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An antibiotic fusaricidin: a cyclic depsipeptide from *Paenibacillus polymyxa* E681 induces systemic resistance against Phytophthora blight of red-pepper

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Abstract In this study fusaricidin, a cyclic depsipeptide isolated from *Paenibacillus polymyxa* E681 (E681), was demonstrated to control Phytophthora blight infection caused by *Phytophthora capsici* in red-pepper. The minimal inhibitory concentration (MIC) of fusaricidin was found to be 16 ppm against *P. capsici*. The disease severity of *P. capsici* was 40% at 0.1 ppm of fusaricidin when compared with water-treated control (81.7%) on post-treatment, whereas the disease severities on pre-treatment were 45% and 83.3% in fusaricidin (0.1 ppm) and water-treated control, respectively, in red-pepper plants. Significant ($P<0.05$) disease suppression was observed on treatment with fusaricidin (0.1 ppm) by foliar spray and soil drench. The disease severity was drastically reduced to 3.3% by soil drench of fusaricidin (1.0 ppm), whereas in water-treated control, the disease severity was 83.3% in the first experiment. Fusaricidin at 0.1 ppm reduced disease severity of *P. capsici* to 27.5% when compared with positive control (43.1%) and water-treated control (66.2%) in the second experiment. Soft rot disease in tobacco was suppressed upon treatment with fusaricidin at 1.0 ppm by leaf infiltration. RT-PCR analyses of *Arabidopsis thaliana* revealed that there was an up-regulation of pathogenesis-related (*PR*) gene expression in wild type *A. thaliana* (*Col-0*), while there was no accumulation of *PR* genes, which implies that the mechanism of protection might be based on a salicylic acid-dependent pathway. This is the first report that fusaricidin exhibits protection against plant pathogens in addition to activity as an antibiotic agent. Hence, E681 can play a role in plant protection by secretion of ISR elicitors including fusaricidin.

Keywords Antifungal assay · Biocontrol agent · *Capsicum annuum* · Defense gene expression · Disease suppression · *Phytophthora capsici* · Soil drench

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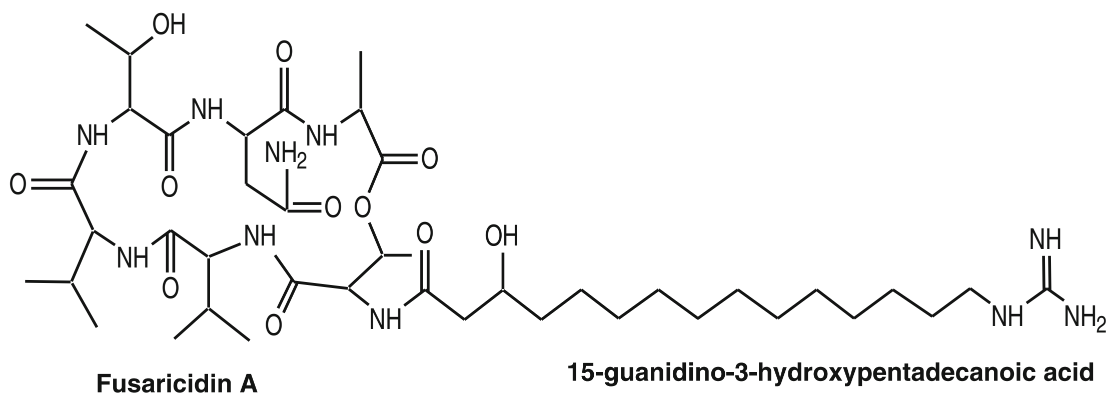
Introduction

Biological control using microorganisms to suppress soilborne plant pathogens has been successfully carried out in several crops (Park *et al.* 1988; Weller 1988). The mechanisms how these diseases were controlled by the microorganisms remained unclear until Homma & Suzui (1989) elaborated the role of antibiotics by suppression of the pathogens. Among the myriads of bacteria thriving in the plant rhizosphere, some spore forming plant growth-promoting rhizobacteria (PGPR),

in particular gram-positive *Bacilli* and *Streptomycetes,* attracted special attention due to their advantages over non-spore-forming bacteria in product formulation and stable maintenance in soil (Emmert & Handelsman 1999). Among these, the genus *Paenibacillus* comprises more than 89 species (Ash *et al.* 1993; Truper 2005). Strains of *Paenibacillus polymyxa* suppress several plant diseases and promote plant growth (Ryu & Park 1997).

