Inoculation Methods to Determine Resistance of *Phalaenopsis amabilis* (L.) Regenerated from Irradiated Protocorms to *Dickeya dadantii*

10.18196/pt.v12i1.16731

Halida Adistya Putri [1*], Dewi Sukma[2], Sudarsono Sudarsono[2], dan Agus Purwito[2]

[1]Oil Palm Education Polythechnic. Jl. Gapura No. 8, Bekasi, West Java, 17520. Indonesia
[2]Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. Jl. Meranti, Bogor, West Java, 16680. Indonesia

[*]Corresponding email: halida.adistya@cwe.ac.id

ABSTRACT

Soft-rot disease (SRD) in Phalaenopsis, caused *Dickeya dadantii*, has resulted in significant losses in the orchid sector in Indonesia. This study aimed to evaluate the inoculation method of *Dickeya dadantii* and identify the resistance response of individual regenerated plantlets of *Phal. amabilis* from irradiated protocorm. A detached leaf assay was used to evaluate the inoculation method and resistance response of SRD. Based on the results of this study, *Dickeya dadantii* bacteria could only infect the leaves through wounding tissue. The density of bacteria that could infect leaf tissue was OD600 = 0.2. All dilution factors tested caused soft rot symptoms in *P. amabilis*. On the other hand, *Vanilla planifolia* only showed symptoms at a dilution factor of 10-0. Four accessions of regenerated plantlets from irradiated protocorms were resistant to SRD. They were from irradiation 5 Gy (IP 05 Gy-23, IP 05 Gy-31, and IP 05 Gy-33) and one accession from the control treatment or without irradiation (IP 0 Gy -1). These results showed that 5 Gy irradiation increased plant resistance to SRD in *Phalaenopsis*. A dose of 5 Gy can potentially produce mutant lines resistant to SRD in *Phalaenopsis* or other plants, too.

Keywords: Detached leaf assay; Soft-rot disease; Mutant

INTRODUCTION

Phalaenopsis amabilis is an important parent in orchid crossbreeding because it is the main white donor in orchid crosses (Tang & Chen, 2007). *Phalaenopsis amabilis* has a wide adaptation because it can be found in many tropical regions like the Philipines, Myanmar, and Thailand. *Phalaenopsis amabilis* is one of Indonesia most important local orchid types because it is widely used as a mother plant for new hybrids (Zahara & Win, 2019). Soft-rot disease (SRD) susceptibility is one of the weaknesses of this orchid. (Sukma et al., 2007; Elina, 2016; Raynalta, 2017). Improvement in this trait is important for orchid breeding activities.

Soft-rot disease (SRD) is a major obstacle to orchid cultivation. SRD attacks various orchid commodities, like *Dendrobium nobile* (Balamurugan et al., 2020), *Phalaenopsis amabilis* (Lubis et al., 2021), and other species (Sukma et al., 2007). Soft-rot disease (SRD) in *Phalaenopsis amabilis* is caused by *Dickeya dadantii* (Sudarsono et al., 2018; Sanjaya et al., 2021). This bacterium is a necrotrophic pathogen (Rigault et al., 2021), which can easily spread symptoms and kill the host plant

Article History
Received: 03 November 2022
Accepted: 19 January 2024

Copyright © by Author
Planta Tropika is licensed under a Creative Commons Attribution-NonCommercial 4.0

(Charkowski, 2018). Symptoms of this bacteria are commonly small water-soaked spots on the leaves and surrounded by yellow halos (Joko et al., 2014). These bacteria are major soft-rot pathogens in *Phalaenopsis* orchid production (Alic et al., 2017). This soft rot disease causes huge losses in orchid cultivation, including orchid species, namely *Phalaenopsis amabilis* (Fu & Huang 2011; Sudarsono et al., 2018), in which *Phalaenopsis* has a high value in orchid commodities (Griesbach, 2002). The incidence of this pathogenic disease is 12-28% in Phalaenopsis orchids in Bali (Suputra et al., 2022).

