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Screening Anti-Southern Rice Black-Streaked Dwarf Virus Drugs Based on S7-1 Gene Expression in Rice Suspension Cells

Dandan Yu,[‖] Zhenchao Wang,[‖] Jing Liu, Mingming Lv, Jiaju Liu, Xiangyang Li, Zhuo Chen, Linghong Jin, Deyu Hu, Song Yang, and Baoan Song*

Supporting Information

ABSTRACT: Southern rice black-streaked dwarf virus (SRBSDV) is a rice pathogen that had an outbreak in southern China in 2010 and caused significant crop losses. Therefore, screening for effective antiviral drugs against SRBSDV is very important. This study used rice suspension cells infected with SRBSDV by polyethylene glycol-mediated uptake for screening antiviral drugs. SRBSDV P7-1, which is coded by the S7-1 gene, has an intrinsic ability to self-interact to form tubules that play an important role in viral infection. Therefore, relative expression level of the SRBSDV S7-1 gene in infected rice suspension cells was assayed by real-time quantitative polymerase chain reaction to evaluate the antiviral activities of various drugs. Dufulin displayed the highest inhibitory activity against SRBSDV S7-1 expression. In addition, changes in peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) activities were determined in inoculated and noninoculated cells. The results showed that both POD and PPO activities increased upon dufulin treatment. Furthermore, the validity of this approach was confirmed in an in vivo experiment in which dufulin was found to effectively inhibit SRBSDV.

KEYWORDS: *SRBSDV, rice suspension cells, S7-1 gene, drug screening, dufulin*

INTRODUCTION

Southern rice black-streaked dwarf virus (SRBSDV), a new member of the genus *Fijivirus* of the family Reoviridae, is a nonenveloped dsRNA virus that has caused significant loss of grain yield in parts of Asia. [1] It was first observed in Guangdong province in China in 2001 and identified in 2008.[2] In recent years, the disease has spread rapidly throughout southern Asia, including Vietnam, Japan, and China.[3,4] SRBSDV is transmitted by white−black planthoppers (WBPH; *Sogatella furcifera* Horváth) in a persistent−propagative manner. SRBSDV is not mechanically transmissible and is currently one of the most damaging rice crop diseases in China. Since 2009, SRBSDV has been causing significant rice yield losses in China. Midlate rice crops are more seriously affected than early-season crops in most districts.[5−7] Approximately 741,000 acres of rice have been infected by SRBSDV in China, and 100,000 acres of rice farm reported crop failure in 2009.[8] In 2010, nearly 2.97 million acres of rice were infected by SRBSDV in China.[9] Thus, SRBSDV poses a significant threat to adequate grain production in the region. Although preventive measures against SRBSDV are being actively researched, no effective model for screening anti-SRBSDV drugs is available to date. Therefore, establishing a rapid, effective, and accurate model for screening anti-SRBSDV drugs is an urgent need.

The icosahedral particles of SRBSDV are 70−75 nm in diameter. The SRBSDV genome contains 10 segments named S1 to S10 in decreasing order of molecular weight.[10] SRBSDV encodes at least five putative structural proteins (P2, P3, P4, P8, and P10) and six putative nonstructural proteins (P1, P6, P7-1, P7-2, P9-1, and P9-2).[3] Molecular characterization of segments S7−S10 of a SRBSDV isolate from maize in northern China showed that Shandong is the northernmost region where SRBSDV is found in China.[11] SRBSDV P9-1 is a component of the viroplasm matrix, which is a putative site of viral replication.[12] SRBSDV P7-1 has an intrinsic ability to self-interact to form tubules along the cell surface, which is important for intercellular movement of the virus in plant cells.[13]

Several groups have used dot enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and reverse transcription loop-mediated isothermal amplification assay to diagnose SRBSDV infection.[14,15] Previously, studies have also used real-time (RT)-PCR to determine the transmission rates of SRBSDV among different host plant species under different temperatures and the transmission characteristics of SRBSDV by rice planthoppers.[16,17] However, screening of anti-SRBSDV agents has not been conducted to date.

Using plant cells or protoplasts to research plant viruses requires advanced technical expertise. Several groups have studied tobacco protoplasts or tobacco cells infected with tobacco mosaic virus, tobacco mosaic virus RNA, cucumber mosaic virus, and cucumber mosaic virus RNA using liposome or poly-L-ornithine and by conducting electroporation.[18−22] Yang et al.[23] used rice suspension cells to study the antiviral activity of *Ailanthus altissima* crude extract in rice strip virus.

