Induced resistance against *Fusarium solani* root rot disease in cassava plant (*Manihot esculenta* Crantz) promoted by salicylic acid and *Bacillus subtilis*

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**ABSTRACT**

Root rot disease of cassava is one of the major diseases in Thailand, widespread incidence of soilborne pathogens has been found to affect more than 80%. The research aims to investigate the effect of elicitors as salicylic acid and *Bacillus subtilis* strain JN2-007 for inducing resistance against root rot disease by *Fusarium solani* in cassava. The experiment was carried out in a susceptible cassava cultivar to test the efficacy of elicitors in the biochemical response of plant defense mechanisms pertaining to hydrogen peroxide (H$_2$O$_2$) and enzyme activities. The results indicated that pathogenicity test of *F. solani* isolate SHRD1 caused the brown lesions around the inoculation point on cassava roots. Subsequently, salicylic acid and JN2-007 reduced mycelial growth of *F. solani* (11.83%–57.73% at day 7), as well as disease severity in the cassava plants at 14 days after the inoculation compared to that of the negative control (28.12%–39.58% compared to 68.75%). Furthermore, salicylic acid at a concentration of 500 µl. L$^{-1}$ could induce H$_2$O$_2$, level of peroxidase, polyphenol oxidase, and catalase activities that were highest at 24 h after pathogen inoculation. The results suggested that elicitors played an important role as a plant defense inducer, leading to reduced Fusarium root rot disease.

**Introduction**

Cassava (*Manihot esculenta* Crantz) has been an important economic crop in Thailand. It has also been used in the production of animal feed, alcohol, biofuel, other pharmaceutical products (FAO 2002; 2015), and as a food crop in Southeast-Asia and Africa (Ubalua and Oti 2007). The increasing demand for food has led to an expansion of cassava cultivation in many countries. The planting area of cassava in Thailand was approximately 1.29 million hectares and recognised as a major cassava producer, annual yielding at approximately 31.2 million tons. Thailand has begun to substitute modified starch produced from imported corn with one produced from local cassava (Charaensatapon et al. 2014; Duchanee 2015; Sangpueak et al. 2018). However, the majority of farmers grow CMR-89 cultivar (susceptible cultivar) because of its high productivity and good adaption to the environment but cassava production is greatly reduced due to attacks by insects and diseases. Among them, Fusarium root rot disease (FRRD) is one of the important cassava diseases worldwide (Harman et al. 1981; Duchanee 2015; Athipunyakom et al. 2019). The damage of *Fusarium* spp. could reduce the cassava yield up to 81% (Theberge 1985; Lozano and Nolt 1994).

The induced defence mechanisms have two forms of systemic defenses including (1) systemic acquired resistance (SAR) and (2) induced systemic resistance (ISR). The SAR requires salicylic acid (SA) as a signal molecule and is associated with the production, defense enzymes, and pathogenesis-related (PR) proteins (Buensanteai et al. 2010). The ISR relies on ethylene (ET) and jasmonic acid (JA) as signalling factors (Walters et al. 2007). The elicitors could be both synthetic or natural compounds and microorganisms. Abiotic elicitors comprise SA, vitamin B1, benzoic acid (BA), chitosan and ascorbic acid (AA) have been widely evaluated against various plant diseases (Eschen-Lippold et al. 2010; Prakongkha et al. 2013a; 2013b). Moreover, biotic elicitors such as *Bacillus* sp. have been frequently assessed to be effective in inducing increased resistance and plant growth against several diseases (Buensanteai et al. 2009; Graham and Myers 2011). Induced resistance mechanisms of elicitors have been announced that callose deposition, hypersensitive response (HR), oxidative burst, accumulation of...
salicylic acid, synthesis of defense enzyme, PR-proteins, and phytoalexins stimulation related to plant defense response against pathogen infection (Sana et al. 2010; Iriti et al. 2011).

The elicitors such as salicylic acid (SA) and Bacillus sp. have been used in research experiment and commercially trial product to control plant pathogens and enhance growth in various plants (Buensanteai et al. 2009; War et al. 2011; Parikh and Adesemoye 2018). SA could induce the production of reactive oxygen species, enzyme activities and pathogenesis-related (PR) proteins in defense mechanism of cassava plants against physiological disorders and biochemical mechanisms (Mostafanezhad et al. 2014). In addition to directly affect plant growth and development through plant growth regulator, Bacillus sp. could colonise roots and trigger plant biochemical and physiological systems to promote growth enhancement (Prathuangwong and Buensanteai 2007; Buensanteai et al. 2012). However, the application of SA and Bacillus are still not many in cassava plants. The aim of this research was to evaluate the efficacy of exogenous SA and Bacillus subtilis strain JN2-007 for inducing resistance against Fusarium root rot disease.