Breeding and variety development is a method to obtain disease-resistant plants. Breeders design a mutant superior using various techniques like somaclonal variations (Ferreira et al., 2020), mutation induction (Jeong et al., 2017), and transgenic plants (Chen et al., 2019). Mutation induction through physical and chemical mutagens is widely used in *Phalaenopsis amabilis* (Raihanun, 2017; Mohammadi et al., 2020; Putri et al., 2021; Azis et al., 2021). Gamma irradiation is one of the physical mutagens widely used to improve genetic diversity, such as resistance to soft rot disease. This study used gamma irradiation to obtain *P.amabilis* resistance to SRD.

Selection to determine *P.amabilis* resistance to SRD is necessary to obtain the mutant line. The detached leaf disk assay for evaluating the resistance response to *Dickeya dadantii* on various *Phalaenopsis* genotypes has been successfully conducted by Elina (2016), Sudarsono et al., (2018), and Sanjaya et al., (2021). Unfortunately, those previous studies used mature plants to evaluate resistance to SRD. However, a new protocol to the inoculation method for young plants from in vitro plantlets is necessary to determine the resistance response in mutant plants. Furthermore, there is limited information about the inoculation method using detached leaf assay for young plants from in vitro plantlets. An effective method of inoculation pathogen for young plants from in vitro plantlets is the main key to success in determining resistance response in mutant plants. In addition to *P. amabilis*, *Vanilla planifolia* was also used to evaluate the inoculation of the soft rot pathogen, which belongs to the *Orchidaceae* family (Flagnan et al., 2018). This experiment irradiated protocorms with gamma irradiation at 0, 5, 10, 15, and 20 Gy and grew them into plantlets. These plantlets were evaluated for their resistance to *Dickeya dadantii* using detached leaf assay. This study aimed to evaluate the inoculation method of soft-rot pathogen and determine the resistance of *Phal. amabilis* from irradiated protocorms against the soft-rot pathogen.

MATERIALS AND METHODS

Experiment 1: Evaluate Concentration and Inoculation of Soft Rot Pathogen (*D. dadantii*)

Experiment 1 was conducted at the Laboratory of Tissue Culture I and Plant Molecular Biology I, Faculty of Agriculture, IPB University, Bogor, Indonesia. The protocol to obtain isolation of *D. dadantii* has been described by Putri et al. (2021).

Inoculum Concentration of D. dadantii

A bacterium isolated from an infected leaf of *Phalaenopsis amabilis* was confirmed as a soft rot pathogen by the Koch Postulate method (Sudarsono et al., 2018; Sanjaya et al., 2021; Putri et al., 2021). A single colony from the selected strain was grown into liquid medium (Lactose Broth/LB) of as much as 15 ml, shaken for 21hour at 100 ppm, and harvested by centrifugation at 8000 rpm for 6

min until the final volume was OD_{600}=0.2 (Putri et al., 2021). The leaf disk assay evaluation method was carried out following Sudarsono et al. (2018). The leaf disc (1-2 cm^2) was taken from the plantlet without irradiation (control) of *Phalaenopsis amabilis* and young leaves of *Vanilla planifolia*. The middle of the leaf discs was wounded using sterilized pins and tested for five concentrations with three times dilutions of *D. dadantii* (Control (sterile ddH2O), 10^{-0} (bacteria stock), 10^{-1}, 10^{-2}, and 10^{-3}). All treatments were repeated three times, and every replication used three leaf discs.