To date, applying chemical pesticides has been the main approach for controlling WBPH. Although several pesticides are effective against SRBSDV, the combination of 25% pymetrozine−thiamethoxam SC and 30% dufulin WP is the most effective treatment for controlling SRBSDV in field trials.[24] The growing realization of SRBSDV as a major rice pathogen has lately driven renewed efforts to screen and develop effective antiviral agents.

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Table 1. Primers for S7-1 in SRBSDV and 18S rRNA in Rice

gene	forward primer 5′−3′	reverse primer 5′−3′
S7-1	AAACGAAATACAAGAAATGAGAAT	ACTACAACTAACGGAACTGA
rice 18S rRNA	ATGGTGGTGACGGGTGAC	CAGACACTAAAGCGCCCGGTA

This paper presents an in vitro method for testing antiviral compounds against SRBSDV based on rice suspension cells. PCR was used to test whether the inoculation procedure was successful. By determining the relative gene expression level of SRBSDV S7-1 in rice suspension cells by quantitative (q)-PCR, moroxydine hydrochloride, ningnanmycin, amino oligosaccharin, dufulin, and some traditional antiviral drugs were evaluated for their ability to inhibit SRBSDV replication. Additionally, changes in peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) activities were determined. To confirm the accuracy of this model, an in vivo experiment was carried out. Our results demonstrate that the new screening method can be used to screen for potential antiviral agrochemicals. To the best of our knowledge, this is the first report on a screening method for new drugs that show antiviral activity against SRBSDV.

MATERIALS AND METHODS

Cells, Virus, and Drugs. Rice suspension cells were derived from germinal vesicles of *Oryza sativa* ssp. *japonica*. Nipponbare was grown in N6 medium (pH 5.8) containing 2 mg/L 2,4-D and 2 g/L sugar at 30 °C and 80 rpm. SRBSDV was extracted with 0.4 M PBS (pH 5.8) from freshly infected rice or maize that had obvious tumor-like protrusion symptoms and then passed through a 0.22 μm filter. To maintain the equivalent incubation quantity, SRBSDV extracts were diluted until the absorption of OD_{490} was determined to 2.0 by using an indirect ELISA method. The drugs used were dufulin, ningnanmycin, amino oligosaccharin, moroxydine hydrochloride, GU188, NK007, Harpin Ea, lentinan, and 14-aminoantofine. All drugs were dissolved in 1‰ Tween-20 or first dissolved in dimethyl sulfoxide and then diluted with 1‰ Tween-20. The concentration of the N6 medium containing the drug was 500 μg/mL.

Cell Viability Assay with Dufulin and Ningnanmycin. Rice suspension cells were treated with dufulin and ningnanmycin separately at 500 μg/mL concentration. After incubation for 14, 20, 24, 28, 36, and 45 h, 900 μL of the rice suspension cells was treated with 100 μL of 0.4% Trypan blue for about 1 min for viewing under an inverted biological microscope. Dead cells were identified as blue spots, whereas viable cells were colorless and transparent. Cell activity was calculated as shown in eq 1:

$$\text{cell activity} = 1 - (\text{dead cells}/\text{total cells}) \times 100\% \quad (1)$$

SRBSDV-Inoculated Rice Suspension Cells. Rice suspension cells were inoculated with SRBSDV as described by Yang et al. with some modifications.[23] The most significant change was the PEG solution and 40% PEG6000 including 0.1 M $CaCl_2$ was used. Our modified protocol utilized 0.2 g of rice suspension cells in 15 mL tubes, which were pelleted at 100 *g* for 3 min. The supernatant was removed, and the cells were placed on ice. Then, 500 μL of fresh N6 medium and 800 μL of SRBSDV extraction were sequentially added and mixed. A total of 500 μL of PEG solution was immediately added, mixed, and vortexed for 25−30 s. The mixture was placed at room temperature for 40 min. The cells were washed twice with sterile water and then washed once with N6 medium. Finally, the cells were cultivated in fresh medium or fresh medium containing drug.

Fluorescent Antibody Staining of Rice Suspension Cells. Four percent paraformaldehyde-fixed rice suspension cells were treated with 0.3% Triton X-100 for 10 min and washed three times with PBS. Then, the cells were incubated with 40 μL of 1:1500 dilution of SRBSDV P9-1 antiserum for 1 h at 37 °C. The rice suspension cells were washed, covered with 20 μL of 1:100 dilution of FITC-conjugated sheep anti-rabbit serum for 1 h at 37 °C in a dark place, and mounted in 50% glycerol. A total of 40−60% cells was counted under fluorescence microscopy.

