

# Direct suppression of a rice bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) by monoterpene (*S*)-limonene

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**Abstract** Rice bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a severe disease of rice plants. Upon pathogen infection, rice biosynthesizes phytoalexins, including diterpenoids such as momilactones, phytocassanes, and oryzalexins. However, information on headspace volatiles in response to *Xoo* infection is limited. We have examined headspace volatile terpenes, induced by the infection of *Xoo*, and investigated their biological roles in the rice plant. Monoterpenes  $\alpha$ -thujene,  $\alpha$ -pinene, sabinene, myrcene,  $\alpha$ -terpene, and (*S*)-limonene and sesquiterpenes cyclosativene,  $\alpha$ -copaene, and  $\beta$ -elemene were detected from 1-week-old *Xoo*-infected rice seedlings, by solid-phase microextraction–gas chromatography–mass spectrometry. All monoterpenes were constitutively released from rice seedlings before *Xoo* infection. However, (*S*)-limonene emission was further elicited after exposure of the seedlings to

*Xoo* in coincidence with upregulation of limonene synthase gene (*OsTPS20*) transcripts. Only the stereospecific (*S*)-limonene [and not (*R*)-limonene or other monoterpenes] severely inhibited *Xoo* growth, as confirmed by disc diffusion and liquid culture assays. Rice seedlings showed suppressed pathogenic symptoms suggestive of resistance to *Xoo* infection after foliar treatment with (*S*)-limonene. Collectively, our findings suggest that (*S*)-limonene is a volatile phytoanticipin, which plays a significant role in suppressing *Xoo* growth in rice seedlings.

**Keywords** (*S*)-Limonene · *Xanthomonas oryzae* pv. *oryzae* · Volatile terpene · A disc diffusion assay · Antibacterial activity

## Introduction

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes bacterial blight disease in rice plant, which severely affects rice production (Mew 1987). *Xoo* enters primarily through secretory tissues, such as hydathodes near the leaf tips, and proliferates within the internal tissues of infected plants. Usually, pathogenic symptoms appear at the tillering stage and a reduction in the yield of 10–20 % (as high as 50 % with severe infection) can occur. Occasionally, infection at seedling stage can cause severe pathogenic symptoms, called kresek, resulting in seedling death within a few weeks (Mew et al. 1993).

Plants have evolved mechanisms to produce a variety of secondary metabolites, including terpenoids, in interaction with environmental stimuli (Arimura et al. 2008; Bajpai et al. 2011; Loreto et al. 1998; Pare and Tumlinson 1999). Terpenes are largely classified into monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20), which are synthesized using geranyl diphosphate, farnesyl diphosphate, and geranylgeranyl diphosphate as substrates, respectively. Monoterpenes and diterpenes are mainly produced in the chloroplast via the 2-*C*-methyl-

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D-erythritol-4-phosphate pathway, while sesquiterpenes are synthesized in the cytoplasm through mevalonate-dependent pathway (Bohlmann et al. 1998; Chen et al. 2011; Lichtenthaler 1999; Tholl 2006). The amount and composition of terpenoids vary with plant species, and their production is related to environmental stimuli. For example, jasmonic acid induces the production of monoterpene linalool, which suppresses the growth of *Xoo* in rice in a concentration-dependent manner (Taniguchi et al. 2014a). Similarly, infection by the rice blast fungus *Magnaporthe oryzae* elevated the level of antifungal sesquiterpene  $\beta$ -elemene in rice; however, this terpene is not effective against *Xoo* (Taniguchi et al. 2014b). The fungus also increases the accumulation of diterpene phytoalexins in blast-resistant rice plants, but not in susceptible plants (Hasegawa et al. 2010). Rice plants biosynthesize non-volatile phytoalexins, such as a flavonoid sakuranetin (Kodama et al. 1992) and diterpenoids, including four groups of phytocassanes A–E, oryzalexins A–F, oryzalexin S, and momilactones A and B in response to biotic stresses (Cartwright et al. 1981; Kato et al. 1973, 1994; Koga et al. 1995). These phytoalexins are mainly produced against *M. oryzae* infection, but rice plants also accumulate atypical diterpenoids against bacterial blight *Xoo*, oryzalide-related compounds, which show anti-*Xoo* activity (Kono et al. 1991; Watanabe et al. 1990, 1992). These non-volatile compounds are constitutively produced and induced by *Xoo* infection and thus can be considered phytoanticipins. Recently, we discovered release of a bouquet of terpenes, mainly (*S*)-limonene, from rice exposed to various abiotic stresses, such as H<sub>2</sub>O<sub>2</sub>, UV-B, and  $\gamma$ -ray (Lee et al. 2015). Although (*S*)-limonene has been considered to play a role in rice seedlings as an antioxidant under oxidative environment, little is known of the biological (physiological) functions of this limonene.

