ORIGINAL ARTICLE

Efficacy of salicylic acid and a *Bacillus* bioproduct in enhancing growth of cassava and controlling root rot disease

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Abstract

The efficiency of a formulated salicylic acid (Zacha 11, 500 mg · l^{-1}) and a *Bacillus* bioproduct (JN2-007, 1 × 10^{7} cfu · ml^{-1}) in controlling cassava root rot disease and enhancing growth was evaluated. The results revealed that cassava stalk soaking and foliage spraying with Zacha 11 formulation or *Bacillus subtilis* bioproduct could increase cassava growth at 60 days after planting under greenhouse conditions. Zacha 11 gave the tallest stem height (11.67 cm), the longest root length (18.91 cm) and the greatest number of roots (49.50). *Fusarium* root rot severity indices of all treated treatments were reduced, and were significantly lower than that of the water control. Plants treated with Zacha 11 and JN2-007 had disease severity reduction of 53.33 and 48.33%, respectively. Furthermore, all treatments increased the endogenous salicylic acid (SA) content in cassava plants at 24 inoculation with significant differences when compared to the untreated samples. The efficacy of Zacha 11 and JN2-007 was evaluated at two field locations, using two different cassava varieties, cv. Rayong 72 and CMR-89. The results showed that all elicitors could suppress root rot disease as well as bacterial leaf blight. Furthermore, the elicitors helped cassava plants cv. Rayong 72 and CMR-89 to increase tuber weight, yield and starch contents, compared to the water control. Thus, it is possible that these formulations could be effective in controlling diseases and increasing cassava productivity.

Keywords: *Bacillus* bioproduct, cassava disease, controlling, growth promotion, salicylic acid

Introduction

Cassava (*Manihot esculenta* Crantz) has been recognized as one of the most important economic crops in Thailand (Chaisinboon and Chontanawat 2011; Jakrawatana *et al*. 2015; Treesilvattanakul 2016). The average cassava yield of the country has been approximately 20 t · ha^{-1}, which is lower than the expected yield. The constraints contributing to yield reduction are many, but those from several fungal, bacterial and viral diseases have been estimated to be as high as 20–80% (Piyachomkwan and Tanticharoen 2011; Camila *et al*. 2018). One of the most severe diseases is cassava root rot caused by a complex of soilborne pathogens, especially *Fusarium* spp., *Lasiodiplodia theobromae*, *Neoscytalidium hyalinum*, *Sclerotium rolfsii* and *Phytophthora* spp. (Charaensatapon *et al*. 2014; Duchanee 2015). Conventional practices for cassava disease control in Thailand have included using chemicals, which are effective to a certain extent. However, chemical control directly affects the health of consumers and the environment (Panuweta *et al*. 2013; Sriket *et al*. 2015). Therefore, resistance elicitors could be better alternatives and have been extensively evaluated to determine their possible use in the management of plant diseases. Plant growth-promoting rhizobacteria (PGPRs), such as *Bacillus subtilis*, can enhance growth and have the ability to induce disease resistance in several economic crops such as soybean, corn, rice and cauliflower (Prathuangwong and Kasem 2004; Prathuangwong and Buensanteai 2007). Exogenous salicylic acid (SA) has also been used as a promoter of plant growth. It generates a wide range of metabolic and physiological responses in plants, thereby affecting the development and growth of plants (Hayat and Ahmad 2007) which can indirectly increase production and harvested plant yield. It can also trigger the SA-dependent signaling pathway in plant defense against pathogens in plant cells and stimulates the induction of plant defense mechanisms in numerous plants (Hayat *et al*. 2010; Le Thanh *et al*. 2017; Sangpueak *et al*. 2018). Even though there have been numerous reports about using several resistance elicitors for controlling economic crop diseases, none of them have been tested on cassava root rot disease (CRRD). Therefore, this research was conducted to evaluate the efficacy of salicylic acid and a *Bacillus* bioproduct in controlling CRRD under both greenhouse and field conditions.

