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Effect of genotype, concentration and timing of salicylic acid application to *Phalaenopsis* against *Dickeya dadantii* infection

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Abstract. *Sanjaya IPW, Sukma D, Sudarsono, Chan MT. 2020. Effect of genotype, concentration and timing of salicylic acid application to* Phalaenopsis *against* Dickeya dadantii *infection. Biodiversitas 21: 4317-4324.* Soft -rot disease caused by *Dickeya dadantii* (Dd) is one of the most devastating infection, affecting the cultivation of *Phalaenopsis* in tropical regions. Salicylic acid (SA) is known as an inducer of resistance against pathogens in some plant species. The study aims to identify the effects of genotypes, concentrations, and timing of salicylic acid application to *Phalaenopsis* against *D. dadantii* inoculation. In experiment 1, eight *Phalaenopsis* genotypes were treated by 60 ppm SA and then inoculated with *D. dadantii*. At a dose of 60 ppm, SA did not increase the resistance to *D. dadantii* in any genotypes. In experiment 2, a representative of a very susceptible genotype (*P. amabilis*) was treated with high SA concentrations (60, 360, and 720 ppm). The results demonstrated that at SA concentrations of 360 and 720 ppm, slightly increase *P. amabilis* resistance to *D. dadantii*. In experiment 3, the most effective SA concentration (720 ppm) was used at 2, 3, 5, or 7 days after SA treatment. The timing of the SA application did not affect the resistance of *P. amabilis* to *D. dadantii*. The present study shows that SA concentrations up to 60 ppm did not inhibit *D. dadantii* infection. Nevertheless, such inhibition was observed at a high SA dose (720 ppm) for a short period.

Keywords: *Dickeya dadantii*, inducer, *Phalaenopsis*, resistance, salicylic acid, soft-rot disease

INTRODUCTION

Phalaenopsis is an important orchid genus used as ornamental plants. This genus is interesting due to variations in color, shape, size, pattern of flower, and long flowering period (Wang and Hsu 1994). However, due to the soft-rot disease found in tropical regions, growers must face a significant problem in *Phalaenopsis* cultivation. Soft-rot disease (SRD) mostly attacks *Phalaenopsis* orchids at elevated temperature and high humidity (Sukma et al. 2017). SRD is controlled by cutting off the infected leaves or application of bactericidal compounds. However, these methods are ineffective. For example, a wound left after cutting provides an easy point of entry for pathogens. Meanwhile, the bactericidal application leaves residues on the plant leaves, which reduces the quality of *Phalaenopsis* as an ornamental plant.

Molecular detection and characterization showed that SRD in *Phalaenopsis* is caused by *Dickeya dadantii* (Sudarsono et al. 2018). Previous studies showed that most of the *Phalaenopsis* species are susceptible, however, several species are comparatively resistant to SRD, i.e., *Phalaenopsis pantherina* is moderately resistant, *Phalaenopsis amboinensis*, *Phalaenopsis javanica* and *Phalaenopsis fimbriata* are fully resistant against SRD (Elina 2016; Raynalta 2017; Sukma et al. 2017). Efforts are made to increase *Phalaenopsis* resistance from SRD to reduce plant losses and related economic losses in nurseries. Conventional breeding approaches for the development of SRD resistant *Phalaenopsis* have not been successful due to the limited sources of resistant species or cultivars for crossing. Because of this, SRD resistant transgenic orchids were produced by using ferredoxin-like protein (pflp) on *Oncidium* (Liau et al. 2003) and *Phalaenopsis* (Chan et al. 2005), and a Wasabi defensin gene (Sjahril et al. 2006) in *Phalaenopsis*. However, the adoption of transgenic plants for commercial production faces many challenges, including regulatory constraints.

Naturally, plants have developed mechanisms of resistance to pathogen infections. Plant resistance involves three central systems, such as pathogen detection, signal transduction, and plant resistance responses (Andersen et al. 2018). Plants exposed to pathogens will activate plant signals in the form of phytohormones such as ethylene (ET), jasmonic acid (JA), and salicylic acid (SA) (Han and Kahmann 2019). Signal transduction is an essential step towards activating the plant's resistance response to the pathogen. SA induces plant resistance through the systemic acquired resistance (SAR) pathway, which activates the physical and chemical resistance mechanisms (Tripathi et al. 2019). Exogenous SA application has been reported to increase the resistance of tomato against TYLCV (Li et al. 2019); rice against *Xanthomonas oryzae* pv. *oryzae* (Leiwakabessy et al. 2018), and *Phalaenopsis* against *D. dadantii* (Firgiyanto et al. 2015).