Inoculation of *D. dadantii*

There were four methods of inoculation used for this experiment, including (A0) the top of the leaf disc without wounding, (A1) the top of the leaf disc with wounding, (B0) the bottom of the leaf disc without wounding, and (A1) the bottom of the leaf disc with wounding. This experiment was arranged in a factorial, completely randomized design. The factors were inoculation methods and five dilutions of *D. dadantii* (Control (sterile ddH_2O), 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) (Figure 1). All treatments were repeated three times, and every replicate used three leaf discs. The inoculated leaf discs were incubated in a plastic box at room temperature (±25°C) with the wetted sponge and sterile ddH_2O to keep the humidity (75%). We recorded symptoms for all treated leaf discs up to 24 hours after inoculation.

Experiment 2: Resistance Response of *Phal. amabilis Regenerated* from Irradiated Protocorms Against Soft Rot Pathogen

This experiment used plantlets of *Phal. amabilis* regenerated from irradiated protocorms after acclimatization at the greenhouse. Irradiation was carried out at the National Nuclear Energy Agency of Indonesia (BATAN). The variables in this experiment were the diameter of the soft-rot symptom, which was recorded up to 24 hours after inoculation. The disease severity index was calculated using the formula by Raynalta (2017) with modified scoring. There were five resistance classes based on the diameter of soft-rot symptoms as follows:

Score (v):
1 = ≤ 1mm
3 = 2 ≤ *i* ≤ 4 mm
5 = 4 < *i* ≤ 6 mm
7 = 6 < *i* ≤ 8 mm
9 = >8 mm

Resistance Classs (RC):
1. Resistance (R) : DS 0-20%
2. Moderate Resistance (MR) : DS 21-40%
3. Moderate Susceptible (MS) : DS 41-60%
4. Susceptible (S) : DS 61-80%
5. Very Susceptible (VS) : DS > 80%

$$DS = \frac{\sum(ni \; x \; v)}{Z \; x \; N} \text{ x } 100\% \qquad (1)$$

DS : disease severity index
N : number of samples observed
Z : maximum score value
ni : number of affected leaf discs in the score of *i*

Data Analysis

The collected data were analyzed using analysis of variance (ANOVA) with the STAR (Statistical Tool for Agricultural Research) application. The Duncan Multiple Range Test (DMRT) evaluated significant treatments with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Effects of Pathogen Concentration on the Detached Leaves of *Phalaenopsis amabilis* (L.) Blume and *Vanilla planifolia*

Bacteria with SRD virulence were isolated. SRD symptoms were similar to rot symptoms on naturally infected leaves (positive method of Koch's postulates). This selected strain was validated using the 16S rRNA marker gene to detect the pathogen, and the sequencing data confirmed the bacteria was *Dickeya dadantii* (Sanjaya et al., 2022). Therefore, this pathogen is confirmed to be associated with soft rot disease in *P. amabilis.* The results showed that soft rot pathogens could infect not only *Phal.amabalis* but also *Vanilla planifolia*. These results indicate that *Vanilla planifolia* can be an indicator plant from the *Orchidaceae* family to SRD other than *P. amabilis*. Soft-rot symptoms in *Vanilla planifolia* were discovered in Liwa Botanical Garden by Mahfut et al. (2020).

Table 1. Effects of dilution factors on diameters of the soft rot symptom in a detached *Phalaenopsis amabilis* (L.) Blume and *Vanilla planifolia* using 9 leaf discs for each dilution at 12 h after inoculation

Dilution Factors	The average on diameters of the soft rot symptom (mm)	
	Vanilla planifolia	*Phalaenopsis amabilis* (L.) Blume
Control	0.00 b	0.00 c
10^{-0}	5.11 a	3.76 a
10^{-1}	0.00 b	3.77 a
10^{-2}	0.00 b	2.23 b
10^{-3}	0.00 b	0.54 c
F-calculated	**	**

Note: Values followed by the same letters in the same column are not significantly different based on DMRT at level α = 5%; ** = significantly different (P < 0.01)

The bacterial density obtained was $OD_{600nm} = 0.2$, effectively infecting *P. amabilis* and *Vanilla planifolia*. Based on the results showed symptoms of soft rot on the leaves of *P. amabilis* in all dilution factor treatments. In contrast, *Vanilla planifolia* leaves showed symptoms only in the 10^{-0} dilution factor (Table 1). *P. amabilis* is very susceptible compared to *Vanilla planifolia* plants, as seen from the diameter of the symptoms. *Vanilla planifolia* only causes symptoms at a dilution factor of 10^{-0}. The concentration limit indicated quorum sensing. Quorum sensing is the process of bacterial chemical communication and mechanism to ensure sufficient numbers of cells to carry out specific biological responses (Mukherjee & Bassler, 2019).