Paenibacillus polymyxa strains are capable of producing several hydrolytic enzymes, including proteases, β-1,3-glucanases, cellulases, xylanase, lipase, amylase and chitinases which play an important role in the biocontrol of plant pathogens (Beatty & Jensen 2002; Raza *et al.* 2008). These strains have been isolated from the rhizosphere of various crops (Choi *et al.* 2008; Petersen *et al.* 1996). *P. polymyxa* has been successful in the control of gray mold in strawberries caused by *Botrytis cinerea* (Helbig 2001); *Fusarium oxysporum* and *Pythium* spp.– causal agents of seedling blight and wilt, root rot of cucumber and water melon (Dijksterhuis *et al.* 1999; Yang *et al.* 2004); sesame damping off (Ryu *et al.* 2006); and to control diseases caused by *Phytophthora palmivora* and *Pythium aphanidermatum* (Timmusk &Wagner 1999). *P. polymyxa* is known to produce two types of peptide antibiotics: one is active against bacteria and the other one is active against fungi and actinomycetes (Beatty & Jensen 2002). This species also synthesizes plant hormones such as auxin and cytokinin (Timmusk & Wagner 1999), solubilizes soil phosphorus, enhances soil porosity (Gouzou *et al.* 1993) and has been used for flocculation and flotation of various minerals (Deo & Natarajan 1998).

Generally, it is difficult to manage diseases caused by *Phytophthora* because of their high aggressiveness and increase in resistance to metalaxyl, an effective systemic compound against oomycetes (Gavino *et al.* 2000); and 95 *Phytophthora* and 100 *Pythium* species which have been previously reported (Erwin & Ribeiro 1996; Kamoun *et al.* 1999). The emergence of insensitive strains, and their persistence, makes the application of a chemical ineffective and rather exacerbates the problem. These issues necessitated us to look for alternative methods for disease control in order to reduce pesticide application to food crops and the concern regarding environmental pollution. Phytophthora blight caused by *Phytophthora capsici* is one of the most devastating soilborne diseases of red-pepper worldwide (Hausbeck & Lamour 2004; Hwang & Kim 1995). Intensive studies have been concentrated on the biology of *P. capsici*, such as evaluation of pepper germplasm for disease resistance, yield-loss assessment, and testing of chemical, biological, and cultural measures of control (Hwang & Kim 1995).



Fusaricidin comprises a group of cyclic depsipeptides with an unusual 15-guanidino-3-hydroxypentadecanoic acid moiety bound to a free amino group (Kajimura & Kaneda 1997). Fusaricidin is produced by *P. polymyxa* PKB1 at the beginning of sporulation (Beatty & Jensen 2002) and gatavalin is produced by *Bacillus polymyxa* spp. and *Colistinus koyama* ATCC 21830 (Nakajima *et al.* 1972). Both products belong to the same or a similar family of peptide antibiotics with very similar HPLC profiles. Two further antibacterial

substances were isolated and purified from culture broth of *P. polymyxa,* namely, gavaserin and saltavalin (Pichard *et al.* 1995). Kajimura & Kaneda (1995) reported the taxonomy, fermentation, isolation, structure elucidation and biological activity of Fusaricidin A, an antibiotic produced by *Bacillus polymyxa* KT-8. However, there is only limited information on the activity of fusaricidin against oomycetes, and there is no report available on disease suppression through ISR. Thus, in the present investigation, we report for the first time the protection exerted by fusaricidin, an antibiotic compound active against Phytophthora blight in red-pepper plants.

Materials and methods

Culturing of microorganisms and the media used The strain *P. polymyxa* E681 (E681) was obtained from the Korean Research Institute of Bioscience and Biotechnology (KRIBB), South Korea. The strain was identified and deposited at Microbial Resources Data Base, KRIBB, South Korea, under lyophilized conditions, and maintained at 4°C until use for mass cultivation. Media used for cultivation and bioassay of the strains were potato dextrose agar (PDA) and potato dextrose broth (PDB) purchased from Difco (Detroit, MI, USA). pH of the medium was adjusted to 7.2 before sterilization. Bennett's agar and Bennett's broth for preservation and liquid culture of the strain contained (per liter) glucose 10 g, yeast extract 1 g, bacto peptone 2 g, beef extract 1 g, agar 20 g (or without agar), 5 g each of thiamine-HCl, riboflavin, niacin, pyridoxine-HCl, inositol, Ca-pantothenate and amino benzoic acid, biotin 25 mg, cycloheximide 50 mg and nalidixic acid 10 mg. Fungi *P. capsici* was grown on V8 juice agar plate at 28°C for 7 days.