Market demand is primarily focused on hybrid *Phalaenopsis* genotypes, a complex genetic background derived from parents with different resistance classes (Sukma et al. 2017). Importantly, the response to exogenous SA applications may be affected by the genetic background of the plants (Leiwakabessy et al. 2017). Therefore, SA evaluation in many species or cultivars is necessary before widespread application in nurseries. Exogenous SA concentration is a critical matter regulating its function as a plant hormone. The 10 ppm SA concentration induced resistance in *Phalaenopsis* hybrid KHM 205 but did not induce resistance in KHM 1138 against *D. dadantii* infection (Firgiyanto et al. 2016). Induction of *Phalaenopsis amabilis* resistance using high SA concentrations (≤85 ppm) can suppress the growth of *Fusarium oxysporum in vitro* (Noviantia 2016). Ozpinar et al. (2017) stated that the negative effects of SA were found when it was applied in high concentrations (>2000 ppm) on *Zea mays*, *Triticum aestivum*, and *Cicer arietinum*. These effects included the inhibition of root and stem growth.

Importantly, the effects of Salicylic acid (SA) on *Phalaenopsis* resistance to SRD due to *D. dadantii* have not been widely investigated. This study aims to evaluate resistance responses of several *Phalaenopsis* genotypes to SA treatment at various concentrations and timing of application to enable the development of an optimal SA regime for stimulating *Phalaenopsis* immunity to SRD caused by *D. dadantii*.

MATERIALS AND METHODS

Study area

The study was conducted at the Laboratory of Plant Molecular Biology I and the Orchid House in the Leuwikopo Experimental Garden, Faculty of Agriculture, IPB University, Bogor, Indonesia, from January to March 2020. The study consisted of three sets of experiments.

Experiment 1: Effect of genotype on the response of *Phalaenopsis* to *Dickeya dadantii* after SA treatment

In this experiment, one-year-old seedlings were used after acclimatization in the greenhouse. Each plant had three to four leaves. A factorial experimental design was implemented with two factors, such as *Phalaenopsis* genotype and SA concentration. The treatment combination was presented in Table 1. The eight *Phalaenopsis* genotypes examined were *Phalaenopsis amabilis* (l.) Blume (PAB), *Phalaenopsis cornucervi* (Breda) Blume &Rchb. F. (PCC), *Phalaenopsis pulcherrima* (Lindl.) J.J.Sm. (PPUL), *Phalaenopsis amboinensis* J.J Smith (PAM), *Phalaenopsis amabilis* x *Phalaenopsis schilleriana* Rchb.f. (PAPS), *Phalaenopsis bellina* (Rchb.f.) Christenson x *Phalaenopsis tetraspis* Rchb.f. (*P.* '*Beltret*'), *Phalaenopsis* '*Luzon*' x *P. mannii* Rchb. F. (*P.* '*Luzman*') and *Phalaenopsis deliciosa* Rchb. F. x *Phalaenopsis pulcherrima* (PDPP). One SA concentrations (60 ppm) was tested. There were sixteen combinations of treatments with three replicates per treatment. Plant materials were kept in the orchid house with a relative humidity of 50%, and they were fertilized once a week with leaf fertilizer and B1 vitamin. The plant leaves were wiped with wet cotton to remove all residues and treated with 60 ppm SA by foliar spray application until run-off at 24 hours before *D. dadantii* inoculation. Leaves sprayed with water (0 ppm SA) were served as control treatment.

Experiment 2: Effect of SA concentration on the response of *Phalaenopsis amabilis* to *Dickeya dadantii*

The experiment used one-year-old *P. amabilis* seedlings that were most susceptible to *D. dadantii*. Three SA concentrations, i.e., 60, 360, and 720 ppm, were used in a one-factor treatment. Spraying with water (0 ppm SA) was used as the control treatment. All treatments were repeated six times, and every replicate used one plant. The *Phalaenopsis* were treated with the SA solution by foliar spraying at 24 hours before *D. dadantii* inoculation. All treated plants were kept in the orchid house with a relative humidity of 50%, and the leaves were prepared for *D. dadantii* inoculation, as described in experiment 1.

Experiment 3: Time of SA application effect on *Phalaenopsis amabilis* response against *Dickeya dadantii*

For this experiment, *P. amabilis* that was most susceptible to *D. dadantii*, was also used. Different timing of SA application was tested in this one-factor treatment. The plants were treated with SA concentration at 720 ppm. The timing of SA treatments was 2, 3, 5, and 7 days before *D. dadantii* inoculation (DBI). Each SA treatment was repeated ten times, and each replicate used one plant. For this experiment, 720 ppm SA was applied by foliar spraying. All plants were kept as described in experiment 1 and 2. Plant leaves were prepared for *D. dadantii* inoculation as previously described.