Indirect ELISA and Western Blot Analysis. Total protein extraction from cells was performed as previously described.[25] Protein extracts were subjected to SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes. The membranes were blocked for 40 min with 5% skim milk solution in blocking buffer (TBST). Then, the membranes were incubated with a 1/1500 dilution of SRBSDV P9-1/P10 antiserum for 1 h at 37 °C, washed, and covered with a 1/30000 dilution of alkaline phosphatase-conjugated sheep anti-rabbit IgG for 1 h at 37 °C. After three additional washing steps of 3 min each, the membranes were stained for approximately 10 min using NBT and BCIP, and the reaction was stopped by washing the blots in distilled water. HRP-conjugated sheep anti-rabbit IgG was used for indirect ELISA.

RNA Extraction, cDNA Synthesis, PCR, and qPCR Analysis. At 0, 14, 20, 24, 28, 36, and 45 h post inoculation, total RNA was extracted using the TRIZOL reagent kit (TakaRa, Dalian, China). OD_{260} and OD_{280} of the RNA extracts were determined using an ultraviolet spectrophotometer, and total RNA purity was estimated by calculating OD_{260}/OD_{280}. All OD_{260}/OD_{280} values of RNA were between 1.8 and 2.2. The concentration of total RNA was calculated according to the dilution ratio and the value of OD_{260}.

cDNAs were synthesized using a cDNA synthesis kit (TakaRa). H_2O was added up to 14.25 μL to the solution containing 2000 ng of RNA and 2 μL of oligo d(T) and heated to 70 °C for 10 min. Then, the solution was rapidly placed on ice for 2 min. Up to 4 μL of MLV buffer, 1 μL of 10 mM each dNTP, 0.25 μL of RRI, and 0.5 μL of MLV were added. The reaction mixture was heated to 42 °C for 1 h and to 70 °C for 15 min.

PCR amplifications were carried out in a final volume of 25 μL containing 5 μL of cDNA, 2.5 μL of 10× PCR buffer, 1.5 μL of $MgCl_2$, 2 μL of 0.25 mM each dNTP, 11.8 μL of H_2O, 0.2 μL of Taq DNA polymerase, and 2 μL of primer pair. S7-1 primers were used for detection. The amplification condition was 5 min at 94 °C and 30 cycles of 30 s at 94 °C (denaturation), 30 s at 55 °C (annealing), and 2 min at 72 °C (extension) followed by 10 min at 72 °C. The whole PCR reaction volume was electrophoresed for 40 min onto 2% agarose gel in TAE buffer with 0.2 μg/mL ethidium bromide.

RT-qPCR was carried out using SYBR *Premix Ex Taq*^{II} (TaKaRa). The reaction solution contained 10 μL of SYBR, 1.6 μL of primer pair, 2 μL of cDNA, and 6.4 μL of H_2O. The gene expression level was quantified in relation to expression of rice 18S rRNA (GenBank accession no. AK059783). The PCR cycle consisted of the following steps: 30 s at 95 °C and 40 cycles of 30 s at 95 °C and 30 s at 60 °C.

Primers were synthesized on the basis of the SRBSDV dsRNA segment 7-1 and rice 18S rRNA by Sangon (Shanghai, China). Sequences of the primers used are shown in Table 1.

Determination of POD, PPO, and PAL Extracts from Rice Suspension Cells and Rice Tissues. The inoculated or non-inoculated rice suspension cells were used to determine the response of POD, PPO, and PAL to 500 μg/mL dufulin. The rice suspension cells were collected 48 h post inoculation, washed with sodium phosphate buffer (0.01 M, pH 5.8), and ground to a fine powder with liquid nitrogen. Then, the cells were homogenized in 2 mL of sodium phosphate buffer (0.01 M, pH 5.9) and centrifuged at 12000 *g* for 15 min at 4 °C. The supernatant was divided into two parts. The first part was used for protein determination, and the other part was used for POD and PPO determination. The buffer used in PAL determination was sodium borate buffer (0.1 M, pH 8.8, containing 5 mM β-mercaptoethanol and 5% polyvinylpyrrolidone). Protein determination was conducted according to the method described by Bradford.[26] Detection of POD, PPO, and PAL was done as described by Liu et

al.[27] Enzymatic activity unit was defined as the increment of an absorbance change unit per minute. The absorbance change units of POD, PPO, and PAL were 0.1 OD_{470}, 0.01 OD_{495}, and 0.01 OD_{290}, respectively. Responses to dufulin in the in vivo experiments were as follows. A total of 15 single rice seedlings were transplanted after soaking in 500 μg/mL dufulin for 10 s, and another 15 single rice seedlings that were not treated with dufulin were used as controls. Dufulin (500 μg/mL) was sprayed on all seedlings in the former group every 3 days. At 9 days post transplantation, POD, PPO, and PAL responses were determined as described before, and the quality of sampling was adjusted to 2 g.