Materials and Methods

Preparation of the salicylic acid elicitor (Zacha)

An exogenous formulation of salicylic acid (Zacha), a product of Bioactive Agro Industry Co. Ltd., was used in this experiment. Zacha is a prototype formulation developed by the Plant Molecular Biology Laboratory, Suranaree University of Technology, Thailand.

Preparation of the bioproduct elicitor and culture conditions of *Fusarium solani*

The bioproduct *B. subtilis* JN2-007 is a product of Bioactive Agro Industry Co. Ltd., and developed by Nikaji *et al*. (2015) from the Plant Molecular Biology Laboratory, Suranaree University of Technology, Thailand. The product was adjusted to 1×10^7 cfu · ml^{-1} concentration with sterile distilled water. *Fusarium solani* isolate SHRD was obtained from stock culture in potato dextrose broth (PDB) with 30% glycerol stored at −80°C in the Plant Molecular Biology Laboratory, Suranaree University of Technology, Thailand. The culture was transferred onto a potato dextrose agar (PDA) plate, then incubated at 28 ± 2°C for 7 days. Subsequently, agar blocks of the growing mycelia were transferred into a PDB flask (125 ml) and incubated in a shaker at room temperature for 3 days, and used for the experiment (Patil *et al.* 2011; Malandrakis *et al.* 2018).

Evaluation of the elicitors' efficacy in promoting plant growth and reducing root rot severity under greenhouse conditions

Stalks of cassava accession number CMR-89 were surface-sterilized with 1% NaOCl for 2 min, followed by washing three times with sterile distilled water and allowed to dry for 5 min at room temperature. The experiment was set up as two identical sets, each set consisted of six treatments including the solution of Zacha 11 elicitor (salicylic acid 500 mg · l^{-1}), JN2-007 elicitor (1×10^7 cfu · ml^{-1}), three positive controls (Laminar, 1×10^9 cfu · mg^{-1} WP; Sarcon, SA 1,000 mg · l^{-1}; and Mancozeb 80% WP) and negative control (water) treatment, four replications. The cassava stalks were soaked in the solutions for 10 min before planting in black plastic pots (30 cm), in four replications with three stalks per each replication. At 15, 30 and 45 days after planting (DAP), the cassava plants were sprayed again with the same solution of each treatment. Subsequently, at 60 DAP, one set of the plants was inoculated with *Fusarium* suspension of 1×10^6 conidia · ml^{-1} by mixing it into the soil at the rate of 100 ml · pot^{-1}, while plants in the other set were uprooted and growth parameters were recorded. Disease severity scores based on the affected root and stem areas were recorded as follows: 1 – no symptoms, 2 – area affected by less than 25%, 3 – area affected by 25–50%, 4 – area affected by 51–75%, and 5 – area affected by more than 75% (Onyeka *et al*. 2005; Wokocha *et al*. 2010; Sompong *et al.* 2012; Duchanee 2015). Then, the percentage of disease severity index was calculated using the slightly modified formula of Le Thanh *et al*. (2017) as follows: Disease severity (%) = [Σ(class frequency × score of rating class)]/[(total number of scored plants) × (maximum disease score)] × 100%. The root rot disease reduction was assessed at 7 days after inoculation (DAI) and was calculated using the formula: Reduced disease severity of the disease = [(DS of the control group – DS of elicitor treated groups/ DS of the control group] × 100%. The severity assessment was done by measuring the affected cassava root and stem area (Sompong *et al.* 2012; Duchanee 2015; Le Thanh *et al*. 2017). The experiment was conducted three times.

Evaluation of endogenous salicylic acid (SA)

Firstly, 0.5 g of cassava leaf samples were soaked in liquid nitrogen and homogenized with 1 ml of extraction buffer (90 : 9 : 1 volume of absolute methanol : glacial acetic acid : distilled water) and centrifuged at 12,000 rpm for 20 min at 4°C. After that, 500 μl of the supernatant was mixed with an equal volume of 0.02 M ferric ammonium sulfate and then incubated for 5 min at 30°C. The absorbance of 530 nm was read by a Bio-Tek microplate reader and then compared with standard references to calculate the endogenous SA content in the sample (modified slightly by Raskin *et al*. 1989; Rozhon *et al*. 2005; Prakongkha *et al*. 2013).