Dickeya dadantii inoculation procedures

The isolate of *D. dadantii* used in experiments 1, 2, and 3 was the same one isolated during previous research conducted by Putri (2019). A single colony was taken using a sterile inoculation loop and cultured on 15 ml of lactose broth (LB) medium. The bacterial culture was incubated in a 100 RPMs shaker for 24 hours at room temperature, then 1 ml of aliquot was sedimented by centrifugation at 8000 RPM for 6 minutes. Subsequently, bacterial pellets were diluted in 1 ml of sterile distilled water, and this was repeated to seven serial dilutions. Each dilution was plated on nutrient agar (NA) medium, and the plates incubated at room temperature for 24 hours. Subsequently, the colonies were counted and converted into CFU ml^{-1}. The *D. dadantii* inoculation was performed through an intact leaf inoculation protocol. Each leaf was pricked with sterilized pins, and 10 µL droplet of *D. dadantii* suspension was deposited in the wounded site. The plants with inoculated leaves were incubated in a closed box at room temperature and 75% humidity.

Table 1. Soft-rot symptom diameter (SD), disease severity (DS), resistance classes (RC) of *Phalaenopsis* genotypes against *Dickeya dadantii* infection after salicylic acid (SA) treatment and *D. dadantii* colony-forming unit (CFU) in the infected leaves

Accessions/ treatments		12 HPI		18 HPI		24 HPI		30 HPI		
		SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	CFUmg^{-1}
P. *'Luzman'*	SA0	0.0f	0d (R)	0.0e	0c (R)	0.0de	0c (R)	0.0f	0c (R)	1 x104
	SA60	1.6ef	20d (R)	4.4de	47b (MS)	7.6d	77a(S)	10.8d	100a (VS)	3 x 104
PAM	SA0	1.5ef	20bc (R)	2.2d	27b (MR)	3.2d	37b (MR)	3.9e	43b (MS)	2 x104
	SA60	1.4ef	20cd (R)	3.4e	40b (MR)	3.6e	40b (MR)	4.4e	47b (MS)	1 x 106
P. *'Beltret'*	SA0	3.0cd	30cd (MR)	6.4c	70a (S)	9.7c	100a (VS)	13.2d	100a (VS)	5 x 105
	SA60	4.2cd	50abc (MS)	8.3cd	90a (VS)	12.4cd	100a (VS)	14.1cd	100a (VS)	2 x 105
PAPS	SA0	2.0de	20c (R)	6.0c	73a (S)	9.6c	100a (VS)	13.0d	100a (VS)	2 x 105
	SA60	2.8de	33bcd (MR)	7.1cde	73a (S)	16.1bc	100a (VS)	19.2bc	100a (VS)	9 x104
PCC	SA0	3.8bc	47ab (MS)	10.1bc	100a (VS)	17.2b	100a (VS)	16.7cd	100a (VS)	4 x106
	SA60	5.5bc	60ab (MS)	11.9bc	100a (VS)	20.7ab	100a (VS)	19.6bc	100a (VS)	3 x106
PAB	SA0	6.7ab	70a (S)	14.5ab	100a (VS)	22.7ab	100a (VS)	22.7ab	100a (VS)	9 x 104
	SA60	5.6ab	60ab (MS)	8.9bc	87a (VS)	18.5abc	100a (VS)	24.2ab	100a (VS)	3 x104
PDPP	SA0	4.8bc	53ab (MS)	10.3bc	100a (VS)	18.5b	100a (VS)	23.2bc	100a (VS)	1 x 105
	SA60	6.1bc	70ab (S)	14.0b	100a (VS)	23.2ab	100a (VS)	28.7a	100a (VS)	2 x 105
PPUL	SA0	6.8a	70a (S)	16.8a	100a (VS)	26.8a	100a (VS)	33.6a	100a (VS)	3 x 106
	SA60	11.2a	97a (VS)	21.2a	100a (VS)	24.4a	100a (VS)	30.3a	100a (VS)	1 x 105

Note: The numbers in a column followed by similar letter are not significantly different in the Tukey test at α : 0.05; HPI: hours post-inoculation; VS: Very Susceptible; S: Susceptible; MS: Moderate Susceptible; MR: Moderate Resistant; R: Resistant

