25 to 28°C and relative humidity ranging from 30 to 60% under natural daylight for approximately two months. Millet with *P. parasitica* was embedded near the tobacco roots in the pots. The inoculated plants were placed at 30°C with 100% relative humidity for 14 days.

RESULTS

Isolation and analysis of the *ZmDEF1* gene

The *ZmDEF1* cDNA isolated from *Z. mays* was 245 bp in length, consisting of a single open reading frame. The ORF encodes a polypeptide of 80 amino acids consisting of a predicted signal peptide of 31 amino acids at the N-terminus domain and a mature peptide of 49 amino acids with a calculated molecular mass of 5.4 kDa and a pI of 8.61. The nucleotide and predicted amino acid sequence of ZmDEF1 is shown in Figure 1. Several plant defensins of previous studies were compared with ZmDEF1 to confer the cleavage site between the signal peptide and mature *Z*mDEF1 (data not shown). The presence of the signal peptide in the primary translation product suggested that ZmDEF1 is destined to the cell wall or the vacuole, locations where many defense-related proteins are found.

The alignment of ZmDEF1 with other plant defensins is shown in Figure 2. ZmDEF1 shares significant identity with Tad1 (63%), EGAD1 (59%) and SD2 (59%), but low identity with Nad1 (36%), alfAFP (35%), Rs-AFP2 (30%), Dm-AMP1 (28%) and Psd1 (26%). ZmDEF1 contained the well conserved eight cysteine residues, which play an important role in protein stabilization (Figure 2). Other conserved residues such as Ser8, an aromatic residue at position 11, Gly13, Glu29 and Gly34 were also found

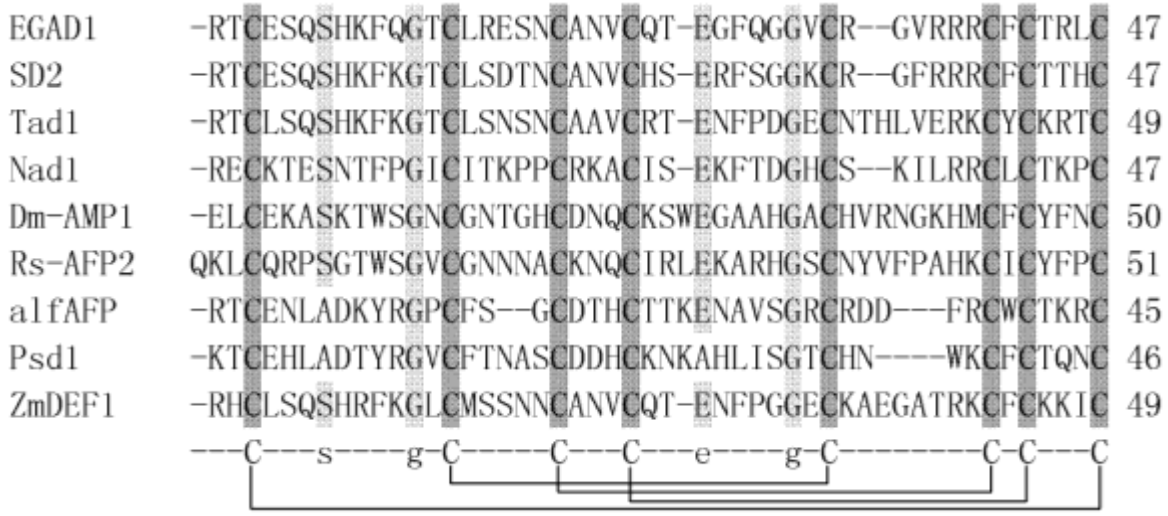

Figure 2. Amino acid sequence alignment of mature plant defensins from several plants. Comparison of the deduced mature ZmDEF1 amino acid sequence with those of other plant defensins. Conserved residues are presented in black boxes, partially conserved residues in gray boxes, while the defensin consensus sequence is shown below the alignment. The disulfide bond connectivities are shown below the consensus sequence by connecting lines. The sequences were aligned using the CLUSTALW2 online (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