In Vivo Assay for the Action of Dufulin against SRBSDV. To confirm the effect of dufulin against SRBSDV, an in vivo experiment was carried out. A single rice seedling was grown in a tube, and SRBSDV was transmitted by WBPH, which had the virus for 24 h. After 24 h, 75 seedlings were immediately transplanted in an insect-proof net. Another 75 seedlings were soaked in 500 μg/mL dufulin for 10 s and then transplanted. Dufulin (500 μg/mL) was sprayed on the seedlings every 10 days. At 30 days post transplantation, the virus-carrying rates were tested by PCR.

RESULTS AND DISCUSSION

Cell Viability Assay with Dufulin and Ningnanmycin. To develop an antiviral screening model, no toxicity to suspension cells must be ensured. We conducted cell viability assays for two classical antiviral agents, namely, dufulin and ningnanmycin (500 μg/mL), to determine their toxic effects. At 14, 20, 24, 28, 36, and 45 h, cells treated with dufulin showed viabilities of 100, 93.36, 97.13, 100, 100, and 97.9%, whereas those treated with ningnanmycin had viabilities of 97.14, 100, 92.55, 98.18, 96.36, and 100%, and cells in the control group exhibited viabilities of 100, 96.03, 86.53, 93.33, 96.67, and 95.79, respectively (Figure 1). Thus, we found no evidence of toxic effects of these antiviral drugs on the suspension cells.

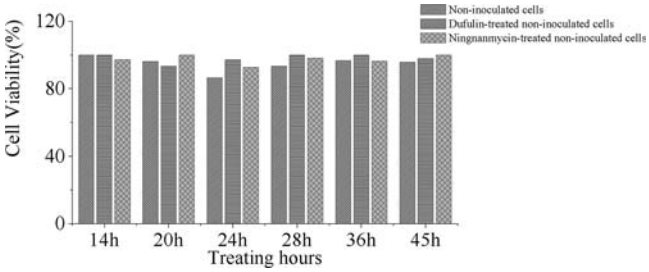

Figure 1. Cell viability assay with dufulin and ningnanmycin.

Efficiency of Inoculation Determined by Cell Immunofluorescence and Western Blot. The proportion of infected cells was determined by staining the samples with antibodies. Our immunofluorescence data indicated that the cells could express viral antigens. Cells showed fluorescence spots upon staining with P9-1 antibody and fluorescein isothiocyanate (FITC) IgG 12−48 h post inoculation with SRBSDV (Figure 2). Similar inclusion body structures were observed under white light. A comparison between the fluorescence of SRBSDV infected and uninfected cells showed that the controls with mock-inoculated cells had no specific fluorescence spots. The proportion of infected cells, estimated as the percentage of cells that showed strong fluorescence spots, was about 40−50%. This proportion increased as the culture time increased. The results showed that SRBSDV can be inoculated in rice cells using polyethylene glycol (PEG). Western blot assays using P9-1 antibody showed that the inoculated rice cells can express SRBSDV P9-1 (Figure 3).

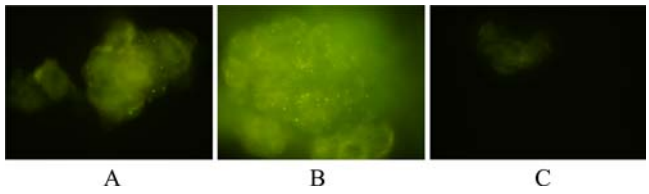

Figure 2. Immunofluorescence staining with anti-SRBSDV serum p9-1 antibody and FITC-labeled sheep anti-rabbit serum: (A) images of cells 12 h post inoculation with SRBSDV; (B) images of cells 48 h post inoculation with SRBSDV; (C) negative control, cells without inoculation but with SRBSDV. The fluorescence spots indicate P9-1 in the virus.