Screening of cyclic depsipeptide producer E681 and culturing Fusaricidin producing strain E681 was screened and identified as a potential biocontrol agent (BCA) according to the method followed by Lee *et al.* (2005). For the preparation of culture broth, E681 was first inoculated in 200 ml erlenmeyer flasks containing 60 ml of the screening medium consisting of (per liter) soluble starch 10 g, glucose 20 g, soybean meal 25 g, beef extract 1 g, yeast extract 4 g, NaCl 2 g, K_2HPO_4 25 g and $CaCO_3$ 2 g. pH of the medium was adjusted to 7.2 before sterilization and cultured on a rotary shaker at 150 rpm and 28°C for 5 days. For scale-up fermentation, the strain was precultured in a 500 ml erlenmeyer flask for 3 days, and a stock solution (3%) was used to re-inoculate in a 5*l* jar fermentor (Korea Fermentor Co., Daejeon, Korea) to produce fusaricidin. The purified fusaricidin was stored at 4°C until further use.

In vitro antifungal activity of fusaricidin The antifungal activity of cyclic depsipeptide, fusaricidin-A, was examined on PDA plates containing fusaricidin at various concentrations (0 to 512 ppm) against major fungal plant pathogens (*Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora capsici*, *Colletotrichum acutatum*, *Pythium ultimum*, *Alternaria alternata*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*) for in vitro primary screening. The mycelial discs of each pathogen, prepared with a sterile cork borer (5 mm), were placed on PDA plates. The plates were incubated at 25°C for 48 h, and then observed for mycelial growth. Antifungal activity is expressed as the minimal inhibitory concentration (MIC) at which no mycelial growth was observed.

Preparation of spore suspension of P. capsici and bacterial pathogen P. carotovorum SCC1 inocula - P. capsici inocula were prepared as described by Ploetz *et al.* (2002). A 5-mm-diam mycelial plug of an isolate was transferred to a V8 agar plate. After 1 week of incubation at 25°C, V8 agar plugs with mycelia were placed into a petri dish containing V8 broth, and allowed to grow for another week at 25°C. The V8 broth was then drained and each plate was washed twice with sterile distilled water (SDW). SDW was added to cover the mycelia on each plate, and then the plates were placed under wide-spectrum light at room temperature for 48 h to induce sporangial development. The sporangia were chilled at 4°C for 45 min to induce the release of zoospores. The zoospores were adjusted to a final concentration of 1×10^5 spores ml^{-1} to use for challenge inoculation under greenhouse conditions. For the preparation of *P. carotovorum* SCC1 (SCC1) inoculum, the bacterial cell suspensions were prepared from 24-h-old culture at 28°C. Ten ml of SDW was poured on tryptic soy agar (TSA) culture plate and scraped with sterile plastic loop and adjusted to a final concentration of 1×10^8 cfu ml^{-1} (OD_{600}=0.8) before challenge inoculation.

Pre- or post-treatment of fusaricidin on red-pepper plants against Phytophthora blight Three-week-old

red-pepper (*Capsicum annuum* L.) cv. Hanbyul seedlings were used in this study. The seedlings were treated with fusaricidin by foliar spray at various concentrations (0.1, 1.0, 10, 100 and 1,000 ppm) in pre- or post-treatments. In pre-treatment, the red-pepper plants were foliar sprayed with fusaricidin 7 days before challenge inoculation with pathogen, whereas in post-treatment, 7 days after pathogen challenge red-pepper plants were treated with fusaricidin by foliar spray. Dimethomorph, a common systemic fungicide, was used as positive control at 1.0 ppm. It is a cinnamic acid derivative and a member of the morpholine chemical family. One week later, the disease severity of Phytophthora blight on red-pepper was assessed during pre-and post-treatments according to the modified method by Sunwoo *et al.* (1996) based on a 0–5 scale: where 0=no visible disease symptoms; 1=leaves slightly wilted with brownish lesions beginning to appear on stems; 2=30–50% of entire plant diseased; 3=50–70% of entire plant diseased; 4=70–90% of entire plant diseased; 5=completely wilted or plant dead. Based on these ratings, percentage of disease severity was calculated.