The bacterial concentration of 10^{-3} still showed symptoms in *P. amabilis* after 12 h inoculation (Tabel 1). Based on these results, the dilution factors need to be increased to evaluate the resistance of the plant. The bacterial concentration of 10^{-5} was the best result for this study because it decreased the spread of symptoms, and the symptom was evaluated until 24 h after inoculation (Table 2). This result corresponds to Sudarsono et al. (2018), who stated that resistance responses of *Phalaenopsis* species should be observed as early as 20 hours after inoculation.

Effects of Wounding and Pathogen Concentration on Detached Leaves of *Phalaenopsis Amabilis* (L.) Blume and *Vanilla planifolia*

Bacterial inoculation without wounding showed no soft rot symptoms at all dilution factors up to 24 hours (Table 2). On the other hand, bacterial inoculation with wounding showed symptoms of soft rot on the top and bottom of the leaves. Wax was present on the top of the Phalaenopsis leaves, but there was no significant difference in the diameters of soft rot between the top and bottom of the leaves. These results indicate that the bacteria can only infect the tissue when there is a wounding to the plant tissuec orresponds to Sudarsono et al. (2018), reporting that soft rot symptoms did not appear in all treatments of *Dickeya dadantii* concentration without wounding in detached leaf assay

Table 2. Effects of wounding and pathogen concentration on diameters of the soft rot symptom in detached leaves of *Phalaenopsis amabilis* (L.) Blume using 9 leaf discs for each treatment combination

Treatments	Dilution Factors					
	Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	----------12 h---------					
A0	0	0.00 b	0.00 c	0.00 b	0	0
A1	0	3.13 a	2.27 b	0.00 b	0	0
B0	0	0. 00 b	0.00 c	0.00 b	0	0
B1	0	2.97 a	2.97 a	3.43 a	0	0
F- calculated	-	**	**	**	-	-
	----------24 h--------					
A0	0	0.00 b	0.00 b	0.00 b	0.000 b	0.00 b
A1	0	8.83 a	8.87 a	6.63 a	4.63 a	3.30 a
B0	0	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
B1	0	8.83 a	9.17 a	6.57 a	5.83 a	4.00 a
F- calculated	-	**	**	**	**	**

Note: Values followed by the same letters in the same columns are not significantly different based on DMRT at level α = 5%; ** = significantly different (P < 0.01). (A0) The top leaf of *Phal amabilis* without wounding. (A1) The top leaf of *Phal amabilis* with wounding. (B0) The bottom leaf of *P. amabilis* without wounding. (B1) The bottom leaf of *P. amabilis* with wounding.