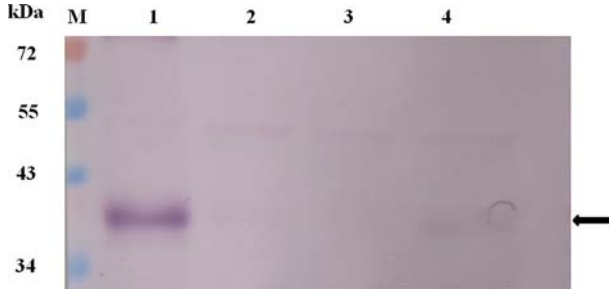

Figure 3. Western blot assay for protein extraction from inoculated rice cells. Lanes: M, prestained protein marker; 1, positive control; 2, protein extraction from rice cells 12 h post inoculation; 3, protein extraction from rice cells treated with dufulin 48 h post inoculation; 4, protein extraction from rice cells 48 h post inoculation.

Screening Drugs Inhibit SRBSDV Replication. Cell-to-cell movement is necessary for the propagation of viral infection in host plants and vectors. Viral infection is limited to the initially infected cell, and systemic disease does not exist in the absence of viral movement. Tubule-forming proteins of plant viruses are usually important in assisting intercellular or cellular transport of viral particles.[28] *Phytoreovirus* and *Fijivirus* are characterized by tubular structures. The rice dwarf virus (*Phytoreovirus*) Pns10 is involved in the formation of tubular structures containing viral particles that enhance viral movement among neighboring insect cells.[29] RBSDV P7-1 (*Fijivirus*) is also a major component of tubules in plant and insect vectors.[30] Furthermore, SRBSDV P7-1 coded by S7-1 has the intrinsic ability to self-interact to form tubules, which have important functions in viral infection.[13] Therefore, we sought to determine the relative expression level of the SRBSDV S7-1 gene in rice suspension cells to evaluate antiviral activity.

The level of S7-1 expression in rice suspension cells without drug treatment was not zero at 0 h post inoculation, indicating that SRBSDV was successfully inoculated. S7-1 expression was low at 0 h and then increased to a maximum at 20 h. However, S7-1 expression rapidly decreased from 20 to 45 h. One explanation is that low S7 gene expression level due to the poor growth status of the rice suspension cell along with increasing virus levels and decreasing nutrient level may have contributed to the sharp decline in cell viability, especially after 24 h (Figure 4).

As in the "inoculation without dufulin treatment" group, virus proliferation occurred in the treatment group "treated with dufulin post inoculation" between 0 and 45 h. However, the proliferation peak occurred at 20 h in the former but at 24 h in the latter group. Virus proliferation decreased gradually thereafter (Figure 4).

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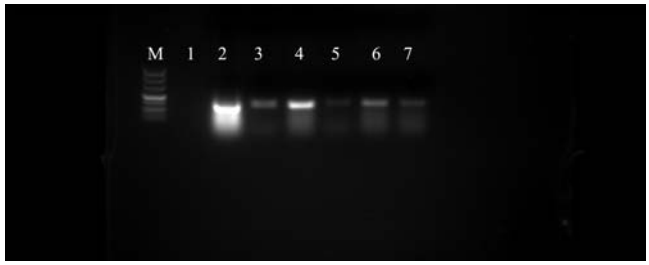

Figure 4. PCR analysis of cells inoculated or not with SRBSDV. Lanes: M, DL500 marker; 1, negative control 1; 2, inoculation 1; 3, inoculation 2; 4, inoculation 3; 5, dufulin-treated 1; 6, dufulin-treated 2; 7, dufulin-treated 3.

One explanation for these observations is that in the treatment group "treated with dufulin post inoculation", SRBSDV proliferation at 0−20 h was initially inhibited by increased activities of defense enzymes owing to dufulin and weak toxicity of the organic solvent DMSO. However, SRBSDV proliferation from 20 to 24 h was rapidly accompanied by adaptability of the treated cells to the mild cytotoxic effects of the organic solvent DMSO, such that the maximum level of virus proliferation was greater than that of the treatment group "inoculation without dufulin treatment" at 24 h. This mechanism of virus proliferation in the presence of dufulin may explain why S7-1 expression ratio was somewhat higher than that without drug at 24 h post inoculation in Figure 5.