Evaluation of fusaricidin for ISR activity against P. capsici and SCC1 To induce the protection exerted by fusaricidin, 3-week-old red-pepper seedlings were treated with fusaricidin by soil drench at various concentrations (0.1, 1.0, 10 and 100 ppm). During 3 weeks of time, a second and third dose of fusaricidin was applied to seedlings by soil drench at 7-day intervals. For the induced protection of red-pepper from *P. capsici*, fusaricidin-treated plants after challenge inoculation with zoospores by soil drench were placed on a greenhouse bench after incubating them in a humidity chamber at 28°C for 24 h. The greenhouse conditions were 22–26°C, with a natural photoperiod. One week later, the percentage of disease severity was recorded as above. For the induced protection of tobacco plants from SCC1, 3-week-old plants were treated with fusaricidin by infiltration, and were challenge inoculated with the suspensions of SCC1. The plants were kept on a greenhouse bench after incubating them in a humidity chamber for 24 h. Three days later, the percentage of disease incidence was recorded.

Defense gene expression in Arabidopsis thaliana through RT-PCR - *Arabidopsis thaliana* wild type, Columbia (*Col-0*) and salicylic acid (SA) insensitive transgenic line (*Nah-G*) were obtained from the Ohio State University Stock Centre, Columbus OH, USA. Two-week-old *Arabidopsis* seedlings were soil drenched with fusaricidin at various concentrations (0.1, 1.0 and 10 ppm), and then the leaf tissues were collected for RNA isolation 7 days after treatment. Total RNA was isolated using easy-spin^{TM} IIP Total RNA Extraction Kit (iNtRON Biotechnology, South Korea). Reverse transcriptase (RT)-PCR was performed according to Kishimoto *et al.* (2005) with Ex *Taq* polymerase (Takara Biomedicals, Otsu, Japan). The reaction mixture contained 0.1 μg of cDNA, 10 pMol each of forward and reverse primers, 250 nM dNTPs, and 0.5 U of Ex *Taq* polymerase in 20 μl of buffer solution. The PCR was carried out in a MJ Research thermal cycler (PTC-100, USA) with the following conditions: 94°C for 5 min followed by 94°C for 1 min, 57°C for 1 min for 25 cycles, followed by 72°C for 10 min for final extension. Primers for defense gene are F-5′-AACCGCCAAAAGCAAACGCA-3′ (*PR-1a*-F), R-5′-TCACGGAGGCACAACCAAGTC-3′ (*PR-1a*-R). Amplified PCR products were analyzed with 1% agarose gel electrophoresis and the gels were documented (LAS-3000, Fuji Photo Film Co. Ltd., Tokyo, Japan).

Statistical analysis Data were analyzed (mean±SE) with SAS JMP software. Significant differences in treatment means on each sample data were determined using LSD at $P=0.05$. All experiments were repeated at least once. For each experiment, data were analyzed separately. Results of one representative experiment are shown.

Results

In vitro antifungal activity In vitro antifungal activity of the cyclic depsipeptide, fuaricidin-A, was assayed against various phytopathogenic fungi that are a common major cause of diseases in agricultural crops. The minimal inhibitory concentration (MIC) values are listed in Table 1. These data reveal that the tested antibiotic agent, fusaricdin, has potent antifungal activity against the fungal pathogens: in particular, the oomycetes of *P. capsici* and *P. ultimum* were inhibited by treatment with fusaricidin at the lowest (16 ppm) concentration.

Effect of fusaricidin on suppression of Phytophthora leaf blight infection The present study identified the

Table 1 In vitro antifungal assay of fusaricidin using minimum inhibitory concentrations (MIC) against major fungal plant pathogens

Fungal pathogen	MIC (ppm)
Rhizoctonia solani	>512
Fusarium oxysporum	128
Phytophthora capsici	16
Colletotrichum acutatum	256
Pythium ultimum	16
Alternaria alternata	256
Botrytis cinerea	32
Sclerotinia sclerotiorum	32