Evaluation of the elicitors' efficacy in promoting plant growth and reducing root rot severity under field conditions

The experiment was conducted under field conditions at Suranaree University of Technology, Nakhon Rat chasima, Thailand, in two locations; DMS: 14°52'44.7"N 102°00'14.6"E and 14°51'43.2"N 102°01'57.5"E. The experimental areas were thoroughly ploughed two times and approximately 45 cm high beds were made. Cassava stalks cv. Rayong 72 and accession number CMR-89 (8–12 months old, 15 cm long) were then planted vertically with 1 × 1 m spacing. Weed management was undertaken and granular fertilizer (15-15-15) was applied at 125 kg · ha^{-1} [1, 2 and 3 months after planting (MAP) to all treatments]. The same concentrations of elicitors tested under greenhouse conditions were also used under field conditions. Each treatment had four replications. Application of the elicitors was done by stalk soaking for 10 min before planting and spraying of cassava foliage (125 l · ha^{-1}) three times at 1, 2 and 3 MAP. Water was used as a negative control (untreated) whereas Laminar (commercial *Bacillus*), Sarcon (commercial salicylic acid) and Mancozeb, served as positive controls. The cassava plants were recorded for severity from natural infection using the disease scores similar to that described in the previous experiment. After that, the number of tubers (tubers · plant^{-1}), tuber weights (kg · plant^{-1}), fresh tuber yields (tons · ha^{-1}) and starch contents (%) were determined at 9 MAP (Terry and Hahn 2009; Polthanee *et al.* 2014; Romkhambut 2015).

Results

Elicitors' efficacy in promoting cassava plant growth and reducing root rot severity under greenhouse conditions

Enhancing effects of the elicitors on growth and reduction of disease severity on cassava accession number CMR-89 cultivars were observed. The cassava plants treated with Zacha 11 (500 mg · l^{-1}) and JN2-007 (1 × 10^{7} cfu · ml^{-1}) had all growth parameters significantly higher than that of the water control (Table 1). At 60 DAP, the cassava plants treated with Zacha 11 and JN2-007 had the tallest stem heights (11.67 and 9.71 cm, respectively), the longest root lengths (18.91 and 17.91 cm, respectively) and the greatest number of roots (49.50 and 47.16, respectively). These results were significantly different from the negative control treatment (4.66 cm, 9.92 cm and 21.83, respectively). The stem height, root length and root quantity of treatments Laminar, Sarcon and Mancozeb were in the middle between Zacha 11 or JN2-007 and the water control. The severity and reduction of *Fusarium* root rot were assessed on cassava roots at 7 days after inoculation. The results indicated that root rot seve-

Table 1. Efficacy of elicitor application in promoting cassava plant growth and reducing *Fusarium* root rot severity under greenhouse conditions

Treatment[1]	Growth parameter[2]			Disease severity index[2] [%]	Disease reduction[2] [%]
	stem height [cm]	root length [cm]	number of roots		
Zacha 11	11.67 ± 1.59 a	18.91 ± 2.31 a	49.50 ± 1.44 a	35.00 ± 5.77 b	53.33 ± 7.70 a
JN2-007	9.71 ± 0.91 a	17.91 ± 1.16 a	47.16 ± 1.52 a	38.75 ± 4.78 b	48.33 ± 6.38 a
Laminar	7.25 ± 1.54 ab	13.75 ± 0.86 ab	40.22 ± 0.453 ab	40.00 ± 4.08 b	46.67 ± 5.44 a
Sarcon	8.83 ± 1.19 ab	13.50 ± 0.90 ab	39.50 ± 1.16 ab	37.50 ± 6.45 b	50.00 ± 8.61 a
Mancozeb	7.33 ± 1.15 ab	13.41 ± 0.90 ab	37.83 ± 1.69 ab	25.00 ± 4.08 a	66.67 ± 5.44 b
Water control	4.66 ± 1.23 b	9.92 ± 1.08 b	21.83 ± 1.80 b	75.00 ± 15.81 c	0