The present study demonstrates profiling of headspace volatile terpenes released from the leaves of *Xoo*-infected rice. Biological functions of major *Xoo*-inducing volatiles, such as  $\alpha$ -pinene, limonene, and  $\alpha$ -copaene, were investigated by disc diffusion and liquid *Xoo* culture assays. Antibacterial limonene was specifically analyzed for its enantiomeric type, and only a stereospecific (*S*)-limonene showed highest anti-*Xoo* activity. Biological activity of (*S*)-limonene was further verified by in planta bioassay with its foliar pretreatment, followed by *Xoo* infection. The work provides fundamental understanding of rice-*Xoo* interactions, which can be helpful in developing a treatment method based on plant-derived natural pesticides to control bacterial blights.

## Materials and methods

### Plant materials and reagents

Rice plants (*Oryza sativa* L. spp. Japonica cv. Dongjin) were grown in half strength Murashige and Skoog (MS) solid

media (10 plants/magenta box) in a magenta box [65 mm (L)  $\times$  200 mm (H)  $\times$  65 mm (W)] and maintained at a temperature of 25 °C with a light/dark cycle of 14:10 h. Authentic standards [i.e.,  $\alpha$ -pinene, (*S*)-limonene, and (*R*)-limonene] were purchased from Sigma-Aldrich, USA, and  $\alpha$ -copaene was purchased from ACC Corporation (American Custom Chemicals Corporation, USA).

### Analysis of headspace volatile compounds by gas chromatography–mass spectrometry

Leaves of 1-week-old rice seedlings were inoculated with *X. oryzae* pv. *oryzae* (*Xoo*) in a magenta box and transferred into a 20-mL glass vial (three seedlings/vial) after 3 days. After 1 day of incubation in the vial, headspace volatiles were collected for 60 min at 25 °C, using a 50:30  $\mu$ m divinylbenzene (DVB)/carboxen (CAR)/polydimethylsiloxane (PDMS)-coated solid-phase microextraction (SPME) fiber (StableFlex 24Ga) (Supelco, USA) for gas chromatography–mass spectrometry (GC–MS) (Agilent GC system, 7890A; MSD, 5957C). The GC–MS analysis was performed with a HP-5MS capillary column (0.25 mm i.d.  $\times$  30 m, 0.25  $\mu$ m film thickness) (Agilent Technologies, USA), maintaining a flow rate of helium as the carrier gas at 1 mL/min. The oven temperature was set to rise from 80 °C (3-min holding time) to 150 °C at a rate of 5 °C/min and a thermal gradient of 10 °C/min up to 250 °C and then 20 °C/min until 300 °C (3-min holding time). The conditions of mass spectrometry and chiral GC–MS were set according to Lee et al. (2015). Relative amount of volatiles was estimated in a percentile by comparing its peak area out of total peak area of all terpene volatiles detected. The enantiomeric identification of limonene was determined using an HP-Chiral-20B chiral capillary column (0.25 mm i.d.  $\times$  30 m with a 0.25- $\mu$ m film) (Agilent Technologies, USA). Retention times and mass spectra of authentic (*R*)-limonene and (*S*)-limonene standards were compared with those of a volatile produced from the experimental examinations. GC program was set to a holding time of 3 min at 80 °C, followed by 1 °C/min gradient to 95 and 30 °C/min gradient up to 240 °C (3-min holding time).

### Cultivation of *Xoo*

*Xoo* was cultured in nutrient broth (NB, 0.5 % peptone and 0.3 % beef extract) and a modified Wakimoto's (WF-P) medium (2 % sucrose, 0.5 % Bacto Peptone, 0.05 % calcium nitrate, 0.082 % sodium phosphate, 0.005 % ferrous sulfate, 1.5 % agar) at 28 °C for 48 h (Karagnilla et al. 1973). For a disc diffusion assay, *Xoo* was cultured in NB until optical density at 595 nm (OD)<sub>595</sub>=1.0 and subsequently incubated on the WF-P solid medium.