potential for fusaricidin as one of the ISR elicitors for protecting red-pepper plants against Phytophthora blight under greenhouse conditions. Fusaricidin treatment significantly ($P<0.05$) reduced Phytophthora leaf blight infection when compared with water-treated control plants. This disease suppression was found to be at the lowest concentration (0.1 ppm of fusaricidin) by foliar spray (Fig. 1). Suppression of Phytophthora blight infection on red-pepper revealed that fusaricidin treatment by foliar spray at 0.1 ppm has brought down the leaf blight infection to 40% in post-treatment, and 45% in pre-treatment when compared with 81.7% and 83.3% disease severities in post-treatment and pre-treatments, respectively, in water-treated control. In the case of soil drench with fusaricidin, there was a significant ($P<0.05$) reduction of disease severity to 3.3% by treatment with fusaricidin at 1.0 ppm concentration when compared to other concentrations of fusaricidin, dimethomorph and water-treated control (Fig. 2). The results demonstrated the role of fusaricidin as a potential ISR agent against Phytophthora infections in red-pepper plants. It is interesting to note that the percentage of disease severity was increased at higher concentrations (10 ppm to 1,000 ppm) of fusaricidin. This indicates that lower concentrations of fusaricidin elicit systemic resistance for protecting the plants from disease. In the second experiment, the study identified 0.1 ppm as an optimum dosage of fusaricidin in reducing disease severity to 27.50% when compared with other, higher, concentrations as well as dimethomorph at 1.0 ppm (43.13%) and 66.25% in water-treated control. Figure 3 shows that there was a reduced disease severity at 1.0 ppm concentration of fusaricidin and is on a par with the chemical fungicide dimethomorph (1.0 ppm) when compared with higher concentrations and water-treated control.

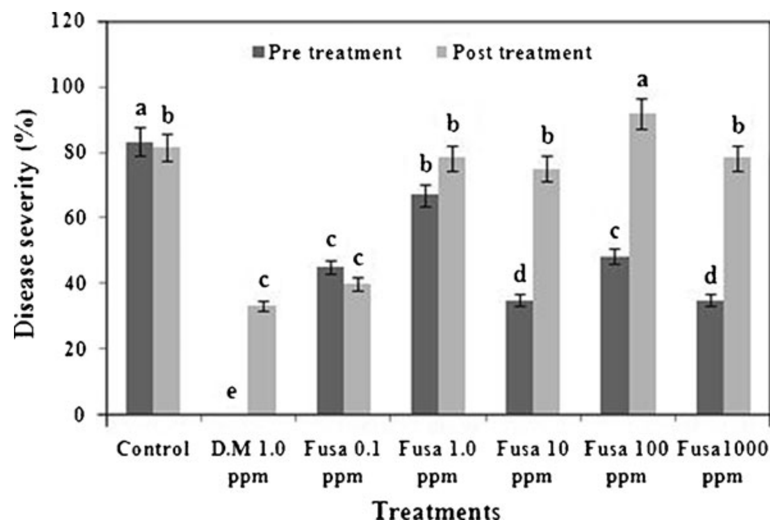


Fig. 1 Suppression of Phytophthora blight in red-pepper plants on pre- or post-treatments of fusaricidin (Fusa) or dimethomorph (D.M) by foliar spray. Distilled water served as untreated control. Red-pepper plants were pre- and post-treated with fusaricidin at various concentrations before and after challenge inoculation of *Phytophthora capsici* spores by soil drench. The plants were kept under greenhouse conditions 24 h after incubating them in a humidity chamber, after which the percentage of disease severity was measured. The experiment was conducted at least twice with 12 replications per treatment with similar results. Bars with the same letter do not differ significantly from each other according to Fischer's least significant difference test ($P=0.05$)

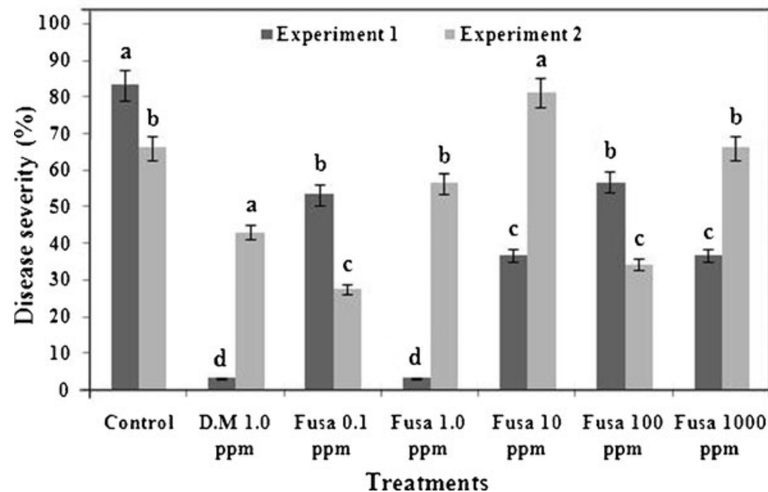


Fig. 2 Suppression of Phytophthora blight on red-pepper plants by soil drench of fusaricidin (Fusa). Dimethomorph (D.M) and distilled water served as positive and negative controls, respectively. Red-pepper plants were treated with fusaricidin at various concentrations, and 3 days later the plants were challenge-inoculated with *Phytophthora capsici* zoospores by soil drench; after 24 h of incubation in a humidity chamber, the plants were kept under greenhouse conditions for 1 week. The percentage of disease severity was measured. Bars with the same letter do not differ significantly from each other according to Fischer's least significant difference test (P=0.05)