Materials and methods

**FRRD causal agents and culture conditions**

*Fusarium solani* isolate SHRD1 (virulent strain) was obtained from stock culture of Plant Molecular Biology Laboratory, Suranaree University of Technology, Thailand, stored in potato dextrose broth (PDB) with 10% glycerol at −80°C. The culture was transferred from PDB onto potato dextrose agar (PDA) plates (potato 200 g L−1, dextrose 20 g L−1, agar 15 g L−1) then incubated at 28 ± 2°C for 7 days. The fungal culture on PDA was used throughout the research (slightly modified from Buensanteai et al. 2009; Duchanee 2015; Sangpueak et al. 2018). *Fusarium* spores suspension was obtained by filtered through layers with sterile distilled water, then adjusted to 106 spores mL−1 by a haemocytometer. Later that, two drops of Tween 20 were added into 100 mL of *Fusarium* spores suspension.

**Preparation of abiotic and biotic elicitors**

**Abiotic elicitor preparation of exogenous SA**

SA (Acros Organics, ThermoFisher Scientific, USA) obtained from the Plant Molecular Biology Laboratory, Suranaree University of Technology, Thailand was used in the research. SA was prepared by dissolving them in sterile distilled water and adjusted to 500 µL−1 (the active ingredient was 6% salicylic acid).

**Bacillus subtilis strain JN2-007 and culture conditions**

*B. subtilis* strain JN2-007 (Plant Molecular Biology Laboratory, Suranaree University of Technology, Thailand), stored in nutrient broth with 10% glycerol at −80°C. Then, transferred to nutrient agar (NA) and incubated at 28 ± 2°C for 48 h. After that, propagated in 250 mL nutrient broth (NGB) at 28 ± 1°C and incubated with constant shaking at 180 rpm for 48 h. The cultures were re-suspended in sterile distilled water and adjusted to densities of 1 × 10⁶ cfu mL⁻¹, 1 × 10⁷ cfu mL⁻¹ and 1 × 10⁸ cfu mL⁻¹ (Buensanteai et al. 2009; Nikaji et al. 2015).

**Pathogenicity test**

Storage roots of healthy cassava plants, susceptible variety (CMR-89), were surface-sterilised with 1% NaOCl for 1 min. Then, the cassava roots were washed out with sterilised water 3 times and blotted dried with a sterile paper towel. To inoculate the fungus *Fusarium*, a 6 mm-diameter hole was cut into the bark using a sterile cork borer. Subsequently, a piece of PDA block with 7 d-old fungal culture was inserted into the hole and sealed with a strip of parafilm. The inoculated roots were kept in moist chambers (light regime of 12 light and 12 h dark, 25°C, RH 90 ± 3%) for one week for the observation (Ismail et al. 2012; Li et al. 2015). Disease severity scores of each fungal isolate were evaluated during the observation using the description described by Machado et al. (2012); Trakunyingcharoen et al. (2013) and Chen et al. (2016).

**Effect of SA and JN2-007 elicitors on FRRD causal agents in vitro**

The experiment was conducted in completely randomised design (CRD) with four replications to evaluate the inhibition effect of SA and JN2-007 elicitors on growth of *F. solani*. A cork borer with a diameter of 5 mm was used to drill agar blocks of the causal fungi and placed on centre of the elicitor-amended petri plate. Stock solutions of SA and JN2-007 were prepared. To assure complete solubility, each stock solution was stirred with a magnet bar in beakers for 30 min. The stock solution was filtered by Whatman papers with filter holes approximately 0.2 µm. The filtered stock solution was then poured into warm medium of PDA at a temperature approximately 55°C–60°C for 2 min, and gently shook, ensured that in this mixed medium
bottle, SA at concentrations of 500 µL⁻¹; or JN2-007 at densities of 1 × 10⁶, or 1 × 10⁷ or 1 × 10⁸ cfu.mL⁻¹. The mixture medium was immediately poured into petri plates with a volume approximately 10 mL per one petri plate. After medium hardened, a hyphal block of causal fungi was put at the centre of each petri plate.