### Analysis of antibacterial activities of terpene hydrocarbons against *Xoo* (a liquid culture assay)

*Xoo* was cultivated in 10 ml NB for 48 h at 28 °C at a constant agitation speed of 180 rpm. The *Xoo* culture was diluted with NB at a ratio of 1:1 (v/v) ( $OD_{595} \approx 1.0$ ). Each terpene (in various concentrations) was then added into the culture and incubated at 28 °C at an agitation speed of 180 rpm. After 24 h, the optical density of the cultures, in the presence (or absence) of terpene hydrocarbons, was recorded using an Infinite™ M200 microplate reader (Tecan, Switzerland) to evaluate the inhibitory activities of the terpenes against *Xoo*.

### Analysis of antibacterial activities of terpene hydrocarbons against *Xoo* (a disc diffusion assay)

*Xoo* was incubated on the solid WF-P medium at 28 °C for 48 h. A sterilized 8-mm paper disc (Advantec, Japan), saturated with  $\alpha$ -pinene, (*S*)-limonene, (*R*)-limonene, and  $\alpha$ -copaene in various concentrations, was placed in the middle of a medium plate from the beginning of incubation. The radius of

inhibition zone around each disc was measured and recorded after 48 h at 28 °C. The experiments were run in triplicate.

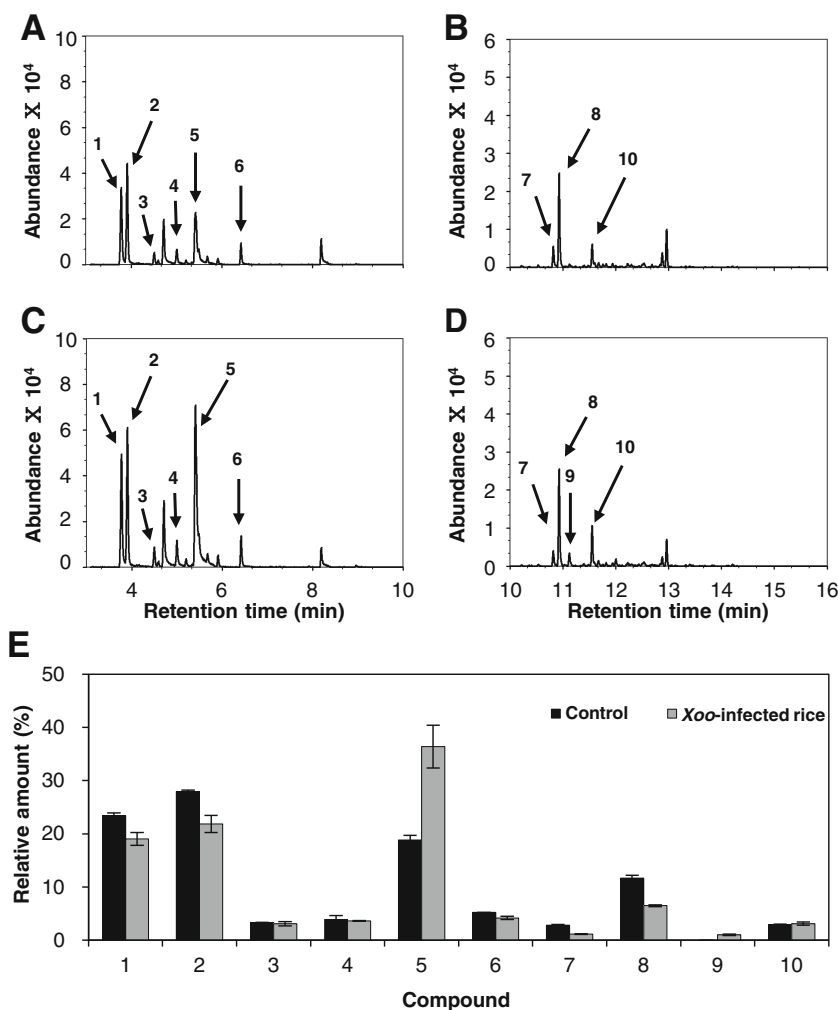
### In vivo leaf infection assay

Limonene solutions in various concentrations (i.e., 0.1, 1, 3, and 5 mM) were sprayed on the leaves of 1-week-old rice seedlings until saturation. After dryness, *Xoo* liquid culture in NB ( $OD_{595} \approx 1.0$ ) was subsequently inoculated on the leaves using the clipping inoculation method (Kauffman et al. 1973). After 1 week of incubation at 25 °C (14:10-h light/dark cycle), the level of in vivo antibacterial activity of limonene was evaluated by comparing the length of blight lesion with that of the control, which involved a spray of 0.01 % ethanol on rice leaves.