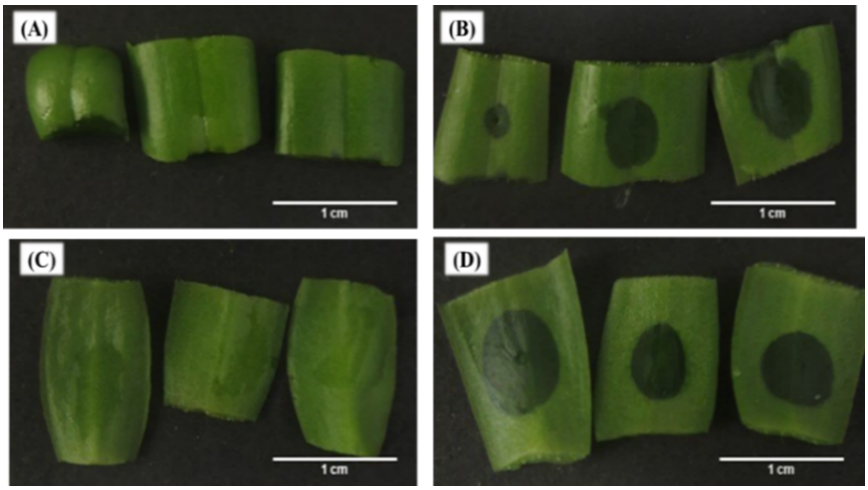

Figure 1. Inoculation of *Dickeya dadantii* on the Top and Bottom of the Leaf of *Phalaenopsis amabilis* (L.) Blume with or Without Wounding at a Dilution Factor of 10^{-5} After 24 h. (A) The Top Leaf of *P. amabilis* Without Wounding. (B) The Top Leaf of *Phal amabilis* With Wounding. (C) The Bottom Leaf of *P. amabilis* Without Wounding. (D) The Bottom Leaf of *P. amabilis* With Wounding.

of *P. amabilis* after 20 hours of inoculation. The lowest dilution (10^{-5}) showed symptoms after 24 hours of bacterial inoculation (Figure 1). *Dickeya dadantii* bacteria spread quickly when the leaf surface tissue is injured (Singh et al., 2019). The lowest dilution (10^{-5}) was used for the resistance test on the plantlets from irradiated protocorms. In this study, we found the best concentration and inoculation method to evaluate resistance response to SRD using detached leaf assay.

Determination of Disease Severity Index and Resistance Class of Plantlets Regenerated from Irradiated Protocorms

Plantlets regenerated from irradiated protocorms were acclimatized to take advantage of natural selection for soft rot disease (SRD). Mortality to soft rot was very high in the field after two months of acclimatization, in which 78% of plants or accessions died due to soft rot. The plants that survived after acclimatization were 27 of the 95 plants of *P. amabilis*. All plants that still survived were tested for inoculation against soft rot pathogens. There are five resistance classes (resistance, moderate re-

Table 3. Disease Severity Index and Resistance Class (RC) of 27 accessions of *Phalaenopsis amabilis* to Soft-rot Disease (SRD) using 3 leaf discs for each accessions

Accessions	DS (%)	RS	DS (%)	RC
	- 12 Jam-		- 24 Jam-	
IP 0 Gy- 1	**0**	**R**	**0**	**R**
IP 0 Gy- 3	41	MS	100	VS
IP 0 Gy-5	48	MS	56	MS
IP 0 Gy-7	22	MR	70	S
IP 0 Gy-8	31	MR	89	VS
IP 0 Gy-11	15	R	78	S
IP 0 Gy-12	33	MR	100	VS
IP 0 Gy-15	22	MR	100	VS
IP 0 Gy-21	63	S	100	VS
IP 05 Gy-23	**0**	**R**	**0**	**R**
IP 05 Gy-25	26	MR	85	VS
IP 05 Gy-31	**0**	**R**	**0**	**R**
IP 05 Gy-33	**4**	**R**	**15**	**R**
IP 05 Gy-38	33	MR	100	VS
IP 05 Gy-41	41	MS	100	VS
IP 10 Gy-47	22	MR	100	VS
IP 10 Gy-50	26	MR	85	VS
IP 10 Gy-53	6	R	72	R
IP 10 Gy-56	0	R	93	VS
IP 15 Gy-69	0	R	41	MS
IP 15 Gy-70	7	R	70	S
IP 15 Gy-71	26	MR	100	VS
IP 15 Gy-74	25	MR	89	VS
IP 15 Gy-83	41	MS	100	VS
IP 15 Gy-84	33	MR	100	VS
IP 20 Gy-93	19	R	100	VS
IP 20 Gy-94	22	MR	88.89	VS
Wild Type	48	MS	78	S