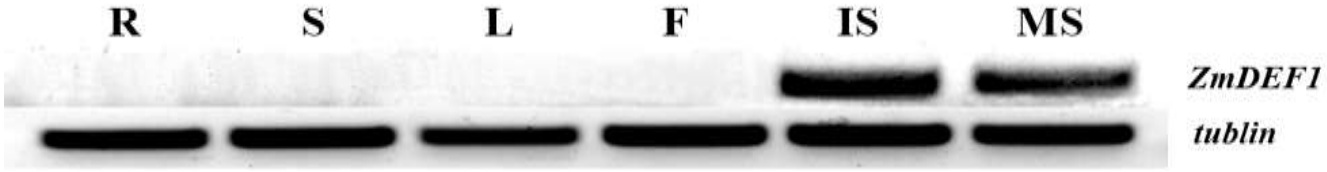

Figure 3. RT-PCR expression analysis of *ZmDEF1* in different organs of *Zea mays*. The specific products of *ZmDEF1* are detected in immature (IS) and mature seeds (MS), but not in roots (R), stems (S), leaves (L) and flowers (F). The *tubulin* gene exhibiting constitutive expression was used as a control.

in the sequence (Figure 2). According to the analysis of sequence performed in this work, ZmDEF1 can be grouped with the Class I defensins.

In addition, to analyze the *ZmDEF1* expression pattern, RT-PCR was performed with total RNA samples extracted from different maize tissues. The data show that *ZmDEF1* transcripts were only detected in immature and mature seeds, but not in roots, stems, leaves or flowers (Figure 3). Furthermore, the result that no *ZmDEF1* transcripts could be detected in seedlings even under induction by MeJa and ABA (data not shown), indicates the ZmDEF1 may play a defensive role only during seed development.

Expression of ZmDEF1 in *P. pastoris*

In the *P. pastoris* expression system, a strain that contains multiple integrated copies of an expression cassette can sometimes yield more heterologous protein than single-copy strains (Cereghino and Cregg, 2000). For screening His^+ recombinants with multiple inserts, all the clones were incubated in 96 well microtiter plate for three times passage until they were all at the same cell density. The cultures were then spotted on YPD plates containing G418 at grads concentration (Figure 4). The recombinants that demonstrated resistance at 4.0 mg/ml G418 were analyzed for the presence of *ZmDEF1* by PCR. Six recombinants were recovered from 224 colonies obtained originally.

Antifungal activity assays *in vitro*

In order to study the antifungal activity of ZmDEF1, the Pichia Expression Kit (invitrogen, USA) was used. ZmDEF1 was produced as a fusion protein with a 6×His tag at C-terminal. The total size of the predicted fusion protein is 7.6 kDa. This roughly corresponds to the molecular size of the expressed ZmDEF1 detected by Western blot (Figure 5). The fusion protein expression in *Pichia* was detected every 12 h. The accumulation of