The observed variables in all experiments were the diameter of soft-rot symptom on inoculated leaves, which was recorded every 6 hours post-inoculation (HPI). Symptom diameter was the basis of disease response assessment for each the tested accession. The calculated disease responses include disease severity (DS), resistance class (RC), and AUDPC (area under disease progress curve). Bacterial populations on leaf samples were recorded at 30 HPI. DS and RC were calculated by applying the formula given by Raynalta (2017) with the modified scoring. The diameter of soft-rot symptom was scored as below:

0 (no symptom); 2 (1.1-2 mm); 4 (3.1-4 mm);
6 (5.1-6 mm); 8 (7.1-8 mm); 10 (> 9 mm)
1 (0.1-1 mm); 3 (2.1-3 mm); 5 (4.1-5 mm);
7 (6.1-7 mm); 9 (8.1-9 mm);

$$\text{Disease severity (DS, \%)} = \frac{\Sigma (ni \times vi)}{Z \times N} \times 100\%$$

ni : 0, 1, 2, 3, 4, 5, 6, 7, 8,9, and 10
vi : disease score in the score of *i*
Z : maximum score value
N : number of samples observed

The calculation of the area under the disease progress curve (AUDPC) was conducted, according to Madden et al. (2007). The bacterial population in leaves showing soft-rot symptom was evaluated through colony-forming units (CFU). Leaf samples (50 mg) of the area between the infected and non-infected zones were taken, ground, and suspended in 1 ml of sterile H_2O. Five times serial dilutions were then prepared, and 3 µL of the suspension was placed on nutrient agar (NA) medium. The cultures were incubated at room temperature overnight, and the colonies were counted as CFU mg^{-1}.

Data analysis

The collected data were analyzed by analysis of variance (ANOVA) using the STAR (Statistical Tool for Agricultural Research) application, and the significant treatments were further tested with the Tukey test at α = 0.05.

RESULTS AND DISCUSSION

Experiment 1: Resistance response of *Phalaenopsis* genotypes to 0 and 60 ppm SA treatments

Statistical analysis showed significant differences in SRD symptom diameter and disease severity among the *Phalaenopsis* genotypes, which can be seen at 12 to 30 HPI. Disease severity results showed different resistance classes among genotypes. All samples exhibited soft-rot symptoms at 12 HPI. In order from the smallest to the largest SRD symptom diameter, the genotypes were *P.* '*Luzman*', PAM, PAPS, *P.* '*Beltret*,' PCC, PDPP, PAB, and PPUL. At 12 HPI, the symptom diameter of *P.* '*Luzman*' and PAM were significantly smaller than that of *P.*' *Beltret*,' PCC, PDPP, PAB, and PPUL. Moreover, the symptom diameter of PAPS was significantly lower than that of PCC, PAB, PDPP, and PPUL (Table 1). On the other hand, the symptom diameter of *P.* '*Luzman*' and PAM showed no significant difference.

At 12 HPI, *P.* '*Luzman*,' PAM, and PAPS were grouped as resistance at control (0 ppm SA). While, PAPS at 60 ppm SA and *P. 'Beltret'* at control (0 ppm SA) were moderately resistant. Meanwhile, the other accessions were grouped as either moderately susceptible, susceptible or very susceptible. The resistance response to *D. dadantii* infection of the PAPS, *P.* '*Beltret*,' PCC, PDPP, PAB, and PPUL decreased at 18 HPI (Table 1).

There were no significant differences in SRD symptom diameter among the different SA treatments in any of the

genotypes at 12 HPI. However, resistance class changes were found in PPUL from susceptible to very susceptible, PAPS from resistant to moderately resistant, and PDPP from moderately susceptible to susceptible after 60 ppm SA treatment at 12 HPI. Observation at 18 HPI showed no significant differences in SRD symptoms among the SA treatments except in PAM which showed significantly wider symptom at 60 ppm SA. Low resistance classes were found in 60 ppm SA treatment in *P.* '*Beltret*' from susceptible to very susceptible, while in *P.* '*Luzman*' from resistant to moderately susceptible. The same pattern was also found in 24 and 30 HPI observations. In general, the results indicated that 60 ppm SA treatment did not increase the resistance response of all *Phalaenopsis* genotypes to *D. dadantii* infection.

At 30 HPI, the genotypes were grouped as either susceptible (S) or very susceptible (VS). *P.* '*Luzman*' was identified as resistant (R) at 0 ppm SA (contol). PAM was moderately resistant (MS) at 0 and 60 ppm SA. In the 60 ppm SA treatment, a significant increase in disease symptom diameter was observed in PAPS, PDPP and *P.* '*Luzman*'.