All culture plates were kept at room temperature for 7 days. PDA plates without SA and JN2-007 elicitors were used as the negative control, and chemical fungicides including carbendazim (500 µg.mL⁻¹; Thaion chemical Co., Ltd., Thailand), mancozeb (2000 µg.mL⁻¹; Sotus home and garden Co., Ltd., Thailand) and prochloraz (200 µL.L⁻¹; Thep watana Co., Ltd., Thailand) were used to inhibit growth of the fungi as a positive control. The mycelial growth was measured in diameter (mm) around the discs (Sobowale et al. 2005; Bandyopadhyay et al. 2006; Wongcharoen 2013) at 5 and 7 days after putting fungal slices (DAPFS). Inhibition percentage was calculated using the formula of Maurya et al. (2014) as follows:

\[
\text{Percent inhibition}(I) = \frac{(C - T)}{C} \times 100
\]

where C = mycelial growth of the pathogen in negative control; T = mycelial growth of the pathogen in elicitor-amended treatment.

The experiment was repeated twice. The treatments having high inhibition effect were chosen to do on following experiment.

**Efficacy of the elicitors in inducing resistance against Fusarium root rot disease under greenhouse conditions**

Cassava stalks of CMR-89 were surface-disinfected with 1% NaOCl for 2 min, followed by washing with distilled water (three times) and let dry for 5 min at room temperature. Then, cassava stalks were soaked for 10 min before planting with elicitors and chemical fungicides (positive controls) that were selected in the previous experiment. After planting, the cassava plants were inoculated with 1 × 10⁶ conidia.mL⁻¹ of *Fusarium* suspension (Oliveira et al. 2013). A 20 mL drop of the *Fusarium* suspension was dropped along bottom portion of each cassava stalk. Subsequently, at 14, 21, and 28 days after planting (DAP), cassava plants were sprayed on their foliage with each elicitor (SA at concentrations of 500 µL⁻¹; JN2-007 at densities of 1 × 10⁶, or 1 × 10⁷ or 1 × 10⁸ cfu.mL⁻¹) with solution volume at approximately 20, 40 and 60 mL/plant. Spraying time of the elicitors was at 4 pm. The greenhouse conditions had light regime of 12 light/12 h dark, 28 ± 2°C, RH 82 ± 6%. After that, cassava plants were investigated for disease severity scale of the Fusarium root rot at 7 and 14 days after the inoculation (DAI) (modified from Sompong et al. 2013; Le Thanh et al. 2017; Thepbandit et al. 2021) and assessed for biochemical changes associated with plant defense accumulation of hydrogen peroxide (H₂O₂) and enzyme activities (peroxidase, polyphenol oxidase, and catalase). All experiments were repeated three times, with similar results in all replications, one leaf per replication with a measurement of H₂O₂ content and enzyme activities for each time.

**Measurement of hydrogen peroxide (H₂O₂) content**

Cassava roots were collected at 0, 12, 24 and 48 h after inoculation (HAI). Briefly, 0.5 g of the root samples were immersed in ice and ground with a sterile mortar and pestle to a suspension then homogenised with 2 mL ice-cold 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3500 g for 30 min at 4°C. Finally, 0.3 mL of the supernatant was dispensed into a new eppendorf tube and 0.3 mL 10 mM potassium phosphate buffer (pH 7.0), 0.6 mL of 1 M potassium iodide (KI) were added and the absorbance was read at 390 nm. Hydrogen peroxide content was calculated from a standard curve and the concentration was expressed as µmol.g⁻¹ FW (Velikova et al. 2000; Njenga et al. 2017).

**Extraction and determination of enzyme activities**

**Estimation of total soluble proteins**

Crude protein extract was extracted by the method as described by Buensanteai et al. (2009); Prakongkha et al. (2013b); Zur et al. (2013); Nair and Umamaheswaran (2016). Cassava roots were collected at 0, 12, 24 and 48 HAI. The plant tissues (0.5 g) were ground in a cold mortar with a pestle using liquid nitrogen that homogenised in 3 mL of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenate was centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was used as the enzyme extract for the assay of peroxidase, polyphenol oxidase, and catalase activities. The protein content was examined using the standard Bradford protein assay (Bradford 1976).