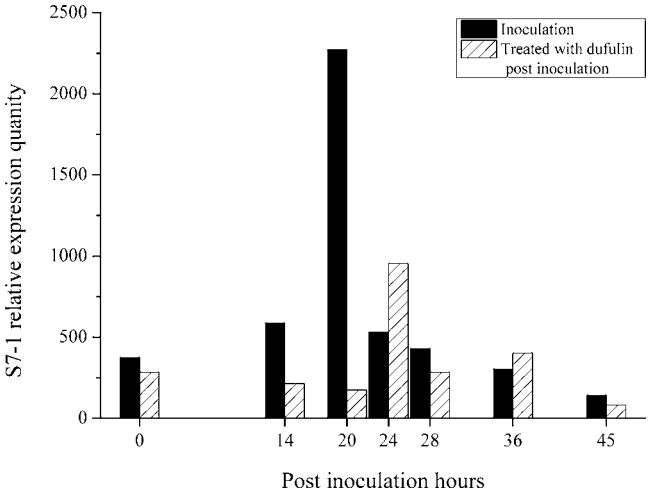

Figure 5. Effect of dufulin on inhibition of the SRBSDV S7-1 levels in rice suspension cells infected with SRBSDV at different times; real-time PCR detection of S7-1 in cells treated with dufulin in comparison with untreated cells. Cells were treated with dufulin after infection with SRBSDV.

Then, the defense activity peaked from 24 to 28 h, and S7 gene expression level was further inhibited by dufulin. However, from 28 to 36 h, with the activity of defense enzymes being decreased, the inhibitory effect against SRBSDV might have weakened, resulting in a gradual increase in the replication level of SRBSDV and S7 gene expression level during this period. Finally, 36 h after inoculation of SRBSDV, S7 gene expression level must have decreased again due to depletion of the nutrient and cellular material of the rice suspension cells. To confirm the specific inhibition of SRBSDV by drugs, accumulation of the SRBSDV S7-1 gene in cells treated with or without drugs was compared by qPCR. Figures 4 and 5 show that the level of S7-1 expression decreased significantly with dufulin treatment in cells 12 h after SRBSDV infection. S7-1 expression was highest at 20 h, after which it rapidly decreased. After 45 h, a decrease in S7-1 level in SRBSDV was observed in the treated cells. Dufulin exhibited the highest inhibitory activity against SRBSDV S7-1 expression, which was reduced to approximately 95.12% after 20 h. The inhibitory effects of amino oligosaccharin, ningnanmycin, and lentinan were 84.84, 84.72, and 83.16%, respectively. Moroxydine hydrochloride and Gu188 exhibited weak inhibition. Table 2 shows the inhibition

Table 2. Inhibitory Effects of Drugs on SRBSDV S7-1 Gene Expression

drug	inhibition (%)	drug	inhibition (%)
dufulin	95.12	NK007	66.77
amino oligosaccharin	84.84	moroxydine hydrochloride	28.72
ningnanmycin	84.72	GU188	26.38
lentinan	83.16	tylocrebrine	0
Harpin Ea	76.31		

rates of the different drugs we tested. Inhibition was calculated as shown in eq 2:

$$\text{inhibition} = (\text{S7-1 expression of inoculation cells} - \text{S7-1 expression of inoculation cells treated with drugs})/\text{S7-1 expression of inoculation cells} \quad (2)$$

Determination of POD, PPO, and PAL Activities. POD, PPO, and PAL activities of the treatment groups of the noninoculation suspension cells with or without dufulin were 180.6015 or 136.9932 U/mg·min (POD), 8.7388 or 3.5126 U/mg·min (PPO), and 12.6381 or 1.8516 U/mg·min (PAL), respectively (Figure 6). Moreover, POD, PPO, and PAL activities in the treatment groups of rice tissues with or without dufulin were 4887.9816 or 3933.5124 U/mg·min (POD), 10.7075 or 8.2464 U/mg·min (PPO), and 21.0941 or 18.5780 U/mg·min (PAL), respectively (Figure 7). Obviously, the activities of POD, PPO, and PAL were increased upon treatment with dufulin, especially in the in vivo experiment.