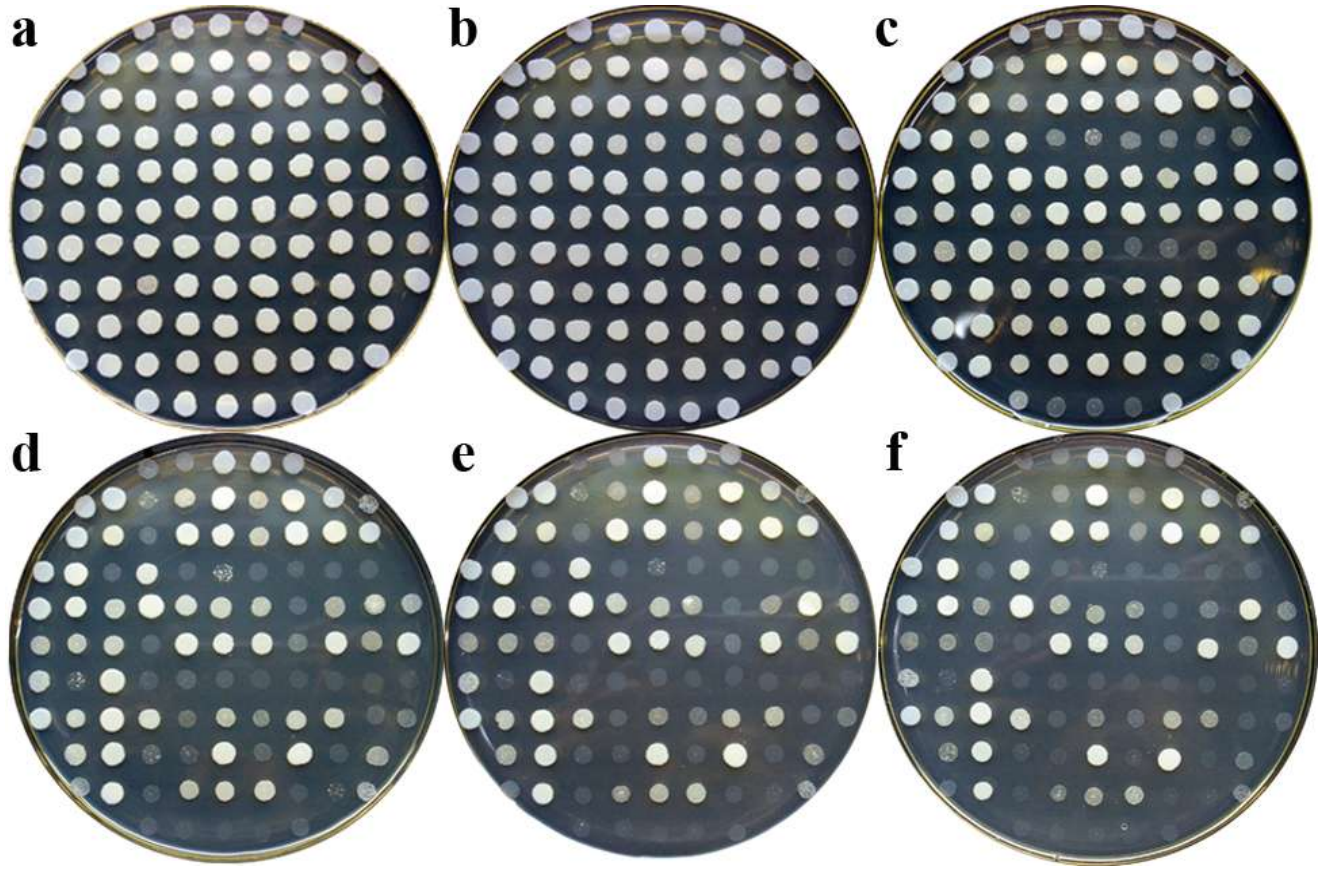

Figure 4. Screening for yeast recombinants containing multiple copies of *ZmDEF1*. The cultures were grown on YPD plates containing G418 at grads concentration. A to F shows the G418 final concentration at 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/ml, respectively.