Disease progression between genotypes in control (0 ppm SA) and 60 ppm SA treatments is shown by the AUDPC graphic (Figure 1). Most genotypes showed the maximum AUPDC at 30 HPI; meanwhile, *P.* '*Luzman*' and PAM showed the lowest disease progression. The SRD symptoms of the genotypes at 30 HPI can be seen in Figure 1. Table 1 presented the CFU data for 30 HPI. The CFU count was the lowest in P. '*Luzman*,' and PAM at control (0 ppm SA). *P.* '*Luzman*,' PAM, and PDPP showed lower CFU count in control (0 ppm SA) compared to that in 60 ppm SA treatments. In contrast, other genotypes had a higher CFU count at 60 ppm SA than that in control (0 ppm SA). *P.* '*Luzman*' at control (0 ppm SA) showed a consistent resistance response at 12, 18, 24, and 30 HPI, however at 60 ppm SA treatment, its resistance response gradually decreases from resistance to very susceptible.

Experiment 2: *Phalaenopsis amabilis* resistance response to SRD caused by *Dickeya dadantii* at different SA concentrations

The soft rot symptom diameter, disease severity and CFU data are presented in Table 2. Different SA concentrations for PAB applied one day before *D. dadantii* inoculation induced a significant difference in soft-rot symptom diameter and disease severity at all time-points. The control (0 ppm SA) treatment produced the highest symptom diameter and disease severity with the lowest resistance class at 12 to 30 HPI. On the other hand, 720 ppm SA treatment reduced the soft-rot symptom diameter and disease severity at 18 HPI with the highest resistance class at 30 HPI. Finally, at 30 HPI showed significant differences in soft-rot symptom diameter among PAB on control (0 ppm SA), 60, 360, and 720 ppm SA. The lowest disease severity at 30 HPI was found at 720 ppm SA treatment.

The AUDPC graphic in Figure 2 showed the lowest disease progression at 720 ppm SA treatment compared to control (0 ppm SA). Figure 2 showed that PAB was more resistant to SRD in the 360 and 720 ppm SA treatment. The SA treatment tended to suppress the *D. dadantii* population on infected leaves, as shown by lower CFU values in 720 ppm SA treatment that that of others. The highest CFU was found at control (0 ppm SA) treatment, and the lowest was at 720 ppm SA treatment, followed by 360 ppm SA. Figure 2 presents the leaf soft-rot symptom performance in four SA concentration treatments.

Experiment 3: *Phalaenopsis amabilis* resistance response to SRD caused by *Dickeya dadantii* for different application times of 720 ppm SA

The results of experiment 3 are presented in Table 3. The best 720 ppm SA concentration was applied to increase PAB resistance at various times before *D. dadantii* inoculation. No significant difference was observed in soft-rot symptom diameter, disease severity, and resistance class at 12 to 30 HPI among the treatments. Application of SA at various times (2, 3, 5, and 7 DBI) increased the disease severity up to 100%. A high bacterial population (8 x 10^6 to 3 x 10^7 CFUmg^{-1}) makes PAB very susceptible to *D. dadantii* infection in all treatments at 30 HPI. All treatments showed the same pattern of AUPDC, and soft-rot symptom diameter, as shown in Figures 3.