POD and PPO activities of inoculation cells treated with dufulin increased by approximately 1.7 times compared to those of cells inoculated only with SRBSDV (Figure 8). The POD activities of inoculated cells, dufulin-treated inoculated cells, and noninoculated cells were 182.71, 309.93, and 820.39 U/mg·min, respectively. POD is an oxidoreductase that is involved in a variety of cellular processes, such as cell wall biosynthesis, and functions in the containment of pathogens.[31] Inoculation of SRBSDV in rice cells resulted in a rapid decrease in POD activity and escape from the cells' self-defense mechanisms. Dufulin may increase POD activity in cells to protect against SRBSDV. PPO is an oxidase that catalyzes the synthesis of lignin and quinonoid compounds, which form a protective barrier to resist pathogen infection. The PPO activities of inoculated cells, dufulin-treated inoculated cells, and noninoculated cells were 2.8, 4.8, and 1.8 U/mg·min, respectively. Dufulin may also increase PPO activity against SRBSDV invasion in cells. PAL is a key enzyme in phenylpropanoid metabolism that has an important function in disease resistance.[32] PAL activity increases to resist pathogen

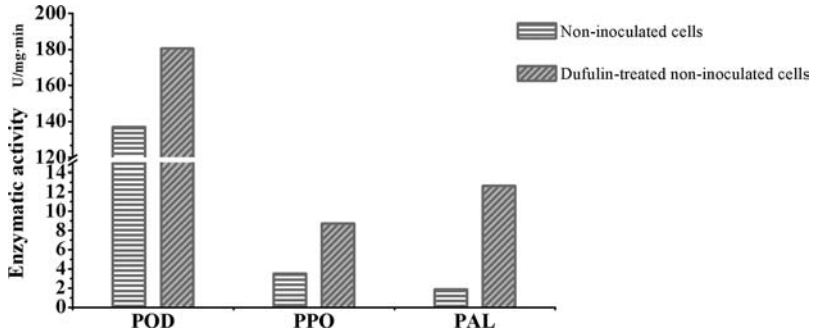

Figure 6. Activities of POD, PPO, and PAL in noninoculated rice suspension cells with or without dufulin.

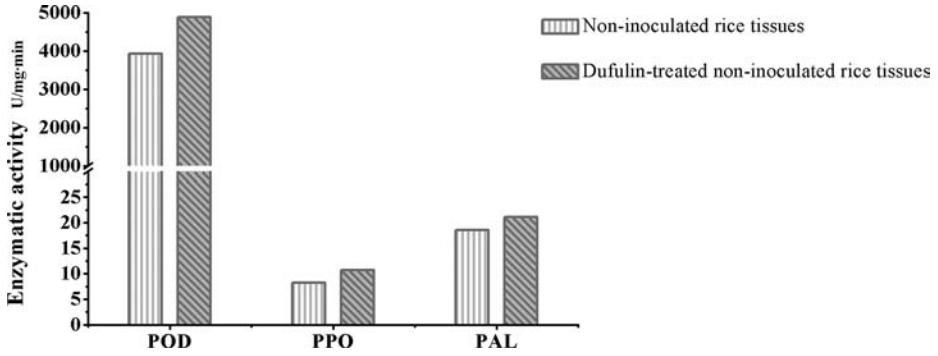

Figure 7. Activities of POD, PPO, and PAL in noninoculated rice tissues with or without dufulin.

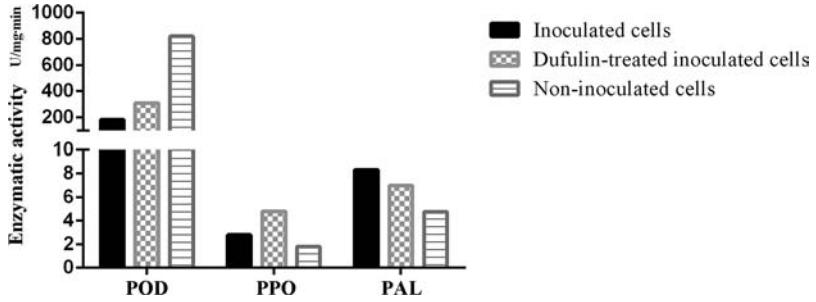

Figure 8. Activities of POD, PPO, and PAL in inoculated rice suspension cells with or without dufulin.

invasion. In this study, the PAL activities of inoculated cells, dufulin-treated inoculated cells, and noninoculated cells were 8.34, 6.98, and 4.74 U/mg·min, respectively. PAL activity in cells increased after SRBSDV inoculation. The changes in POD, PPO, and PAL activities demonstrated that dufulin is effective in inhibiting SRBSDV in rice cells.

The suppression of SRBSDV S7-1 by dufulin may be attributed to its cytotoxic effects. However, the change in S7-1 gene expression at different times in rice suspension cells treated with dufulin indicated that the cells were alive. Both POD and PPO activities increased after dufulin treatment to protect against SRBSDV, indicating that dufulin was effective. Likewise, PAL activity increased in cells after SRBSDV inoculation, which verified that the inoculation was successful.