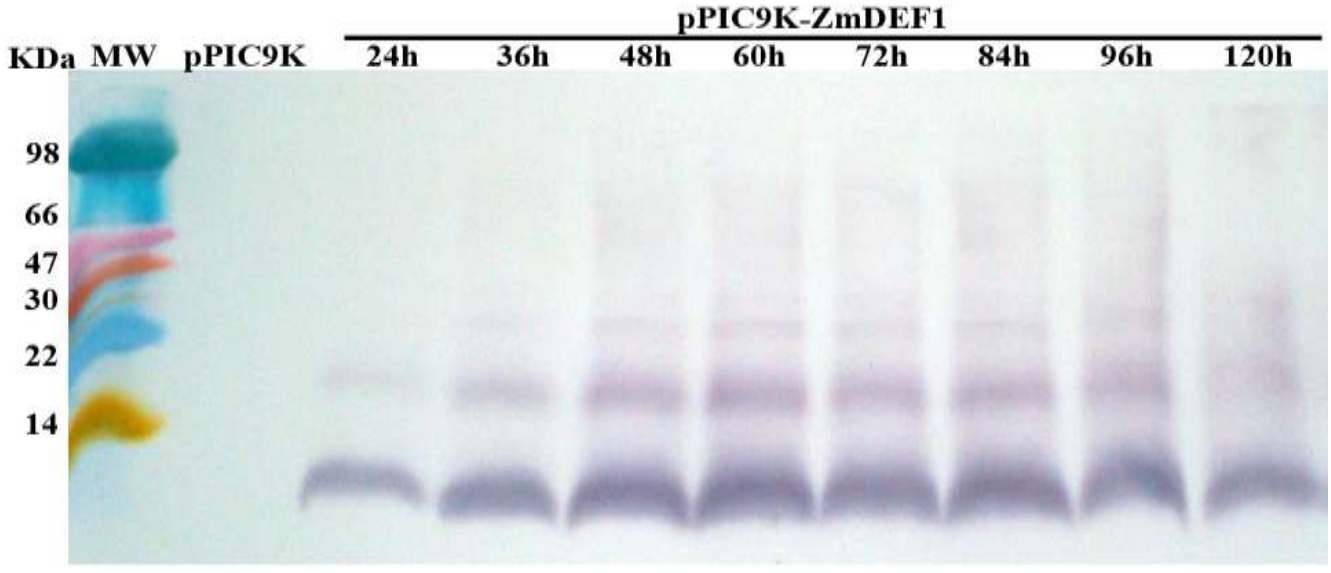

Figure 5. Immunoblot detection of ZmDEF1 expression in *P. pastoris*. Detection of the fusion protein ZmDEF1 expressed in *P. pastoris* using the anti-His antibody. Denaturing Tricine-SDS-PAGE followed by immunoblotting was performed on the total protein extracts from 1 ml supernatant. *P. pastoris*/pPIC9K was used as a negative control. Pre-stained protein standards (MW) were included for estimation of the molecular mass (kDa). The accumulation of ZmDEF1 was detectable after 24 h induction, reaching a maximum after 60 h induction.

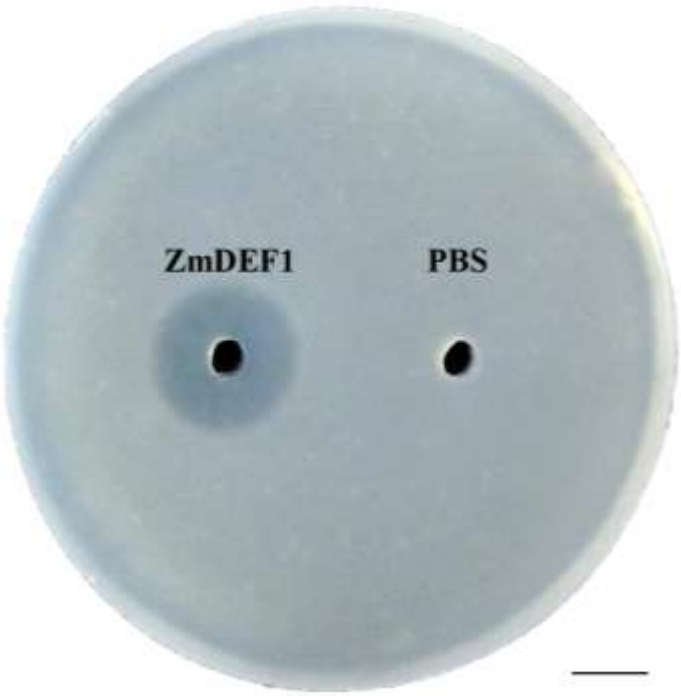

Figure 6. Antifungal activity of ZmDEF1 on *P. parasitica*. The spores of *P. parasitica* were incubated on PDA plates with a crude extract of the fusion ZmDEF1 protein for 3 days. PBS was utilized as a negative control. Bar =1 cm.

ZmDEF1 was detectable after 24 h, reaching a maximum after 60 h and maintained up to the 96 h. After 108 h, the level of ZmDEF*1* expression was reduced (Figure 5). Thus, a three day growth culture was harvested and purified to yield the maximum concentration. The yield of the purified fusion protein was approximately 258 µg/ml of original culture. The purified fusion protein was used to test activity against *P. parasitica*. Compared to the controls, the expressed ZmDEF1 showed an activity against spores germination and hyphal growth of the fungal pathogen, *P. parasitica* (Figure 6).

Ectopic expression of ZmDEF1 in tobacco confers resistance to *P. parasitica*

Transgenic tobacco plants were generated via Agrobacterium-mediated transformation, using the neomycin phosphotransferase II gene (NPTII) as a selectable marker. Transgenic tobacco lines were randomly selected and screened by PCR, using primers specific to *ZmDEF1* gene. The *ZmDEF1* transcript in seedlings of transgenic plants was detected by RNA gel blot. The results show that exogenous *ZmDEF1* gene was expressed in all examined transgenic plants, and was particularly highly expressed in two lines, oxZmDEF1-1 and oxZmDEF1-8 (Figure 7), while phenotypic abnormalities were not observed in any transgenic lines as compared to wild-type.

We also examined the effects of the overexpression of *ZmDEF1* on resistance of the transgenic plants to the fungal pathogen, *P. parasitica var. nicotianae*. Disease resistance was evaluated on the T1 transgenic lines oxZmDEF1-1 and oxZmDEF1-8, as well as the control line, which transformed with an empty vector. Disease symptoms appeared on detached leaves of the control plants in the form of lesions 5 to 6 days after inoculation, and consequently lead to the appearance of yellowish necrotic lesions 10 days after inoculation. On the contrary, symptoms were not detected on leaves of transgenic lines (Figure 8). Subsequently, a whole plant assay was carried out. The disease symptoms started to appear on the control plants at 6 days after infection, but no symptoms were observed on the transgenic plants. By 8 days after infection, the control plants had severe disease symptoms such as leaf wilting and stem rot, and eventually died within 14 days (Figure 9). However, both of the transgenic lines remained relatively healthy with only a slight yellowing of the bottom leaves that had contact with the infecting fungus. We also observed a difference in the severity of tissue damage between control and *ZmDEF1* transgenic plants. The longitudinal section of stem showed that all the tissues of control plants, including epidermis, cortex and pith, were black and rotten, while the corresponding part of transgenic plants remained green (Figure 9I and J).