Note: DS= Disease Severity; RC= Resistance Class; R= Resistance; MR= Moderate Resistance; MS= Moderate Susceptible; S= Susceptible; VS= Very Susceptible

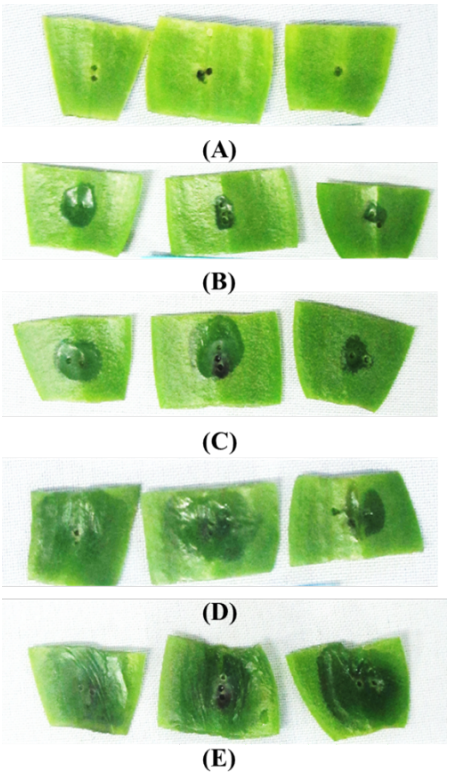

Figure 2. Resistance class (RC) plants of *Phalaenopsis amabilis* from irradiated protocorms. (A) Resistance (R). (B) Moderate Resistance (MR). (C) Moderate Susceptible (MS). (D) S= Susceptible (S). (E) Very Susceptible (VS).

sistance, moderately susceptible, susceptible, and very susceptible) from the five resilience criteria modified by Raynalta (2017).

Table 3 presents the disease severity and resistance class (RC) plants of *P. amabilis* regenerated from irradiated protocorms. The resistance class (RC) was used as a reference after 24 h inoculation because after 24 h, DS has reached 100%. Four accessions were resistant (DS = 0-20%) to SRD, in which three accessions came from the 5 Gy dose (IP 05Gy-23, IP 05Gy-31, IP 05Gy-33) and one accession from the control treatment (IP 0Gy-1). This results showed that a dose of 5 Gy can potentially increase the resistance of *P. amabilis*. In addition to gamma-ray irradiation, somaclonal variation is also indicated to increase the resistance of *P. amabilis*. It was seen in control plants without irradiation treatment, which were included in the resistant classification. The representative of detached leaf assay to SRD can be seen in Figure 2. Resistant accessions did not show symptoms of soft rot, but there were necrosis and chlorosis symptoms. Symptoms of chlorosis and necrosis appeared on Arabidopsis thaliana (bos1 mutant) leaves after being inoculated with *Dickeya dadantii*, where necrosis is an effective mechanism for plant defense against the spread of *Dickeya dadantii* (Kraepiel et al., 2011).

In this study, gamma-ray irradiation through protocorm proved to be effective in increasing plant resistance to SRD. Several studies have reported various methods to increase the resistance of orchids to diseases, such as Nisaq et al. (2021), who succeeded in improving disease resistance of *Phalaenopsis* orchids by spraying nano silica (0, 7.5, 15, 22.5, and 30 ppm) where this treatment at

a concentration of 30 ppm could induce a resistance response and stimulate the growth of *Phalaenopsis* orchids; Nurcahyani et al., (2019) used fusaric acid (FA), which can increase the resistance of *Phalaenopsisis* orchids to *Fusarium oxysporu,* and Chuang et al., (2020) used microbial metabolites from *Pseudomonas aeruginosa* strain Y1 to increase the resistance of *Phalaenopsis* orchid plants. Obtaining mutant plants that are resistant to SRD by induced gamma-ray mutation on the protocorm of *Phalaenopsis* orchids has not been widely carried out, where this species of orchid is very susceptible to *Dickeya dadantii* bacteria (Sukma et al., 2017). Mechanisms and regulation of resistance of the four resistant accessions need to be evaluated further, especially on genes that play a role in resistance to soft rot disease.