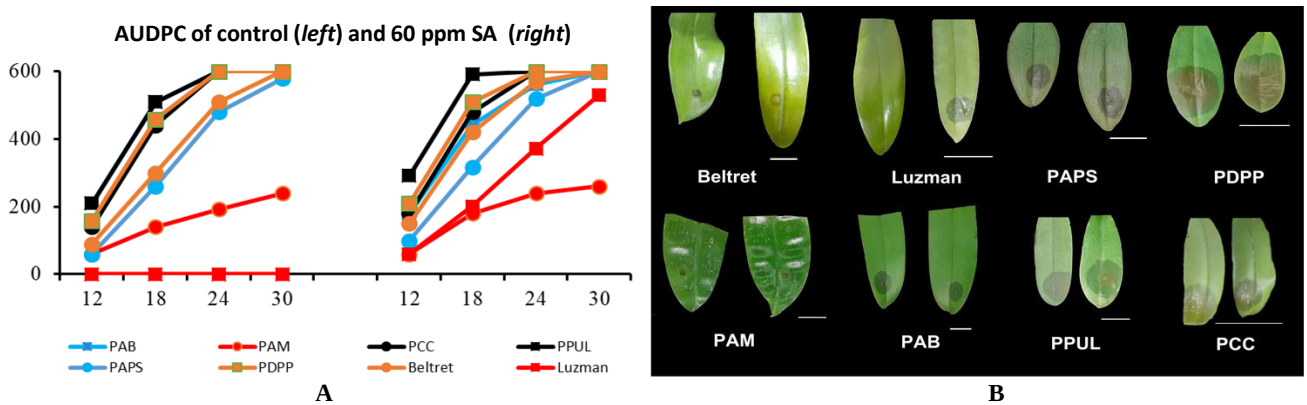
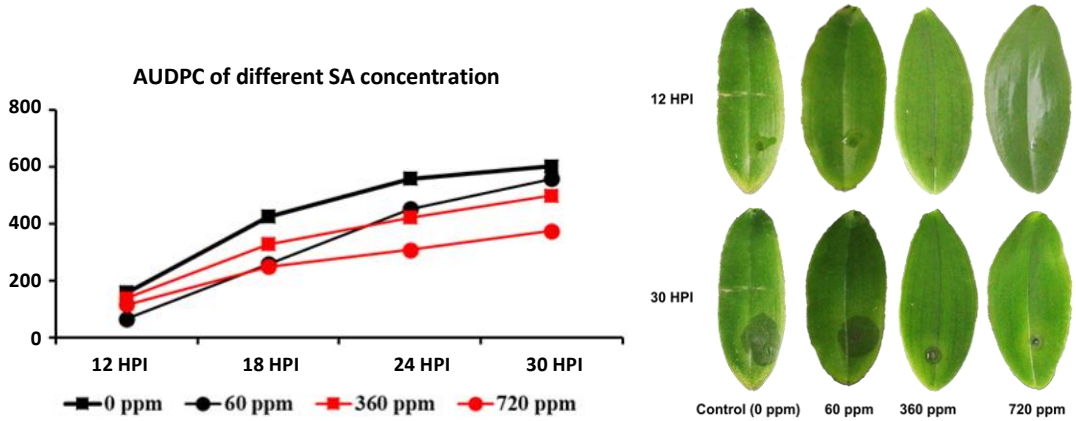
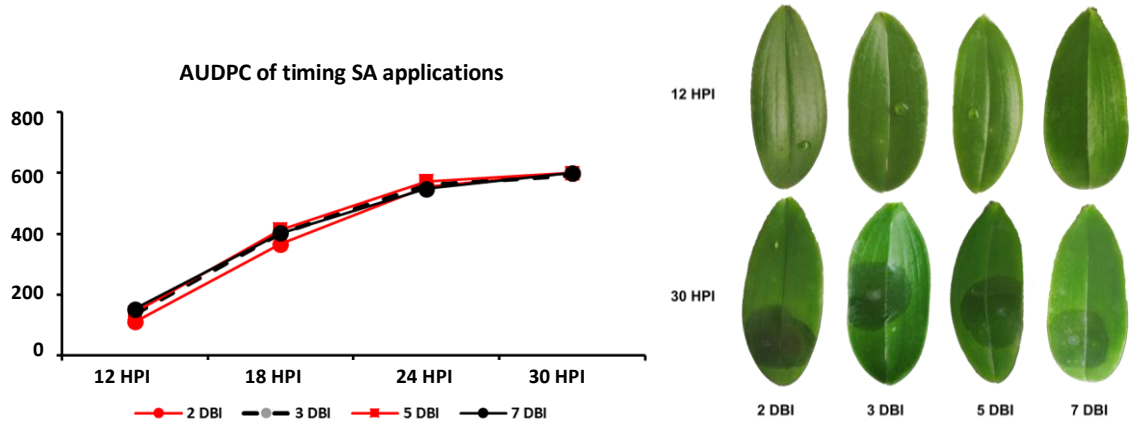


Figure 1. The AUDPC (area under the disease progress curve) (A) and soft-rot disease symptom (B) of *Phalaenopsis* genotypes after control (0 ppmSA) (*left*) and 60 ppm SA treatments (*right*) at 30 hours post – *Dickeya dadantii* inoculation. Scale bars represent 2 cm



A **B**

Figure 2. The AUDPC (area under the disease progress curve) (A) and soft-rot disease symptom (B) in leaves of *Phalaenopsis amabilis* at different salicylic acid concentrations



A **B**

Figure 3. The AUDPC (area under the disease progress curve) (A) and the soft-rot disease symptoms (B) at different timing of SA application in *Phalaenopsis amabilis*

Table 2. Soft-rot symptom diameter (SD), disease severity (DS), resistance classes (RC) of *Phalaenopsis* genotypes against *Dickeya dadantii* infection after salicylic acid treatment and *D. dadantii* Colony forming unit on infected leaves