### Real-time PCR and quantitative real-time PCR

The total RNA was extracted in accordance with the manufacturer's instruction using an RNeasy Plant Mini Kit (Qiagen,

**Fig. 1** Volatile terpenes from rice seedlings analyzed using SPME–GC–MS [extracted ion chromatograph ( $m/z=93.1$ )]. Monoterpenes from *A* control and *C* *Xoo*-infected rice seedlings, sesquiterpenes from *B* control and *D* *Xoo*-infected rice seedlings, and *E* relative amount of 10 representative volatile terpenes in control and *Xoo*-infected rice seedlings: 1  $\alpha$ -thujene, 2  $\alpha$ -pinene, 3 sabinene, 4 phellandrene, 5 limonene, 6  $\alpha$ -terpinolene, 7 cyclosativene, 8  $\alpha$ -copaene, 9  $\beta$ -elemene, and 10 (*E*)-caryophyllene. Values are means  $\pm$  SE ( $n=3$ )



USA), followed by complementary DNA synthesis using the M-MLV reverse transcriptase (Enzymomics, Republic of Korea). For reverse transcription-polymerase chain reaction (RT-PCR) with *OsTPS20*, 1-week-old seedlings grown in a magenta box were infected with *Xoo* and only the aerial plant parts were collected. *OsACT* (*Os11g0163100*) and *OsUBQ* (*Os01g0328400*) were used as internal control genes. A PCR reaction was performed as follows: 95 °C (3 min), 30 cycles of 95 °C (30 s), 55 °C (30 s), 72 °C (30 s), and 72 °C (10 min). For quantitative RT-PCR, 1-week-old seedlings were foliar sprayed with 5 mM of (*S*)-limonene (Fig. 4c) or were infected with *Xoo* on the leaves (Fig. 4b), harvesting at 24 h or 1 week after the treatments. Filter-sterilized 0.01 % ethanol (*v/v*) was sprayed as a control. The assay was performed using the SYBR premix reagent (Takara, Japan) with the Corbett Rotor-Gene 6000 (Corbett Life Science, Australia). The abundance of transcripts was calculated with  $2^{-\Delta\Delta C_T}$  method using the Rotor-Gene 6000 software pre-installed in the instrument (Livak and Schmittgen 2001). *OsACT* was used as an internal control. The primers used are listed in Table S2. The PCR reaction was carried out as follows: 95 °C (10 min), 40 cycles of 95 °C (10 s), 55 °C (15 s), and 72 °C (40 s), followed by 72 to 95 °C (1 °C/5 s).

### Statistical analysis

Experimental data were analyzed using the Statistical Package for Social Sciences (SPSS)/Predictive Analytics Software (PASW) Statistics version 18 (Chicago, USA). Statistical significance was assessed using Duncan's multiple range test at  $p < 0.01$ .

## Results

### Volatile terpenes from rice seedlings against *Xoo* infection

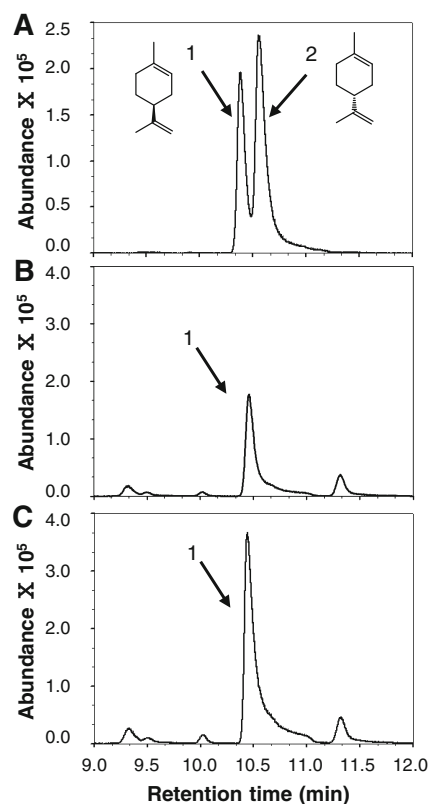
Various terpenes released from 1-week-old rice seedlings infected with *Xoo* are shown in Fig. 1. Rice seedlings without *Xoo* infection were treated as control. There were no significant differences between control and *Xoo*-infected rice seedlings in the species of the volatiles [i.e., monoterpenes:  $\alpha$ -thujene,  $\alpha$ -pinene, sabinene, phellandrene, limonene, and  $\alpha$ -terpinolene; sesquiterpenes: cyclosativene,  $\alpha$ -copaene, and (*E*)-caryophyllene], except for de novo production of  $\beta$ -elemene in infected seedlings (Fig. 1(A–D)). However, the infection treatment affected the amount of the volatiles, particularly limonene, which comprised  $\approx 40$  % of the total peak area of terpene volatiles (Fig. 1(E), Table S1).  $\beta$ -Elemene was the only terpene volatile to be de novo emitted from the *Xoo*-infected rice seedlings.