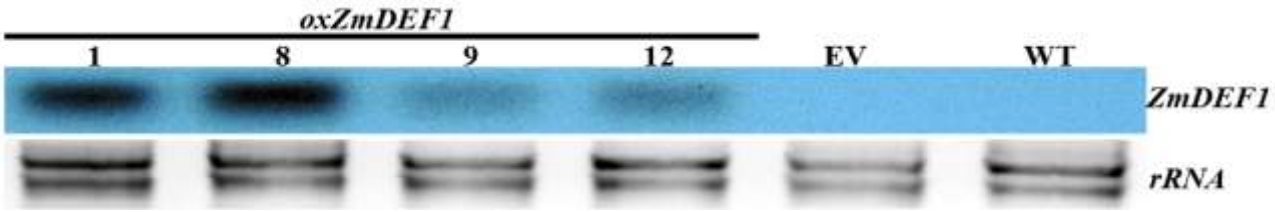

Figure 7. Northern blot analysis of *ZmDEF1* in leaves of transgenic tobacco. Total RNA was hybridized with a radioactively labeled *ZmDEF1* probe. 1, 8, 9, 12: transgenic tobacco lines oxZmDEF1-1, oxZmDEF1-8, oxZmDEF1-9 and oxZmDEF1-12. EV, regenerated plants transformed with plasmid pBI121. WT, wild type.

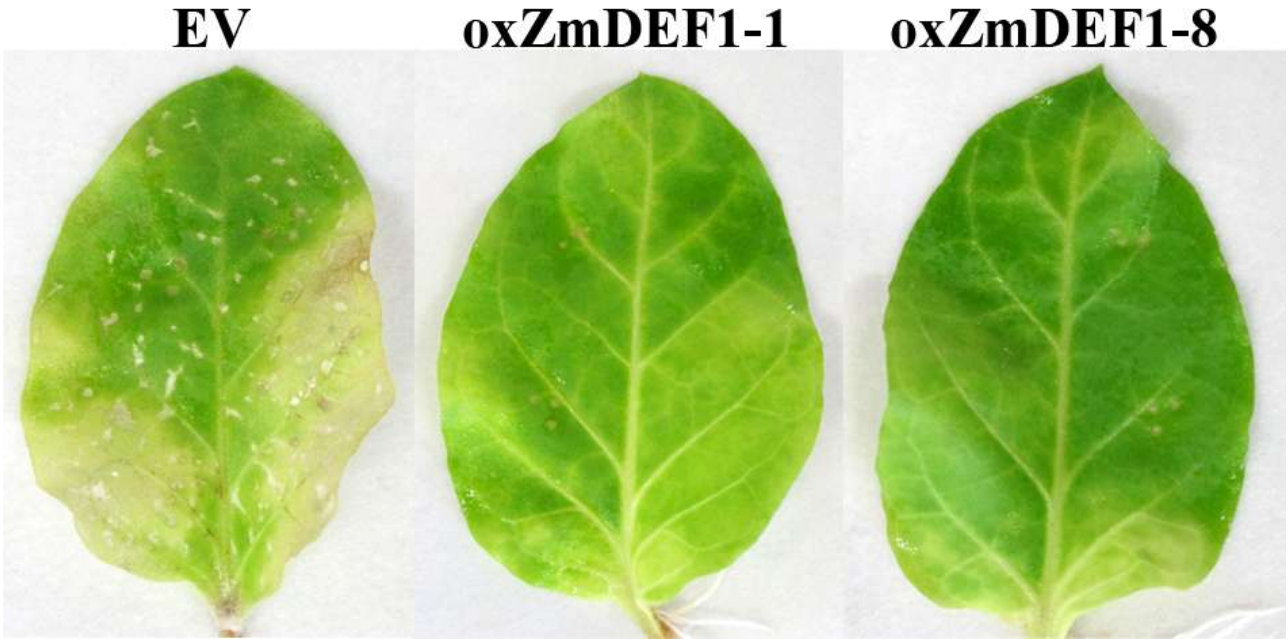

Figure 8. Fungal resistance test of detached leaves of transgenic tobacco expressing *ZmDEF1*. The results were photographed ten days after inoculation with *Phytophthora palmivora*. EV, the T1 plants transformed with plasmid pBI121.