**Peroxidase activity (PO)**

The activity of PO was determined as described by Hammerschmidt et al. (1982) and Abou-Zeid et al. (2018). The reaction mixture consisting of 1.5 mL of 0.05 M pyrogallol and 0.1 mL of enzyme extract was taken in a 96-well plate. To initiate the reaction, 0.5 mL of 1% H₂O₂ was added. The change in absorbance was recorded at 420 nm at 1 min intervals for 3 min from zeros of
incubation at room temperature. The boiled enzyme served as blank. The enzyme activity was expressed as an units.g$^{-1}$.FW.min$^{-1}$.

**Polyphenol oxidase (PPO)**
The reaction mixture consisting of 1.5 mL of 0.1 M sodium phosphate (pH 7.0) and 0.1 mL of enzyme extract was taken in a cuvette. Then, 0.2 mL of catechol (0.01 M) was added to initiate the reaction. The change in absorbance was recorded at 495 nm at 1 min intervals for three min and the results were expressed as an units.g$^{-1}$.FW.min$^{-1}$ (Mayer et al. 1965; Abou-Zeid et al. 2018).

**Catalase (CAT) activity**
CAT activity was assayed in the root samples that collected from healthy and inoculated cassava plants (slightly modified by Njenga et al. 2017). Reaction mixture consisting of 0.5 mL of 0.1 M phosphate buffer (pH 7.0), 0.2 mL of enzyme extract, and 0.1 mL of 1% H$_2$O$_2$ was incubated at 28 ± 1°C. At the start of the enzyme reaction, the absorbance of the mixture was set zero at 230 nm in a spectrophotometer, and changes in the absorbance were recorded at 1 min intervals for 3 min. Values of CAT activity was expressed as µmol of H$_2$O$_2$ oxidised.mg$^{-1}$ of protein.min$^{-1}$.

**Statistical analysis**
The data was analysed and subjected to Analysis of Variance (ANOVA) using SPSS software, version 16. The significance of treatments were determined by the magnitude of F value ($p \leq 0.01$). Treatment means were separated by Least significant difference (LSD).

**Results**

**Pathogenicity test**
The results showed that inoculated roots had infected symptoms of *Fusarium*, occurring internally in the tissues after 12 h (Figure 1). The symptoms included brown or black lesions around the inoculation point and turns into a necrotic lesion. Symptoms were not observed in control cassava roots. Therefore, *Fusarium solani* isolate SHRD1 was used on the following in vitro and in vivo experiments.

**Effect of elicitors on inhibiting Fusarium mycelial growth**
Inhibition of *Fusarium* mycelial growth was assessed at 5 and 7 DAPFS. The results revealed that SA at a concentration of 500 µL$^{-1}$ had a low reduction on mycelial growth of *F. solani* with 15.18% and 11.83% at 5 and 7 DAPFS, respectively, both values significantly higher than those of the negative control at 0.00%. For the second elicitor as JN2-007 on different concentrations, the mycelial growth inhibition for evaluated showed a high reduction in mycelial growth of *F. solani*, gained more than 50% at both 5 and 7 DAPFS, and statistically higher than those of SA and the negative control. Treatments of positive control including Carbendazim, Mancozeb and Prochloraz showed better mycelial growth inhibition than other treatments, gained 55.44%–75.50% during two observation time points (Table 1).

**Effect of elicitors on Fusarium root rot severity and on inducing H$_2$O$_2$ content and enzyme activities**
Elicitors were evaluated for their ability to induce protection in cassava plants against Fusarium root rot disease. The results showed that stalk soak and foliar sprays with the elicitors helped cassava plants and significantly reduced the severity of Fusarium root rot disease at 7 and 14 DAI compared to those of the negative control (Table 2). At 7 DAI, treatments treated with SA or JN2-007, had disease severities at 15.62%–18.75%, significantly higher than that of negative control at 43.75%, but statistically nonsignificant to those of positive controls (9.50%–15.62%). Efficacy of SA or JN2-007 prolonged until 14 DAI, shown by low values of disease severity at this observation time point. At 14 DAI, disease severities of treatments including SA, JN2-007 at 1 × 10$^6$ cfu.mL$^{-1}$, JN2-007 at 1 × 10$^7$ cfu.mL$^{-1}$ or 1 × 10$^8$ cfu.mL$^{-1}$, Carbendazim, Mancozeb and Prochloraz were 21.00%–39.58%, statistically lower than that of the negative control at 68.75%. Among three treatments of JN2-007, the JN2-007 at 1 × 10$^6$ cfu.mL$^{-1}$ showed lowest disease severity than that of JN2-007 at 1 × 10$^7$ cfu.mL$^{-1}$ or JN2-007 at 1 × 10$^8$ cfu.mL$^{-1}$ at 7 and 14 DAI. Similarly, in the group of positive controls, disease severities of Mancozeb was always lower than that of Carbendazim or Prochloraz at 7 and 14 DAI (Table 2). Therefore, four treatments including SA, JN2-007 at 1 × 10$^6$ cfu.mL$^{-1}$, Mancozeb and Negative control were further investigated.