Effect of fusaricidin on disease suppression of soft rot in tobacco Further, the disease suppression development in transgenic tobacco against soft rot disease was assessed by leaf infiltration of fusaricidin. Infiltration of fusaricidin at 1.0 ppm recorded reduced disease severity (22%) when compared with the chemical elicitor BTH at 0.1 ppm (18.3%) and by 85% in water-treated control (Fig. 4). On the other hand, with fusaricidin at 0.1 ppm there was also significant reduced disease severity of 30%. There was greater disease severity at higher concentrations (100 and 1,000 ppm).

Effect of fusaricidin on defense-related gene expression in A. thaliana through RT-PCR In order to ascertain the enhancement of ISR activity of fusaricidin, RT-PCR

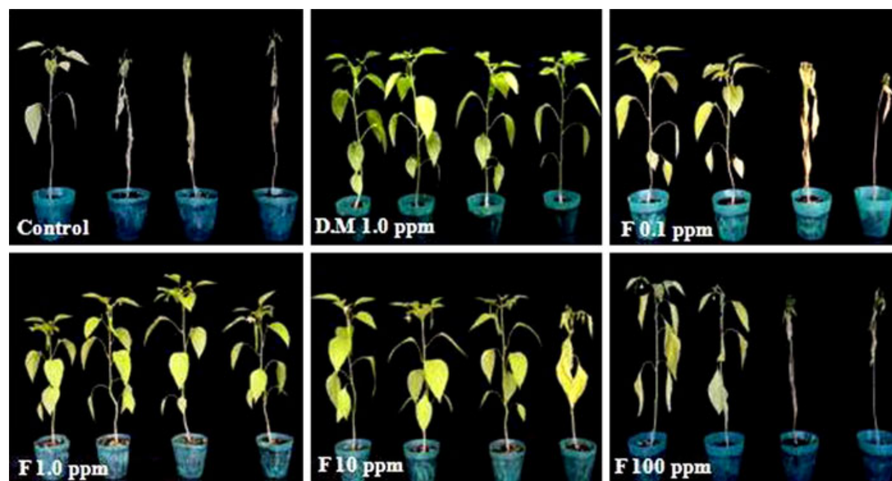


Fig. 3 Induced suppression of disease development in red-pepper plants against *Phytophthora capsici* by soil drench with fusaricidin (F) at various concentrations under greenhouse conditions. Percentage of disease severity was recorded 7 days after pathogen challenge with *P. capsici* zoospore suspensions by soil drench. Fusaricidin at lower concentrations (1.0 and 10 ppm) induced the suppression of disease development. Dimethomorph (D.M) and distilled water served as positive and negative controls, respectively. The experiment was repeated at least once with 12 replications per treatment with similar results