[1]Zacha 11, JN2-007, Laminar (commercial *Bacillus* 1 × 10^{9} cfu · mg^{-1}), Sarcon (commercial salicylic acid SA 1,000 mg · l^{-1}), Mancozeb (80% WP) and water used as a negative control. The elicitors and mancozeb were applied to cassava Acc. No CMR-89 by stalk soaking and foliage spray at 15, 30 and 45 days after planting. *Fusarium solani* inoculation was done by mixing into the soil at 60 days after planting; [2]means in the column followed by the same letter are not significantly different according to Duncan's multiple range test at $p = 0.05$. Each value represents a mean of four replicates

rity on cassava treated with Zacha 11 (35.00 ± 5.77%) or JN2-007 (38.75 ± 4.87%) were not different from those treated with commercial elicitors but were significantly lower than that of the negative control (75.00 ± 15.81%). Mancozeb had the highest percentage of root rot reduction, which was significantly higher than the other treatments. The disease reduction of Zacha 11 was approximately 53.33%, which was higher than that of JN2-007 (48.33%) (Table 1).

The accumulation of endogenous salicylic acid (SA)

There was an increase of endogenous SA content in cassava plants at 24 h after inoculation in all treatments (Fig. 1). The endogenous SA content in cassava treated with Zacha 11 elicitor at a concentration of 500 mg · l^{-1} was significantly increased by 69.95 µg · g^{-1} of fresh weight compared to the negative control (58.08 µg · g^{-1} of fresh weight).

Effects of the elicitors on growth, diseases and yield of cassava under field conditions

In location 1, disease severity indices of brown leaf spot and anthracnose on cassava cv. Rayong 72 were not different in all treatments, indicating no effect of elicitors and Mancozeb on these two diseases (Table 2). For bacterial blight and root rot diseases, the indices were 11.33 and 14.67%, respectively, on plants with the negative control treatment, while symptoms of the two diseases were not observed on the elicitors and

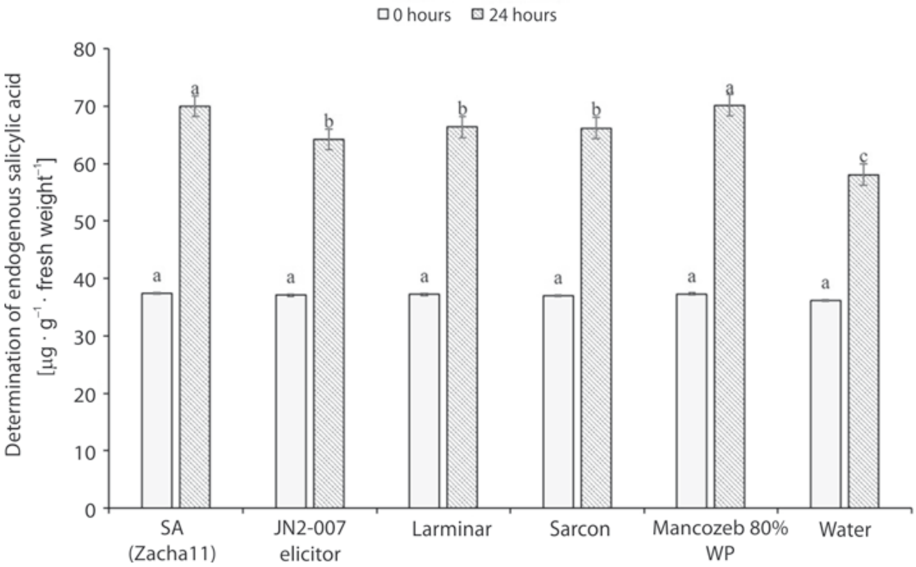

Fig. 1. Effectiveness of elicitors on the accumulation of endogenous salicylic acid in cassava accession number CMR-89

Table 2. Efficacy of elicitor treatments on severity of naturally occurring diseases on two cassava cultivars under field conditions of location 1 in Nakhon Ratchasima