Effect of elicitors were also characterised on changes of resistance elements including H$_2$O$_2$ content and enzyme activities inside cassava treated plants. Firstly, H$_2$O$_2$ content in all treated-treatments increased till 24 HAI and the highest value was observed in SA at 1.73 µmol.g$^{-1}$ FW, and then decreased at 48 HAI (Figure 2). Secondly, all treated-treatments showed an increased level of PO activity after pathogen inoculation. The highest PO activity of 2.54 units.g$^{-1}$ FW min$^{-1}$ was observed in SA at 24 HAI (Figure 3). Thirdly, results in
Figure 4 revealed that the polyphenol oxidase activity was increased in SA treatment that roots infected with *F. solani* strain SHRD1 at 24 HAI was 8.54 units.g\(^{-1}\).FW.min\(^{-1}\), showing significant (\(p \leq 0.01\)) difference compared to the uninfected control. Likewise, the activity of CAT was increased in challenged inoculation with *Fusarium* root rot and less in healthy plants. The highest CAT activity in SA treatment at 24 HAI was 5.54 \(\mu\)mol.mg\(^{-1}\) of protein.min\(^{-1}\) (Figure 5).

### Discussion

To our knowledge, this is the first report on the in vitro mycelial growth inhibition of SA against *F. solani* causing root rot in cassava. In the research, SA reduced the mycelial growth of *F. solani* isolate SHRD1 at concentrations 500 \(\mu\)l.L\(^{-1}\) as compared to the negative control. Similar researches also indicated that the mycelial growth of *F. solani* with different crops was inhibited at concentrations of 12 and 18 mg.mL\(^{-1}\), respectively.

### Table 1. Percent inhibition on mycelial growth of *F. solani* in elicitors amended culture plates.

| Treatments       | Concentration | Day 5       | Day 7       |
|------------------|---------------|-------------|-------------|
| Salicylic acid   | 500 \(\mu\)L\(^{-1}\) | 15.18±0.04\(a\) | 11.83±0.05\(b\) |
| *Bacillus subtilis* JN2-007 | \(1 \times 10^6\) cfu mL\(^{-1}\) | 66.93±0.00\(b\) | 57.73±0.00\(c\) |
|                 | \(1 \times 10^7\) cfu mL\(^{-1}\) | 63.34±0.07\(b\) | 55.55±0.06\(d\) |
| Carbenazim       | 500 \(\mu\)g mL\(^{-1}\) | 66.85±0.01\(b\) | 68.78±0.01\(c\) |
| Mancozeb        | 2000 \(\mu\)g mL\(^{-1}\) | 71.35±0.09\(b\) | 75.50±0.00\(b\) |
| Prochloraz       | 200 \(\mu\)L\(^{-1}\) | 55.03±0.01\(b\) | 55.44±0.01\(b\) |
| Negative control |               | 0.00±0.00\(b\) | 0.00±0.00\(b\) |
| F-test           |               | **           | **           |
| CV (%)           |               | 17.30        | 28.00        |

\(^{1}\)Inhibition percentage of salicylic acid 500 \(\mu\)L\(^{-1}\), *B. subtilis* strain JN2-007 at different densities, carbenzazim (500 \(\mu\)g mL\(^{-1}\)); mancozeb (2000 \(\mu\)g mL\(^{-1}\)) and prochloraz (200 \(\mu\)L\(^{-1}\)) were recorded at 5 and 7 days after putting fungal slices. Mean ± SE (standard error) followed by the same letter do not significant differences (\(p \leq 0.01\)) according to LSD test.