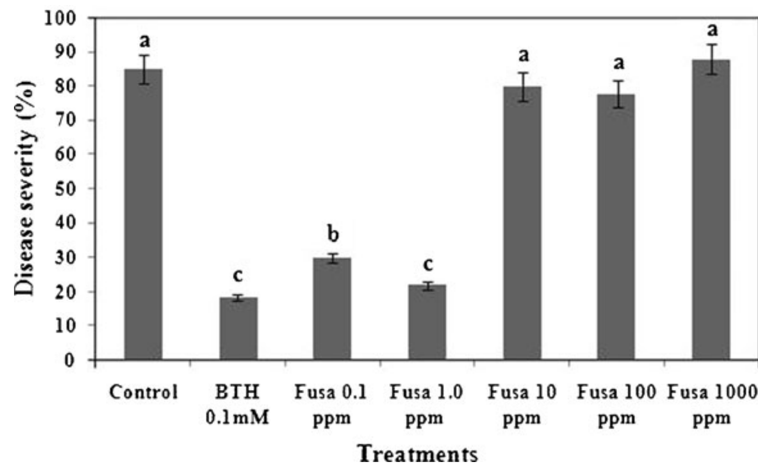


Fig. 4 Suppression of disease development in tobacco plants against soft rot disease caused by *Pectobacterium carotovorum* SCC1 by treatment with fusaricidin (Fusa). Benzothiadiazole (BTH) and distilled water served as positive and negative controls, respectively. Tobacco plants were leaf infiltrated with fusaricidin at various concentrations. Three days after challenge inoculation with *P. carotovorum* SCC1 suspensions, the plants were observed for disease incidence and percentage was calculated. The experiment was repeated at least once with 12 replications per treatment with similar results. Bars with the same letter do not differ significantly from each other according to Fischer's least significant difference test (P=0.05)

analysis for defense gene transcript in *Arabidopsis* was carried out to confirm the activation of the defense gene *PR-1a* by mRNA accumulation in the leaves of *A. thaliana* wild type (*Col-0*). Gene expression studies were performed with Arabidopsis gene-specific primers for the defense-related genes at 12 or 24 or 48 h after challenge inoculation with SCC1 (Fig. 5). The *PR-1a* gene was up-regulated in wild type (*Col-0*) by treatment with fusaricidin at 1.0 ppm concentration at 24 h, while there was minor expression in the transgenic line (*nah-G*) at the same time, and no expression at 12 and 48 h after pathogen challenge, which implies that the salicylic acid (SA)-dependent pathway was elicited by fusaricidin treatment in *Arabidopsis* plants. This molecular evidence of fusaricidin (1.0 ppm)-mediated ISR was supported by Fig. 4, where the same level of treatment induced the suppression of disease development of SCC1 in tobacco plants. Collectively, the disease suppression by treatment with fusaricidin derived from E681 might be due to systemic resistance rather than to direct antagonism.

Discussion

In the present study, we demonstrated the potential of fusaricidin for suppressing the disease development of Phytophthora blight caused by *P. capsici* in red-pepper plants in vitro and under greenhouse conditions as well. Importantly, the MICs of the cyclic depsipeptide fusaricidin has been found similar to or better than those of previous antifungal proteins and peptides (De Lucca *et al.* 2005; Diz *et al.* 2006). Plant protection by the elicitation of ISR has been successfully demonstrated in many crops by treatment with various biocontrol agents against major pathogens. A research effort all over the world in this direction has led to successful development of biotic and abiotic agents that can protect the plant either directly by antagonism mechanisms or indirectly through induced systemic resistance in host plants against invading pathogens. Some of the classical examples of chemical inducers for innate improvement in host defense are 2,6-dichloroisonicotinic acid, benzothiadiazole, methyl jasmonate and probenazole (Von Rad *et al.* 2004). The results demonstrated that the fusaricidin tested in the present investigation effectively inhibited *P. capsici* and SCC1 infections in red-pepper and tobacco, respectively. This is the first report on the cyclic depsipeptide antibiotic fusaricidin derived from *Paenibacillus polymyxa* E681 (E681) in suppression of disease development through ISR in plants. Previously, aminoglycoside antibiotic compounds had been reported to reduce the intensity of disease caused by oomycetes (Xiao *et al.* 2002), including *P. infestans* (Lee *et al.* 2005), The commercial aminoglycoside antibiotics such as neomycin, ribostamycin, streptomycin, including fusaricidin, were tested against *P. infestans*. In