### Chiral analysis for limonene emitted from the *Xoo*-infected rice seedlings

To determine the chirality of the limonene overproduced by *Xoo* infection, we collected the headspace volatiles from the *Xoo*-infected rice seedlings and analyzed using solid-phase microextraction (SPME)–chiral GC–MS. Interestingly, optical isomer of the limonene, which was (*S*)-limonene, was the same as that of the limonene produced by oxidative abiotic stresses (Fig. 2).

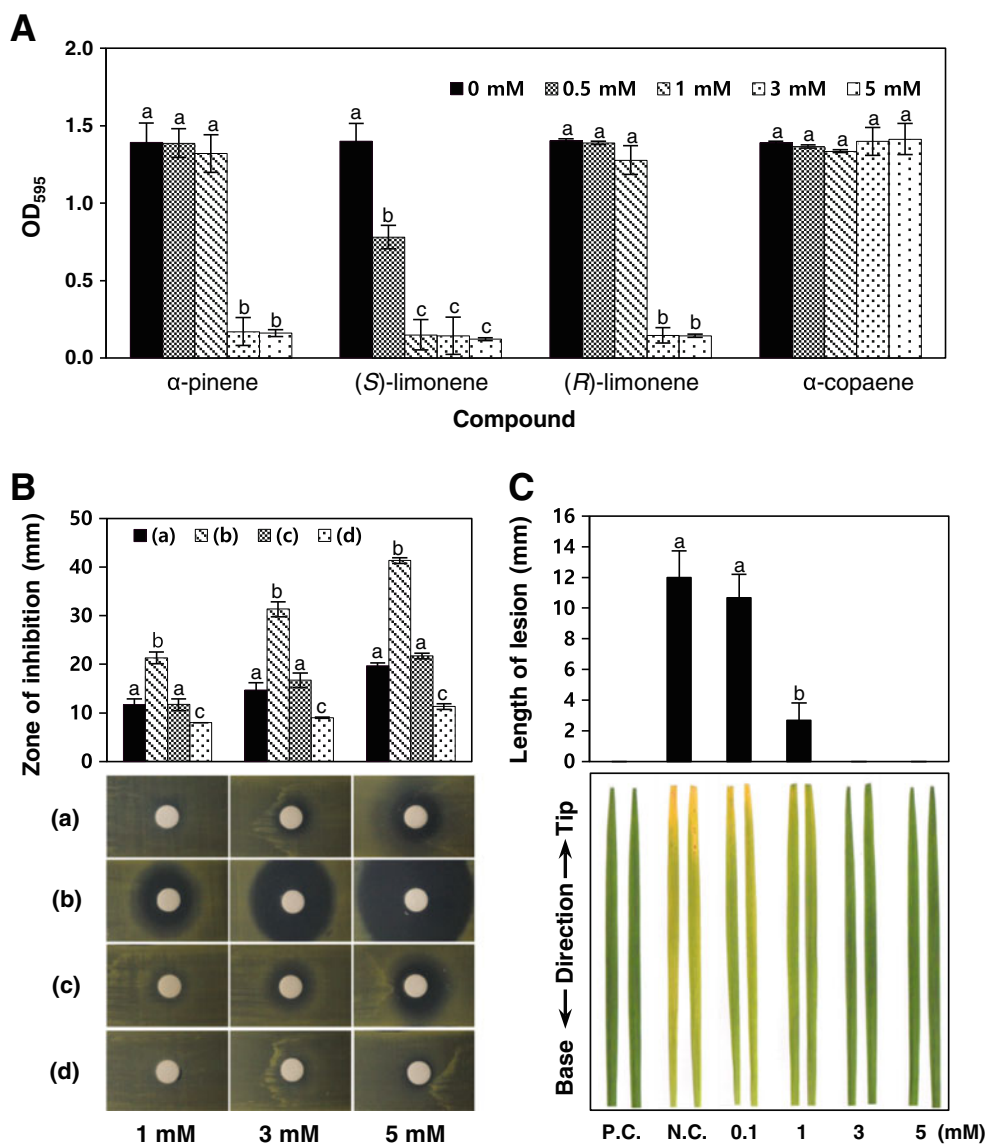
### Screening of volatile terpenes for inhibitory effect on *Xoo* growth