Treatment[1]	Disease severity index[2] [%]							
	Cassava cv. Rayong 72				Cassava accession number CMR-89			
	brown leaf spot	anthrac-nose	bacterial blight	root rot	brown leaf spot	anthrac-cnose	bacterial blight	root rot
Zacha 11	33.33	42.67	0.00 a	0.00 a	14.67 ab	23.33	0.00 a	0.00 a
JN2-007	37.33	50.67	0.00 a	0.00 a	17.33 ab	27.33	0.00 a	0.00 a
Laminar	21.33	42.00	0.00 a	0.00 a	15.33 ab	22.67	0.00 a	0.00 a
Sarcon	20.00	39.67	0.00 a	0.00 a	15.00 ab	20.00	0.00 a	0.00 a
Mancozeb	18.67	37.33	0.00 a	0.00 a	7.33 a	17.33	0.00 a	0.00 a
Water control	38.00	51.33	11.33 b	14.67 b	25.33 b	37.33	7.33 b	24.67 b

[1]cassava plants were treated with Zacha 11, JN2-007, Laminar (commercial *Bacillus* 1 × 10^9 cfu · mg^{-1}), Sarcon (commercial salicylic acid 1,000 mg · l^{-1}), Mancozeb (80% WP) and water used as a negative control, under field conditions at the Suranaree University of Technology, Nakhon Ratchasima, Thailand; DMS: 14°52′44.7″N 102°00′14.6″E; [2]means in the column followed by the same letter are not significantly different according to Duncan's multiple range test at $p = 0.05$. Each value represents a mean of four replicates

Mancozeb treated cassava. On cassava cv. CMR-89, severity indices of brown leaf spot with the elicitor treatments including Zacha 11 (14.67%), commercial SA (15.33%) and JN2-007 elicitors (17.33%), were in the middle, but there was no statistical difference between the negative control (25.33%) and the Mancozeb treated plants (7.33%). No differences were observed for anthracnose disease severity on cassava of all treatments. Similar to what was observed on Rayong 72, bacterial blight and root rot diseases were not found on the elicitor-treated CMR-89, while plants in the negative control treatment showed 7.33 and 24.67% disease severity, respectively (Table 2). In location 2, severity indices of brown leaf spot on Rayong 72 did not differ between all treatments. However, for anthracnose, plants treated with Zacha 11 had the lowest severity index (50.00%), which was significantly different than the negative control (72.67%) (Table 3). For bacterial blight and root rot, the results were similar to those from location 1. No diseases were found with the treatments of elicitors and Mancozeb, but severity indices of 7.33 and 23.33% were recorded on plants in the negative control treatment, respectively. The reactions of treated cassava cv. CMR-89 to brown leaf spot and anthracnose disease severities of all elicitor treatments were not significant or significantly different from that of the negative control treatment. However, indices of elicitor treatments of bacterial blight and root rot were 0%, which was statistically lower than those of the negative control (Table 3).

For growth and yield parameters in location 1, in Rayong 72, Laminar and Sarcon gave the tallest plants (165.93 and 165.47 cm) but there was no statistical difference to other treated ones, except that of the negative control treatment which gave the lowest height (150.27 cm). In terms of tuber quantity in Rayong 72, JN2-007 gave the highest number of tubers per plant (8.80), seconded by Zacha 11 (8.60) and commercial SA (8.53), followed by Mancozeb (6.80), which were significantly higher than that of the negative control (2.33). There were no differences between tuber weights (5.11–7.84 $kg \cdot plant^{-1}$) and yields (51.07–78.40 $t \cdot ha^{-1}$) of plants treated with elicitors and Mancozeb, but they were all significantly higher than that of the negative control (2.11 $kg \cdot plant^{-1}$ and 21.07 $t \cdot ha^{-1}$, respectively). For the starch content, Zacha 11 treated plants gave the highest starch percentage (25.20%), which was significantly different from the negative control treatment by 14.17%. CMR-89, Zacha 11, JN2-007, Laminar, Sarcon and Mancozeb treatments did not significantly affect the plant height of CMR-89 (131.53, 124.00, 129.80, 124.50 and 125.00 cm, respectively) but the treated plants were all significantly taller than those in the negative control treatment. There were no differences of tuber weight per plant and tuber yield in plants of all treatments including those of the negative control. Zacha 11 treated plants also gave the highest starch content compared to those of the other treatments (Table 4). Similar results were also found in location 2, in which elicitor treatment seemed to have less effect on CMR-89's height, but commercial SA appeared to give the highest Rayong 72 plants (147.13 cm) compared to that of the negative control (122.60 cm) (Table 5). In terms of yields, Rayong 72 plants in all treatments gave significantly higher tuber numbers per plant than the negative control treatment but no differences were found in CMR-89 in all treatments including that of the negative control. Likewise, there were no differences in all other yield parameters of CMR-89 treated plants compared to that of the negative control; but