SA Treatments	12 HPI		18 HPI		24 HPI		30 HPI		
	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	CFU mg^{-1}
0 ppm	4.1^{a}	48^{a} (MS)	6.5^{a}	53^{a} (S)	10.3^{a}	88^{a} (VS)	14.6^{a}	93^{a} (VS)	6 X 10^{6}
60 ppm	1.6^{b}	20^{b} (R)	5.4^{ab}	58^{a} (MS)	$7.6a^{b}$	83^{a} (S)	10.8^{ab}	90^{a} (VS)	1 X 10^{7}
360 ppm	3.9^{a}	42^{a} (MS)	5.2^{ab}	57^{a} (MS)	6.4^{b}	70^{ab} (S)	7.6^{bc}	80^{ab} (S)	4 X 10^{6}
720 ppm	2.7^{ab}	35^{ab} (MR)	3.8^{b}	40^{ba} (MR)	5.1^{b}	53^{b} (S)	5.8^{c}	60^{b} (S)	2 X10^{5}

Note: The numbers in a column followed by similar letter are not significantly different in the Tukey test at $\alpha = 0.05$; HPI: Hours Post-Inoculation; VS: Very Susceptible; MS: Moderate Susceptible; MR: Moderate Resistant

Table 3. Soft-rot symptom diameter (SD), disease severity (DS), resistance classes (RC) of *Phalaenopsis amabilis* leaves due to *Dickeya dadantii* infection after different timing of salicylic acid treatments and *D. dadantii* colony-forming unit on infected leaves

Treatments	12 HPI		18 HPI		24 HPI		30 HPI		
	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	SD (mm)	DS% (RC)	CFU mg^{-1}
2 DBI	3.8^{a}	37^{a} (MR)	8.1^{a}	85^{a} (VS)	13.2^{a}	100^{a} (VS)	19.3^{a}	100^{a} (VS)	3 x 10^{7}
3 DBI	4.0^{a}	45^{a} (MS)	8.6^{a}	89^{a} (VS)	14.1^{a}	98^{a} (VS)	20.2^{a}	100^{a} (VS)	3 x 10^{7}
5 DBI	4.4^{a}	47^{a} (MS)	9.1^{a}	91^{a} (VS)	14.8^{a}	100^{a} (VS)	21.5^{a}	100^{a} (VS)	8 x 10^{6}
7 DBI	4.6^{a}	51^{a} (MS)	9.6^{a}	83^{a} (VS)	14.8^{a}	100^{a} (VS)	21.6^{a}	100^{a} (VS)	1 x10^{7}

Note: The numbers in a column followed by similar letter are not significantly different in the Tukey test at $\alpha = 0.05$; HPI: Hours Post-Inoculation; VS: Very Susceptible; MS: Moderate Susceptible; MR: Moderate Resistant

Discussion

Incontrol (0 ppm SA), almost all of the *Phalaenopsis* genotypes showed rapid development of soft-rot symptom, but it was slower in the *Phalaenopsis* '*Luzman*' and PAM genotypes. Sukma et al. (2017), and Firgiyanto et al. (2016) reported PAM (*P. amboinensis*) to be the most resistant *Phalaenopsis* species against *D. dadantii* when compared to *Phalaenopsis pantherina, Phalaenopsis schilleriana, Phalaenopsis cornu-cervi, Phalaenopsis modesta, Phalaenopsis gigantea, Phalaenopsis pulcherrima, Phalaenopsis fimbriata, Phalaenopsis bellina, Phalaenopsis amabilis*, hybrid genotypes *Phalaenopsis* 'KHM 205', 1126, 1318, 2249, and AMP 17. The result presented in this study confirmed that *P. amboinensis* was a more resistant genotype, as reported in the previous research (Firgiyanto et al. 2016; Sukma et al. 2017).