DISCUSSION

Plants have developed various defense mechanisms against pathogen attack. Defensins are one class of antimicrobial proteins that fight off the foreign pathogens (García-Olmedo et al*.,* 1998). All plant defensins descryibed to date have a signal peptide marking the protein for extracellular secretion (Thomma et al*.,* 2002). In the present study, we cloned a cDNA encoding a novel plant defensin ZmDEF1, from *Z. mays* germinated seeds. The predicted protein would have a 31 amino acid signal peptide (Figure 1). The mature peptide of ZmDEF1 contains all the conserved residues reported for plant defensins (Lay and Anderson, 2005). Therefore, ZmDEF1 is likely a novel member of defensins family.

The majority of plants defensin genes are expressed in the seeds of various monocot and dicot species (Bohlmann, 1994; Broekaert et al*.,* 1997). They have been shown to be present as part of normal development or maturation, perhaps as a static defense against pathogens (Thomma et al*.,* 2002). Balandin et al*.* (2005) found that the transcripts of *ZmESR-6* were restricted to the embryo surrounding region (ESR) of the kernel, but the protein accumulated in the placentochalaza-cells at the grain filling phase. The function of ZmESR-6 was thought to protect the germinating kernel from pathogens. Similar to ZmESR-6, the *ZmDEF1* mRNA preferentially accumulates in immature and mature seeds (Figure 3), suggesting a defensive role in protecting kernels during seed development. Jasmonate has been shown to be an effective inducer of other defensins (Epple et al*.,* 1997; Thomma et al*.,* 1998). However, the expression of *ZmDEF1* could not be induced in seedlings after treatment with ABA or MeJa (data not shown). This result is consistent with a specialized role of ZmDEF1.

More also, we chose the methylotrophic yeast *P. pastoris* expression system to study the antifungal activity of ZmDEF1 *in vitro*. A major advantage of *P. pastoris,*

Figure 9. Tobacco plants challenged with Black Shank *P. parasitica*. Pictures were taken eight days after *P. palmivora* inoculation. a, c, e, g and i show transgenic plants, while b, d, f, h and j, show controls, the T1 plants transformed with plasmid pBI121. Compared with control, the transgenic tobacco plants were still green after pathogen infection.

over bacterial expression systems, is that the yeast has the potential to perform many of the post-translational modifications typically associated with higher eukaryotes, such as the processing of signal sequences, folding, disulfide bridge formation, certain types of lipid addition and glycosylation (Cereghino and Cregg, 2000). Eight cysteine residues present in ZmDEF1 mature protein play an important role in protein stabilization by formation multiple disulfide bridges. *P. pastoris* is believed to be more effective in promoting disulfide bonding than the

Escherichia coli (Cregg et al., 1993). In our previous work, we found that the fusion ZmDEF1 expressed using a prokaryotic system showed no antifungal activity (data not shown) possibility due to improper folding of the recombinant protein. However, the recombinant ZmDEF1 peptide created using the *P. pastoris* expression system exhibits an inhibitory activity on *P. parasitica* growth (Figure 6).

Due to an alarming increase of resistance of microorganisms to classical antibiotics, the introduction and expression of antimicrobial peptides like plant defensins in crops is emerging as an intriguing biotechnological application for enhancing disease resistance (Punja, 2001; Osusky et al*.,* 2000; Jha and Chattoo, 2010). In this work, a new plant defensin gene, *ZmDEF1*, was introduced into tobacco by Agrobacterium-mediated transformation. Results indicate that constitutive expression of the *ZmDEF1* gene under the control of the CaMV 35S promoter in tobacco results in enhanced resistance against *P. parasitica* (Figures 8 and 9). Thus, the antifungal activity of the defensin ZmDEF1 and its availability for genetic engineering should make it useful as gene source for engineering transgenic plants resistant against phytopathogenic fungi.

ACKNOWLEDEGMENTS

This work was supported by National Natural Science Foundation (Grant No. 31171258) and the projects (No. 2008ZX08009-003 and 2008ZX08003-002) from the Ministry of Agriculture of China for Transgenic Research.

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