Table 3. Efficacy of elicitor treatments on severity of naturally occurring diseases on two cassava cultivars under field conditions of location 2 in Nakhon Ratchasima

Treatment[1]	Disease severity index[2] [%]							
	Cassava cv. Rayong 72				Cassava accession number CMR-89			
	brown leaf spot	anthracnose	bacterial blight	root rot	brown leaf spot	anthracnose	bacterial blight	root rot
Zacha11	34.00	50.00 ab	0.00 a	0.00 a	32.00	68.67 ab	0.00 a	0.00 a
JN2-007	34.00	72.67 c	0.00 a	0.00 a	36.67	67.33 ab	0.00 a	0.00 a
Laminar	31.33	60.67 bc	0.00 a	0.00 a	37.33	75.33 b	0.00 a	0.00 a
Sarcon	32.67	61.33 bc	0.00 a	0.00 a	29.33	67.07 ab	0.00 a	0.00 a
Mancozeb	26.67	36.00 a	0.00 a	0.00 a	26.67	62.00 a	8.00 ab	0.00 a
Water control	45.33	72.67 c	7.33 b	23.33 b	41.33	79.33 b	8.67 b	23.33 b

[1]cassava plants were treated with Zacha 11, JN2-007, Laminar (commercial *Bacillus* 1×10^9 $cfu \cdot mg^{-1}$), Sarcon (commercial salicylic acid 1,000 $mg \cdot l^{-1}$), Mancozeb (80% WP) and water used as a negative control, under field conditions at the Suranaree University of Technology, Nakhon Ratchasima, Thailand; DMS: 14°51′43.2″N 102°01′57.5″E.

[2]means in the column followed by the same letter are not significantly different according to Duncan's multiple range test at $p = 0.05$. Each value represents a mean of four replicates

for Rayong 72, plants treated with Zacha 11 gave the highest tuber weight per plant (4.95 kg), tuber yield (49.47 kg · ha^{-1}), and starch content (20.23%) compared to those treated with other elicitors and that of the negative control (1.73 kg, 17.33 kg · ha^{-1}, and 14.80%, respectively) (Table 5).

Discussion

From the greenhouse experiment, the results clearly show that cassava stalk soaking and foliage spraying with 500 mg · l^{-1} of SA in Zacha 11 formulation or a bioproduct of JN2-007 *B. subtilis* could increase cassava plant growth at 60 DAP. Zacha 11 and JN2-007 treated cassava plants cv. CMR-89 gave significantly higher growth parameters than those treated with commercial products and Mancozeb. Such effects were also observed under field conditions but were more pronounced in Rayong 72 than in CMR-89. Under the field conditions of location 2, Rayong 72 treated with Zacha 11 gave the highest values of all yield parameters assessed except that of stem height. Such findings indicate differential responses of cultivars to the elicitor treatment both in terms of types, formulation, and concentration of the active ingredients. It was clearly seen in stem height in that commercial SA, which

Table 4. Effect of elicitors on plant height, number of tubers, tuber weight, fresh tuber yields and starch content on two cassava cultivars under field conditions of location 1 in Nakhon Ratchasima