### Table 2. Efficacy of elicitors on Fusarium root rot severity in cassava caused by *Fusarium solani* isolate SHRD1.

| Treatments       | Concentration | Disease severity (%)\(^{1}\) |
|------------------|---------------|-----------------------------|
|                  |               | 7 DAI \(^{2}\) | 14 DAI \(^{2}\) |
| Salicylic acid   | 500 \(\mu\)L\(^{-1}\) | 15.62±3.12\(a\) | 28.12±3.12\(a\) |
| *Bacillus subtilis* JN2-007 | \(1 \times 10^6\) cfu mL\(^{-1}\) | 16.67±3.12\(a\) | 29.22±3.13\(a\) |
|                  | \(1 \times 10^7\) cfu mL\(^{-1}\) | 18.75±3.60\(a\) | 34.37±3.12\(a\) |
| Carbenazim       | 500 \(\mu\)g mL\(^{-1}\) | 12.37±3.12\(a\) | 25.87±5.98\(a\) |
| Mancozeb        | 2000 \(\mu\)g mL\(^{-1}\) | 9.50±0.00\(a\) | 21.00±0.00\(a\) |
| Prochloraz       | 200 \(\mu\)L\(^{-1}\) | 15.62±5.98\(a\) | 28.12±5.98\(a\) |
| Negative control |               | 43.75±8.07\(b\) | 68.75±11.96\(b\) |
| F-test           |               | **           | **           |
| CV (%)           |               | 15.30        | 17.00        |

\(^{1}\)Disease severity scale of the Fusarium root rot was recorded at 7 and 14 days after the inoculation (DAI). Mean ± SE (standard error) followed by the same letter do not significant differences (\(p \leq 0.01\)) according to LSD test.

\(^{2}\)DAI: Days after inoculation.

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**Figure 1.** The symptoms of pathogenicity test in cassava roots caused by *F. solani* isolate SHRD1 at 0, 12, 24 and 48 h after inoculation (H).
El-Mohamedy et al. (2014) found that sprays with SA at 100 mM for 48 h before root inoculation, and their results showed a reduction of disease severity and root rot incidence caused by *F. solani*, *S. rolfsii* and *R. solani*. Kumar and Bains (2018) reported that in most cases, a higher concentration of disease severity and root rot incidence caused by *F. solani*, *S. rolfsii* and *R. solani*. Kumar and Bains (2018) reported that in most cases, a higher concentration

(Kendra and Hadwiger 1984). El-Mohamedy et al. (2014) found that sprays with SA at 100 mM for 48 h before root inoculation, and their results showed a reduction of disease severity and root rot incidence caused by *F. solani*, *S. rolfsii* and *R. solani*. Kumar and Bains (2018) reported that in most cases, a higher concentration
than 0.5 mM of SA could decrease the mycelial growth of *F. mangiferae*. This finding was in accordance with those of Jendoubi et al. (2015) who reported that the different concentrations of SA (0.1, 0.2 and 0.3 mM) had significantly inhibited growth of *F. oxysporum* f.sp. *lycopercisi* and *F. oxysporum* f.sp. *radicis-lycopercisi* compared to the control. Also, for other pathogens such as *F. oxysporum*, *R. solani*, *R. stolonifer*, *S. rolfsii*, *M. phaseolinae*, *Pythium* sp., and *Phytophthora* using SA at a minimum concentration of 2.5 mM (Panahirad et al. 2012; El-Mohamedy et al. 2013). Moreover, in some reports, SA acts as a toxic chemical and inhibited the mycelial growth of other fungal pathogens. This strengthens the hypothesis that the signal transduction pathways activated by SA, their expression of systemic acquired resistance, rather than inhibiting the fungus directly (Metraux et al. 2002; Fragnière et al. 2011; Qi et al. 2012). Furthermore, the application of salicylic acid at a concentration of 1 mM by seed soak and foliar spray in rice plants cv. KDM105 reduced disease severity significantly over that of the control treatment (Le Thanh et al. 2017). Similarly, Thepbandit et al. (2021) studied the SA as SA-Ricemate for the control of leaf blight disease, reduced disease severity by 60% at three weeks post-inoculation.