The inhibitory effect of various volatiles [i.e.,  $\alpha$ -pinene, (*S*)/(*R*)-limonene, and  $\alpha$ -copaene] on *Xoo* growth is shown in Fig. 3a.  $\beta$ -Elemene was not included due to the absence of any antibacterial activity (Taniguchi et al. 2014b).  $\alpha$ -Copaene did not affect the growth of *Xoo* in the liquid cultures at all concentrations, and only the higher concentrations (3 and 5 mM) of  $\alpha$ -pinene and (*R*)-limonene significantly inhibited *Xoo* growth. Lower treatment concentrations of the two volatiles had no visible impact on *Xoo* growth (Fig. 3a), while (*S*)-



**Fig. 2** Chiral analysis of limonene emitted from the rice seedlings using SPME–chiral GC–MS [extracted ion chromatograph ( $m/z=93.1$ )]. **A** Limonene authentic standards, limonene from **B** control plant, and **C** *Xoo*-infected seedlings: **1** (*S*)-limonene and **2** (*R*)-limonene

**Fig. 3** Biological activities of representative volatile terpenes against *Xoo*. **a** A liquid culture assay. **b** A disc diffusion assay of *a*  $\alpha$ -pinene, *b* (*S*)-limonene, *c* (*R*)-limonene, and *d*  $\alpha$ -copaene. **c** In vivo leaf infection assay. *PC* positive control (0.01 % ethanol spray only), *NC* negative control [*Xoo* infection without (*S*)-limonene pretreatment]. Images were photographed after detaching the leaves. Different letters above bars indicate significant difference among the treatments ( $p < 0.01$ , Duncan's multiple range test). Values are means  $\pm$  SE ( $n = 3$ )



limonene was highly effective in inhibiting *Xoo* growth at all treatment concentrations (Fig. 3a).

#### Inhibition of *Xoo* growth in a disc diffusion assay

The antibacterial activity of these compounds was also confirmed by a disc diffusion assay (Fig. 3b). (*S*)-Limonene strongly suppressed *Xoo* in a concentration-dependent manner:  $21.3 \pm 1.2$  mm zone of inhibition at 1 mM,  $31.3 \pm 1.5$  mm zone of inhibition at 3 mM, and  $41.3 \pm 0.6$  mm zone of inhibition at 5 mM (Fig. 3b). However, inhibitory effect of (*R*)-limonene, the opposite enantiomer of (*S*)-limonene, was almost half compared to that of (*S*)-limonene. The effect of  $\alpha$ -pinene was similar to that of (*R*)-limonene, and  $\alpha$ -copaene had little or no effect on *Xoo* inhibition.

#### Suppression of *Xoo* growth by (*S*)-limonene pretreatment

The strong inhibitory activity of (*S*)-limonene against *Xoo* as observed in vitro was verified in planta by comparing the length of blight lesions in intact rice leaves, sprayed with various concentrations of (*S*)-limonene (Fig. 3c). After 7 days of incubation, progressions of pathogenic symptoms from the tip to the base of the leaves were observed, where the leaves were not treated with (*S*)-limonene as a negative control (Fig. 3c). (*S*)-Limonene treatment largely inhibited the *Xoo* growth in a concentration-dependent manner as evident from the shorter length of lesions ( $10.7 \pm 1.5$  mm at 0.1 mM and  $2.7 \pm 1.2$  mm at 1 mM) compared to the negative control leaves ( $12.0 \pm 1.7$  mm). No obvious pathogenic symptoms were observed beyond 3 mM of (*S*)-limonene treatment (Fig. 3c).