Treatment[1]	Cassava cv. Rayong 72[2]					Cassava accession number CMR-89[2]				
	plant height [cm]	number of tubers [tubers · plant^{-1}]	tuber weight [kg · plant^{-1}]	yield [t · ha^{-1}]	starch content [%]	plant height [cm]	number of tubers [tubers · plant^{-1}]	tuber weight [kg · plant^{-1}]	yield [t · ha^{-1}]	starch content [%]
Zacha 11	159.53 ab	8.60 bc	6.57 b	65.73 b	25.20 c	131.53 b	9.87 b	4.76	47.60	20.40 c
JN2-007	157.67 ab	8.80 c	7.84 b	78.40 b	17.47 ab	124.00 b	8.73 b	4.33	43.33	13.97 ab
Laminar	165.93 b	8.53 bc	5.95 b	59.53 b	18.10 ab	129.80 b	9.93 b	3.72	37.20	15.80 b
Sarcon	165.47 b	8.55 bc	5.84 b	58.36 b	17.39 ab	124.50 b	8.87 b	3.32	33.24	15.63 b
Mancozeb	160.87 ab	6.80 b	5.11 b	51.07 b	19.43 b	125.00 b	9.47 b	2.92	29.20	14.47 ab
Water control	150.27 a	2.33 a	2.11 a	21.07 a	14.17 a	109.87 a	4.60 a	2.36	23.60	12.30 a

[1]cassava plants were treated with Zacha 11, JN2-007, Laminar (commercial *Bacillus* 1×10^9 cfu · mg^{-1}), Sarcon (commercial salicylic acid 1,000 mg · l^{-1}), Mancozeb (80% WP) and water used as a negative control, under field conditions at the Suranaree University of Technology, Nakhon Ratchasima, Thailand; DMS: 14°52′44.7″N 102°00′14.6″E.

[2]means in the column followed by the same letter are not significantly different according to Duncan's multiple range test at $p = 0.05$. Each value represents a mean of four replicates

Table 5. Effect of elicitors on plant height, number of tubers, tuber weight, fresh tuber yields and starch content on two cassava cultivars under field conditions of location 2 in Nakhon Ratchasima

Treatment[1]	Cassava cv. Rayong 72[2]					Cassava accession number CMR-89[2]				
	plant height [cm]	number of tubers [tubers · plant^{-1}]	tuber weight [kg · plant^{-1}]	yield [t · ha^{-1}]	starch content [%]	plant height [cm]	number of tubers [tubers · plant^{-1}]	tuber weight [kg · plant^{-1}]	yield [t · ha^{-1}]	starch content [%]
Zacha 11	132.40 ab	8.40 b	4.95 c	49.47 c	20.23 b	134.27 ab	9.40	2.93	29.33	17.80
JN2-007	129.80 ab	8.20 b	3.88 bc	38.80 bc	15.73 a	140.47 b	9.27	2.92	29.20	15.03
Laminar	147.13 c	7.20 b	3.00 ab	30.00 ab	15.00 a	128.53 a	8.73	2.85	28.53	17.20
Sarcon	132.00 ab	7.09 b	3.15 ab	31.49 ab	15.05 a	128.43 a	7.57	2.47	24.36	15.48
Mancozeb	138.27 bc	6.67 b	2.60 ab	26.00 ab	14.87 a	130.13 a	7.67	2.59	25.87	15.80
Water control	122.60 a	3.49 a	1.73 a	17.33 a	14.80 a	125.00 a	7.47	2.35	23.47	13.77

[1]cassava plants were treated with Zacha 11, JN2-007, Laminar (commercial *Bacillus* 1×10^9 cfu · mg^{-1}), Sarcon (commercial salicylic acid 1,000 mg · l^{-1}), Mancozeb (80% WP) and water used as a negative control, under field conditions at the Suranaree University of Technology, Nakhon Ratchasima, Thailand; DMS: 14°51′43.2″N 102°01′57.5″E.