Studying the Effect of Dufulin on SRBSDV by in Vivo Experiments. Figure 9 shows that 20 days post transplantation, the virus-carrying rate of rice was 40% and the dufulin treatment rate was 15%. Inhibition of dufulin against SRBSDV infection in vivo was 62.5%. After 45 days, the rice infected with SRBSDV showed obvious symptoms, including dwarf wrinkled leaves, as shown in Figure 10.

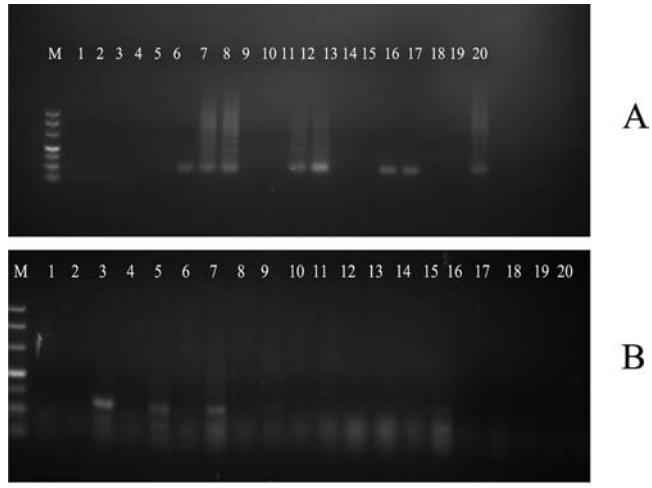

Figure 9. Virus carrying rates: (A) rice seedlings not treated with dufulin; (B) rice seedlings treated with dufulin.

Results of the in vivo experiments show that dufulin is an effective drug for controlling SRBSDV and also indicate that

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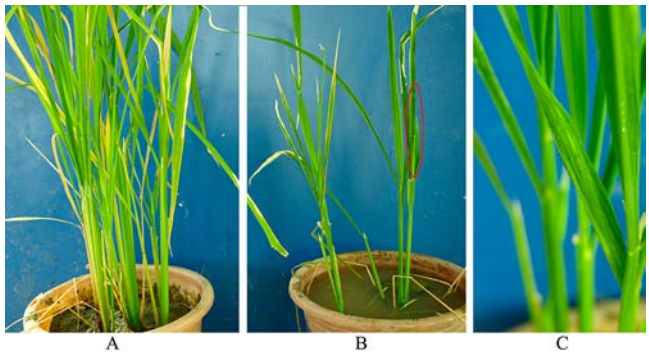

Figure 10. Comparison of rice infected with SRBSDV: (A) rice treated with dufulin; (B) rice not treated with dufulin showing wrinkled leaves; (C) wrinkled leaves marked in panel B.

screening anti-SRBSDV drugs based on S7-1 gene expression in rice suspension cells can be an effective approach. The inhibition rate of SRBSDV infection was 62.5% in vivo. The fact that we observed an inhibition of SRBSDV proliferation rate of 95.12% in rice cells indicated that screening of antiviral drugs at the gene level can prevent failure. Various factors may account for a systemic response in plants in an in vivo experiment. Thus, the high efficacy of dufulin may be due to the single-factor condition in rice cells.

In summary, our study aimed to screen for effective antiviral agents against SRBSDV in rice. The relative expression level of the SRBSDV S7-1 gene in rice suspension cells was utilized to evaluate the antiviral activity of several drugs by qPCR assays. This methodology is generally applicable in screening for antiviral drugs and offers several advantages over other conventional methods, including short duration, quantitative measurement, and easy standardization.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Information on the materials and methods of electrophoresis, western blotting, and determination of enzyme activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

SRBSDV, southern black streak dwarf virus; WBPH, white−black planthoppers; POD, peroxidase; PPO, polyphenol oxidase; PAL, phenylalanine ammonia-lyase; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; RT-qPCR, real-time quantitative polymerase chain reaction; HRP, horseradish peroxidase; FITC, fluorescein isothiocyanate; OD, optical density; cDNA, complementary DNA; RRI, RNase inhibitor; TBST, Tris-buffered saline Tween-20; PEG, polyethylene glycol; NBT, nitro blue tetrazolium; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; IgG, immunoglobulin G; S7-1, strand 7-1; P9-1, protein 9-1

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