## Transcript analyses of (*S*)-limonene synthase (*OsTPS20*) and pathogenesis-related genes

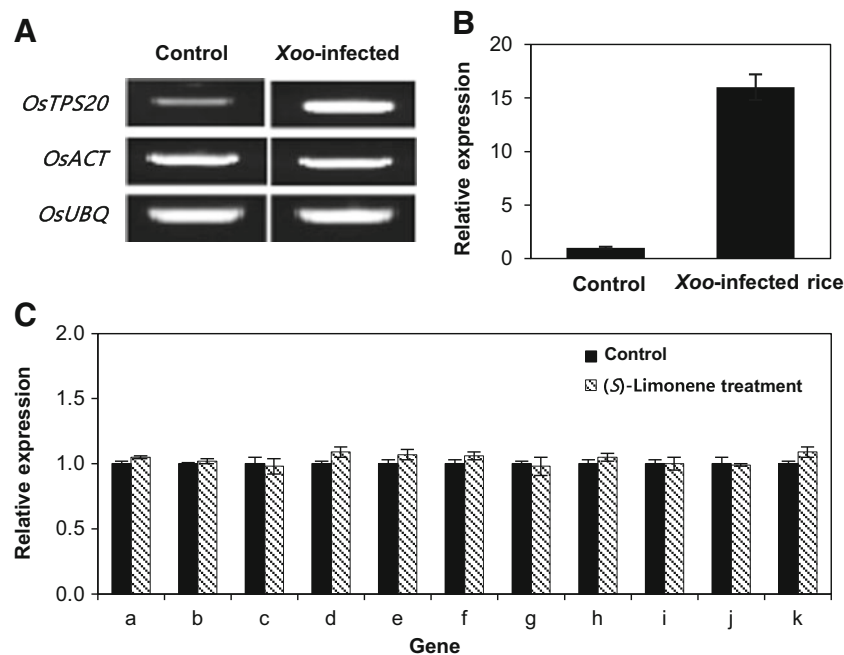
*Xoo* infection in rice seedlings caused a strong induction of *OsTPS20* transcript, with 16-fold increase (Fig. 4a, b). We consider that the suppressive effect of (*S*)-limonene against *Xoo* might be derived from an innate defense system activated by (*S*)-limonene treatment. Representative 11 antibacterial pathogenesis-related (PR) genes [i.e., *Os01g0124400* (trypsin inhibitor), *Os01g0194300* (NPR1-like protein), *Os01g0801500* ( $\beta$ -1,3-glucanase), *Os02g0833900* (peroxidase), *Os04g0350100* (protease inhibitor), *Os04g0493400* (chitinase), *Os06g0691200* (thaumatin-like protein), *Os07g0129200* (PR1a protein), *Os07g0677100* (peroxidase), *Os11g0114900* (nonspecific lipid transfer protein), and *Os11g0701800* (class III chitinase)] were considered (Taniguchi et al. 2014a), and their expressions were determined following (*S*)-limonene treatment. As there was no increase in the steady-state transcript levels with (*S*)-limonene treatment for all the genes, it is very likely that (*S*)-limonene interacts directly with *Xoo*.

## Discussion

To delineate the functionality of the volatiles inducibly (or abundantly) released against *Xoo*-infected rice plants, we

firstly examined the relative amount of volatiles from the rice plant and found that (*S*)-limonene was a major monoterpene to be induced and  $\alpha$ -copaene was the most abundant sesquiterpene (Figs. 1 and 2). We have demonstrated that (*S*)-limonene was able to significantly suppress *Xoo* growth both in a liquid culture assay with 0.5 mM (*S*)-limonene (Fig. 3a) and in a disc diffusion assay with 1 mM (*S*)-limonene (Fig. 3b) in concentration-dependent manners. The anti-*Xoo* activity of (*S*)-limonene was further tested by spraying this limonene on leaves prior to infecting leaves with *Xoo*. Interestingly, suppression of pathogenic symptoms by 1 mM (*S*)-limonene treatment progressed in a concentration-dependent manner—no visible symptoms over 3 and 5 mM treatments (Fig. 3c). The results obtained provide new insights into the protective role of headspace volatiles against rice bacterial blight. Previously, non-volatile phenolic compounds, free radicals, or lignin-like polymers correlating with the activity of peroxidase have been implicated in plant defense against *Xoo* infection (Reimers et al. 1992), together with the presence of fibrillar materials, enveloping bacterial blights (*Xanthomonas campestris* pv. *oryzae*) in rice vessels of incompatible interactions (Horino and Kaku 1989).