[2]means in the column followed by the same letter are not significantly different according to Duncan's multiple range test at $p = 0.05$. Each value represents a mean of four replicates

has twice as much SA (1000 mg · ml^{-1}) as Zacha 11 (500 mg · ml^{-1}), gave the tallest stem height to Rayong 72 but not to CMR-89. The higher concentration of SA recommended in the commercial SA formulation seemed to have no additive effect on tuber yield and starch content of Rayong 72 because the 500 mg · ml^{-1} SA rate in our formulation performed better in terms of increasing the yield. A lower concentration of SA not only saved the cost of treatment but also lowered the risk of SA phytotoxicity. Salicylic acid has been known to be phytotoxic to many crops when used at high concentrations. Enhancing effects of exogenous SA application on plant growth have been reported in several crops (Gharib 2006; Gawade and Sirohi 2011; Khandaker *et al.* 2011). Therefore, our findings can add cassava to the list of such crops. Also, salicylic acid is a plant hormone which plays an important role in plant defense and has a key role in the signal transduction pathways. SA increases in the area around wound lesions and remains high in plants having acquired resistance which are common plant biochemical responses associated with systemic acquired resistance (SAR) activation. Moreover, these defense genes associated with defense enzymes in plant cells were activated by early and secondary signaling transduction molecules and then triggered resistance in the plant against pathogen infection (Vallad and Goodman 2004; Buensanteai *et al.* 2009; Hinarejos *et al.* 2016).

From our greenhouse experiment, all the tested elicitors were equally effective in reducing *Fusarium* root rot severity, but were slightly less effective than that of Mancozeb. However, if we consider the benefit of SA and *Bacillus* as growth or yield promoters, as seen in our field experiment, as well as the drawback of using chemical fungicides, such marginal effects of fungicides can be ruled out. Under field conditions because the *Fusarium* was not inoculated, the root rot symptoms observed on plants in the negative control treatment could have been from many other soil-borne pathogens. The absence of root rot and bacterial blight symptoms observed on the elicitor treated plants indicated that the nature of SA and *Bacillus* in inducing disease resistance is broad-spectrum to cassava diseases. It has been shown that exogenous SA application could decrease leaf symptoms caused by *Glomerella cingulata* in apple plants (Zhang *et al.* 2016). Chávez--Arias *et al.* (2020) also reported that the foliar application of SA elicitor (100 mg · l^{-1}) in cape gooseberry seedlings reduced disease severity and vascular wilt caused by *F. oxysporum* f. sp. *physali*. Exogenously applied salicylic acid (0.2 mM) reduced *Rizoctonia solani* infection by 73% on potato tubers in the greenhouse (Hadi and Balali 2010).

Similarly, *Bacillus* spp. are well documented for their ability to enhance plant growth and induce systemic resistance (Kloepper *et al.* 2004; Ryu *et al.* 2004). In addition to directly affecting plant growth and development through plant growth regulators, *Bacillus* spp. could colonize roots and trigger plant biochemical and physiological systems to promote growth (Prathuangwong and Kasem 2004; Prathuangwong and Buensanteai 2007). These results are similar to those of Song *et al.* (2014) who suggested that the *Bacillus* species had promising potential as a microbial agent for the biocontrol of ginseng root rot caused by *Fusarium cf. incarnatum*. Hinarejos *et al.* (2016) showed that *B. subtilis* IAB/BS03 could reduce disease severity of two foliage diseases including *Botrytis cinerea* and *Pseudomonas syringae* on tomato. Root dip treatment with *B. subtilis* formulations showed a considerable increase in root length with *B. subtilis* (33 cm) and chlorothalonil (28.5 cm) when compared to untreated control (15 cm). Growth promotion was better with root dip application while better disease control was achieved with seed application. A 66 and 84% reduction in incitation of disease was noticed with soil and seed application methods (Narasimhan and Shivakumar 2016). Soaking and foliar spray application of salicylic acid and beneficial bacteria increased yields of many crops according to Hadi and Balali (2010) on potato, Javaheri *et al.* (2012) on tomato, Jonathan *et al.* (2015) on cassava and Yildirim *et al.* (2006) on cucumber. These increases in yields may be closely linked to the increase in plant growth characteristics, i.e., plant height, number of roots, root weight and yields.

In general, the elicitor formulations of Zacha 11 and JN2-007 could increase cassava growth, tuber weight, yield and starch contents, suppress root rot disease as well as bacterial leaf blight.

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