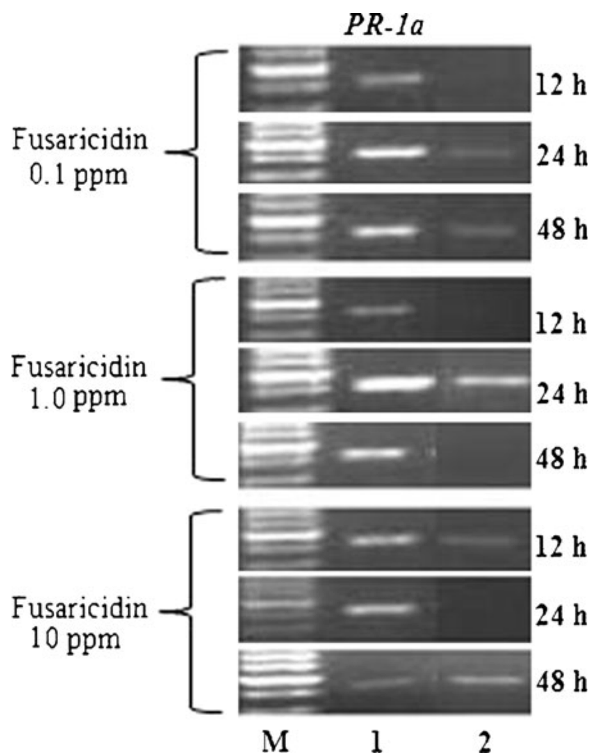


Fig. 5 Elicitation of defense-related gene expression in *Arabidopsis thaliana* wild type, *Col-1* (*Lane 1*), and transgenic line, *nahG* (*Lane 2*), by treatment with fusaricidin at different concentrations (0.1, 1.0 and 10 ppm). The expression of pathogenesis-related (*PR*) gene *PR-1a* analyzed by RT-PCR was examined at 12 or 24 or 48 h after challenging with pathogen. Amplified products were separated by gel electrophoresis and visualized by ethidium bromide staining. The experiment was conducted two times with similar results. *M*=100 bp DNA ladder

addition, recently the role of hyaluronic acid from *Streptomyces* spp. as a potential ISR agent in cucumber and tomato plants against major economically important diseases has been established by Park *et al.* (2007). Previously Kajimura & Kaneda (1995) also discussed the isolation, structure and biological activity of fusaricidin from *P. polymyxa*. The present study demonstrated the potential of biochemical agent fusaricidin for the induced protection of red-pepper from *P. casici*. The fact that there was greater disease suppression of Phytophthora blight on treatment with fusaricidin by soil drench compared with foliar spray implies that the mechanism of disease suppression is through systemic rather than direct antagonism. This also suggests that soil application of fusaricidin at 0.1 ppm is an ideal dosage for triggering the host defense in red-pepper plants.

A naturally soilborne bacterium, *P. polymyxa,* possesses several desirable properties as a biocontrol active agent against plant-pathogenic fungi, and exhibits resistance to several fungicides and herbicides approved for use on canola crops in Canada (Ramarathnam & Fernando 2006). Strains of *P. polymyxa* have also been shown to produce a wide range of antibiotic peptides, which may give them a growth advantage in the competitive soil environment. Their antagonistic activity against fungi and gram-positive bacteria, and the fact that they have no effect on gram-negative bacteria, distinguishes this second group of peptides from the first group (Katz & Demain 1977). The fusaricidin group of antibiotics seems to be less diverse as compared with the Polymyxin-Colistin-Circulin group. However, both groups still lack complete structural and molecular characterization. The occurrence of Phytophthora isolates resistant to metalaxyls and oxadixyls in field crops has posed new challenges to manage this important group of plant pathogens causing significant yield loss in several crop species (Parra & Ristaino 2001). The extensive and indiscriminate use of metalaxyl has led to the rapid development and widespread distribution of metalaxyl-resistant strains of *Phytophthora* spp. throughout the world (Deahl *et al.* 1993; Goodwin *et al.* 1996). The occurrence of metalaxyl resistance to *Phytophthora* species in potato and pepper fields has resulted in devastating late blight problems due to the failure of disease control in most of the production areas (Parra & Ristaino 2001; Ristaino & Johnston 1999).

Thus, this study brought out the importance of antifungal and anti-oomycete activity of fusaricidin, exhibiting induced protection from Phytophthora leaf blight of red-pepper and soft rot of tobacco through systemic resistance which can be used in large-scale field application in the future. Thus, fusaricidin plays a significant role as an effective ISR elicitor against Phytophthora blight of red-pepper. Earlier reports documented only the antifungal activity of fusaricidin against this major group of plant pathogens (Choi *et al.* 2004; Kajimura & Kaneda 1995), but this study identified the role of fusaricidin as one of the potential ISR elicitors against Phytophthora leaf blight pathogen. Molecular evidence of *PR* gene expression demonstrated that the soil drench of fusaricidin was effective in establishing the systemic resistance in leaves of activation of the defense gene in transgenic *Arabidopsis* treated with fusaricidin, and proved that

fusaricidin activated the defense genes to protect the plants from *P. capsici* through ISR. Based on our findings, the E681 derivative, cyclic depsipeptide fusaricidin, can play a role in inducing resistance for protecting plants against invading plant pathogens in red-pepper plants. Thus, it might serve as an alternative approach to chemical fungicides, and for investigating the biochemical changes in plant responsiveness to pathogen challenge.

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