The biological activities of terpenes appear to be specific not only to plant species and pathogen types but also to enantiomeric types and concentrations of terpenes. For instance, limonene in transgenic orange fruits, where the concentration of limonene was minimized by antisense expression of a



**Fig. 4** Transcript levels of *OsTPS20* and pathogenesis-related (PR) genes in response to *Xoo* infection. **a** Steady-state transcript level of *OsTPS20*; quantitative real-time PCRs for **b** *OsTPS20* and **c** PR genes. **a** *Os01g0124400* (trypsin inhibitor), **b** *Os01g0194300* (NPR1-like protein), **c** *Os01g0801500* ( $\beta$ -1,3-glucanase), **d** *Os02g0833900* (peroxidase), **e** *Os04g0350100* (protease inhibitor), **f** *Os04g0493400* (chitinase),

**g** *Os06g0691200* (thaumatin-like protein), **h** *Os07g0129200* (PR1a protein), **i** *Os07g0677100* (peroxidase), **j** *Os11g0114900* (nonspecific lipid transfer protein), and **k** *Os11g0701800* (class III chitinase). *OsACT* and *OsUBQ* were used as internal controls. Values are means $\pm$ SE ( $n=3$ )

limonene synthase, activated defense mechanisms against the fungus *Penicillium digitatum* (Pers.) Sacc. and *Xanthomonas citri* subsp. *citri* (Rodríguez et al. 2014). Conversely, high amounts of limonene in orange peels attracted more pests and pathogens, rather than repelling or inhibiting them (Rodríguez et al. 2011). Importantly, our study demonstrates that stereospecificity of the compound could be a key determinant of biological functions; only the specific enantiomer, (*S*)-limonene, and not (*R*)-limonene, retains a strong inhibitory activity against *Xoo*.

Rice constitutively releases (*S*)-limonene at low levels, but the level of this limonene is elevated during abiotic oxidative stresses (Lee et al. 2015) or biotic bacterial blight infection (in the present study), mainly biosynthesized by OsTPS20 (Lee et al. 2015). Rice bacterial blight infection usually occurs at the tillering stage, and the symptoms peak at the flowering stage. However, rice plants are most susceptible at the seedling stage (less than 21-day-old plants) and damage to plants from the disease at this stage of plant growth is more severe, with kresak symptoms observable (Mew et al. 1993). Therefore, it is critical to control rice bacterial blight at the early stage of plant development to ensure good yield of rice. The findings of our study point to the possibility that (*S*)-limonene can be used as an alternative environment-friendly chemical to manage the disease at the seedling stage. Furthermore, the development of rice breeding program for rice cultivars producing an appropriate amount of (*S*)-limonene will also be a valuable strategy.

Oxygenated monoterpenes have been considered as potential antibacterial agents and extensively screened against numerous bacterial strains (Kotan et al. 2007). Therefore, we determined the accumulation of carveol or carvone, which are oxygenated limonene derivatives. However, we did not observe any accumulation of these compounds under the experimental conditions of our work. In contrast, for direct suppression of *Xoo* by (*S*)-limonene as observed in our study, monoterpene alcohol linalool confers resistance indirectly to *Xoo* infection by inducing the expression of PR genes in rice (Taniguchi et al. 2014a). Because (*S*)-limonene treatment per se did not induce the expression of any of the PR genes (Fig. 4c), we assume that rice might have a dual-defense mechanism that controls *Xoo* growth, using (*S*)-limonene as a direct contact phytoanticipin and using linalool to activate innate defense systems. At least in rice, (*S*)-configuration of the compounds seems to be important for proper functioning of the mechanisms, as linalool was also documented to be (*S*)-enantiomer in rice (Yuan et al. 2008). Future functional analyses of many other volatiles (individually or in combination) may uncover further details on the interaction of rice with *Xoo* in respect of chemical defense. It would also be of interest to generate transgenic rice plant by overexpressing *OsTPS20* to determine whether endogenously accumulated

(*S*)-limonene induces the expression of defense-related genes or the vapor phase of the volatile could also inhibit bacterial blight.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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