SA concentration of 60 ppm was not able to induce resistance in the *Phalaenopsis* genotypes but instead increased disease severity in PAPS, PDPP, and *P.'Luzman*'. Leiwakabessy et al. (2017) have reported that the IR64 rice variety had the lowest leaf blight symptom progression due to the Xoo (*Xanthomonas oryzae* pv. *oryzae*) pathotype VIII at 5 mM SA (equivalent to 690.60 ppm), while in Conde and Ciherang varieties, it was at 10 mM (equivalent to 1381.21 ppm). A lower SA concentration was observed to induce resistance in one rice variety, but a higher concentration was required in other varieties, indicating that different genotypes respond differently to specific SA concentrations. Klessig et al. (2018) reported that SA regulates plant growth and development in a concentration-dependent manner, where a high level of SA stimulates ROS accumulation resulting in oxidative stress and cell death (Poór 2020). To find the best SA concentration for inducing resistance in very susceptible *Phalaenopsis* against SRD, we applied different SA concentrations to *P. amabilis* one day before *D. dadantii* inoculation. Higher SA concentrations of 360 and 720 ppm induced greater PAB resistance against SRD compared to 60 ppm. These results are in accordance with the report by Leiwakabessy et al. (2017), who found that the 10 mM (equivalent to 1381.21 ppm) SA concentration had greater resistance against *X. oryzae* than 5 mM (equal to 690.60 ppm) SA concentration in the Conde and Ciherang rice varieties. At low concentrations, SA induces plant immunity only to a small degree and may not elicit a response at all. However, at high concentrations, it directly induced defense-related gene expression (Conrath et al. 2006; Klessig et al. 2018).

Plant resistance to pathogens is controlled by several factors, including physical and biochemical resistance mechanisms (Chandrakanth et al. 2018; Saeed and Tahira 2019), as well as passive and active resistance. Active resistance is induced by plant recognition of pathogen attacks (Bacete et al. 2018). This recognition has signaled mainly in the form of hormones such as SA (Andersen et al. 2018). SA is known to induce plant resistance through the SAR pathway activating pathogen-related (PR) proteins (Ding and Ding 2020; Malik et al. 2020). Application of 60 ppm SA in Experiment 1 elicited a wider symptom diameter compared to control in PAPS, PDPP, and *P.* 'Luzman'. SA stimulates ROS accumulation, resulting in oxidative stress and cell death (Kapoor et al. 2019; Huang et al. 2019). However, the host cell hypersensitive response provides a suitable environment for *D. dadantii*, which is a necrotrophic pathogen (Mengiste 2012).

SA regulates plant growth, development, and induces plant defense-related gene expression (Klessig et al. 2018; Conrath et al. 2006). It induces peroxidase, chitinase, 1,3-glucanase, thaumatin, proteinase inhibitors, endo-proteinase, endo-chitinase, lysozyme activity, thionine, and lipid transfer proteins (Ali et al. 2018; Devi et al. 2017; Bektas and Eulgem 2015). Some of these enzymes may be capable of retarding *D. dadantii* infection by suppressing bacterial growth. In this study, a lower CFU value was found in plants treated with 720 ppm SA, probably the results of inhibition by the previously mentioned antimicrobial polypeptides (Ali et al. 2018; Devi et al. 2017; Bektas and Eulgem 2015). The timing of the SA application before pathogen inoculation has been considered to affect its resistance. Li and Zou (2017) and Chandrasekhar et al. (2017) found that exogenous application of SA at three days before inoculation induced resistance in *Botrytis cinereal* infected tomato plants and *Ralstonia solanacearum* infected chili.

High bacterial populations were observed in susceptible and very susceptible plants in all experiments. A high CFU counts indicate a higher bacterial activity, supports quorum sensing to release the enzymes that degrade cell wall, and elicit the soft-rot symptoms (Joshi et al. 2020; Potrykus et al. 2017; Khoiri et al. 2017). The highest SA concentration (720 ppm) applied to PAB showed the lowest CFU value. Presumably, in addition to increasing ROS activity, SA also increased the expression of other biochemical and physical resistance genes and suppressed the growth of pathogens (Ali et al. 2018; Devi et al. 2017).

Even though high SA concentration (720 ppm) induced resistance to PAB against SRD caused by *D. dadantii*, the resistance only lasted a short time. Furthermore, SA could promote negative effects on plants. SA reduces plant fitness by reducing growth and impairing seed set in wheat (Heil et al. 2000).

In conclusion, most of the genotypes were found to be susceptible or very susceptible to *D. dadantii*. There were only two potentially resistant genotypes, named *P. amboinensis* and *P.* 'Luzman' (hybrid). The application of 60 ppm SA did not increase the resistance of the genotypes and even decreased resistance to *D. dadantii* in some cases. A high SA concentration (720 ppm) was applied to *P. amabilis*, which slightly increased the resistance to *D. dadantii* for a short period. The timing of SA treatment (2, 3, 5, 7 days) before *D. dadantii* inoculation did not alter *P. amabilis* resistance. Further study is necessary to clarify the function of SA in the resistance mechanism of *Phalaenopsis* to *D. dadantii* through the comparison of SA dynamics in resistant versus susceptible genotypes under non-SA and SA exogenous application.

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