The second elicitor *B. subtilis* strain JN2-007 at 1×10^6, 1×10^7 or 1×10^8 cfu mL^{-1} had good ability on inhibit *Fusarium* root rot disease, shown by low disease severity at 16.67%–39.58% during two observation time points. These results of this research are in line to some other studies. In 2015, Khedher et al. reported that *B. subtilis* V26 was a good biological control agent against *Rhizoctonia solani* on potato. In mung bean, Hashem et al. (2017) showed that the plants treated with *B. subtilis* BERA 71 had disease severity at 1.13%, significantly lower than that of the control (3.88%). Samaras et al. (2021) showed that *B. subtilis* MBI600 could help tomato plants to have low disease severity of *F. oxysporum* f.sp. *radicis-lycopersici* (approximately 2.5%), compared to the control (3.5%). *Bacillus* spp. promote plant growth, inducing resistance against several pathogens in some plants and being the most promising biocontrol agents. Besides, it uses for disease incidence reduction that an environmentally friendly strategy for crop production (Miljaković et al. 2020). In black pepper (*Piper nigrum* L.), Nguyen et al. (2021) revealed that the application of bio-products by *B. velezensis* KN12, *B. amyloliquefaciens* DL1, *B. velezensis* DS29, *B. subtilis* BH15, *B. subtilis* V1.21 and *B. cereus* CS30 had their ability to suppresses root diseases (*Phytophthora* and *Fusarium*) and promote black pepper growth and yield in the Central Highlands of Vietnam.

After treating elicitors such as SA or JN2-007 by stalk soak and leaf sprays, the elicitors activate defence mechanism inside cassava plants. Many kinds of defence
elements take part in plant defence, leading to higher and quicker responses in induced cassava plants when pathogens infect. Among these defence elements, H$_2$O$_2$ content, CAT, PO and PPO are important defence ones. Hydrogen peroxide acts as a local signal molecule for the induction of protective plant defense mechanisms (Quan et al. 2008). In this research, the H$_2$O$_2$ content in elicitors treated plants was higher compared to the non-treated plants, and the one inoculated with $F. solani$ strain SHRD1 has increased H$_2$O$_2$ levels at 24 h after inoculation. Several growth-promoting bacteria have been reported to possess antioxidant activity (Radhakrishnan et al. 2017; Rai et al. 2017). $B. subtilis$ has also been shown to possess an adaptation mechanism against H$_2$O$_2$ and Li et al. (2008) revealed that biocontrol of $Ralstonia$ solanacearum in tomatoes, through a role in increasing activities of PAL, PPO, POD, and SOD, as well as in induction of systemic resistance in tomato against early and late blight by inducing defense-related enzymes such as PPO, POD, and SOD. Our result suggests that SA triggers an internal signal involved in oxidative bursts required for inducing defense-related genes. Similar results have been found in salicylic acid accumulation in SA-treated rice against $Xanthomonas$ oryzae pv. oryzae (Le Thanh et al. 2017). Hao et al. (2014) reported that defense induction of $Salvia$ miltiorrhiza by SA contributed to enhance the production of H$_2$O$_2$. $Fusarium$ root rot infection in cassava plants led to increase in PO, PPO, and catalase activity in SA-treated plants was recorded after 24 h of inoculation. Among these defense molecules, PO and PPO are recognised to be involved in the strengthening of the plant’s cell walls by the accumulation of lignins which leads to protection against different invading pathogens (Acharya et al. 2011; Chandra et al. 2015). An increase in PO and PPO activity due to fungal infections is reported in many plants. CAT are important antioxidant enzymes mainly involved in the removal of reactive oxygen species. The defense enzymes induced upon plants treated with SA and its growth promotion activity might have reduced the disease incidence in cassava. Moreover, H$_2$O$_2$ content and four enzyme activities related to the defense system were performed in the inoculated cassava with the pathogen as well as the induction agent defense system SA elicitors in the early hours.

In conclusion, induced resistance in the susceptible cassava cultivar CMR-89 to $F. solani$ isolate SHRD1 by exogenous SA and $B. subtilis$ strain JN2-007 treatment was characterised by various known defence responses including increased hydrogen peroxide (H$_2$O$_2$) and enzyme activities (peroxidase, polyphenol oxidase, and catalase). These defence responses together provided effective suppression of the $F. solani$, we plan to study the proteome and transcriptome of cassava plants treated with elicitors and inoculated with cassava root rot complex diseases. These findings will help researchers to better understand and indicate that biochemical changes in cassava root treated with SA and $B. subtilis$ played an important role in the enhancement of
defense mechanisms leading to reduced Fusarium root rot severity in cassava and will help farmers to protect cassava yields from losses related to disease infection.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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