CONCLUSION

The pathogen of SRD was not only successfully inoculated on *Phal.amabilis* but also on *Vanilla planifolia* leaves. Test results of the inoculation method with leaf disk assay showed that the dilution factor and the process of bacterial inoculation greatly affected symptoms on the leaves. The bacterial concentration of 10^{-5} was the best result for this study, and symptoms of SRD only occurred with wounding. Four accessions classified as resistant were three from 5 Gy irradiation dose and one control plant. Protocorm irradiation at a dose of 5 Gy has the potential to produce mutant plants that are resistant to SRD. It is necessary to analyze further resistant accessions for resistance genes that play a role in SRD.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Research and Technology and Higher Education Indonesia for a grant competence project No. 079 / SP2H / LT / DRPM / II / 2016, under the coordination of Dr. Dewi Sukma, S.P., Msi.

REFERENCES

Alic, S., Naglic, T., Tsuek-Znidaric, M., Peterka, M., & Dreo, T. (2017). Putative new species of the genus *Dickeya* as major soft rot pathogens in *Phalaenopsis* orchid production. *Plant Pathology, 66*, 1357-1368. https://doi.org/10.1111/ppa.12677

Azis, S, A., Sukma, D., Syukur, M., & Azmi, K, K. (2021). Development of new *Phalaenopsis* cultivars using colchicine. *Acta Hortic, 1340*, 223-230. https://doi.org/10.17660/ActaHortic.2022.1340.35

Balamurugan, A., Kumar, A., Sakthivel, K., Ashajyothi, M., Sahu K, P., & Karthikeyan, M. (2020). Characterization of *Dickeya fangzhongdai* causing bacterial soft rot disease on *Dendrobium nobile* in India. *Eur J Plant Pathol, 158*, 773-780. https://doi.org/10.1007/s10658-020-02094-7

Charkowski, A, O. (2018). The changing face of bacterial soft-rot diseases. *Annual Review of Phytopathology, 56*(1),1-20. https://doi.org/10.1146/annurev-phyto-080417-045906

Chen, T, Y., Pai, H., Lee, S, C., Lin, T, T., Chang, C, H., Hsu, C, F., & Lin, N, S. (2019). Dual resistance of transgenic plants against *Cymbidium mosaic virus* and *Odontoglossum ringspot virus. Scientific Reports, 9*(1), 1-12. https://doi.org/10.1038/s41598-019-46695-7

Chuang, H. W., Tseng, T, S., Hsieh, H, Y., Kao, T, C., & Chen, G, H. (2020). Common cellular events implicated in the regulation of cold stress tolerance and soft rot resistance induced by metabolites of *Pseudomonas aeruginosa* in *Phalaenopsis* orchids. *Advanced Chemicobiology Research, 1*(1), 5-21. https://doi.org/10.37256/acbr.11202272

Elina, J. (2016). *Respon ketahanan anggrek Phalaenopsis terhadap penyakit busuk lunak akibat infeksi Dickeya dadantii* [Graduate School Thesis IPB University].

Ferreira, M, S., Moura, E, R., Lino, L, S, M., Amorim, E., Santoso-Serejo, J, A., & Haddad, F. (2020). Selection of somaclonal variants of the cultivar 'Prata-Anã' for resistance to *Fusarium oxysporumf*. sp.*cubense* race 1. *Rev. Bras. Frutic, 42*(6), 1-10. http://dx.doi.org/10.1590/0100-29452020620

Flagnan, N, S., Ospina-Calderon, N, H., Agapito, L, T., Mendoza, M., & Mateus, H, A. (2018). A new species of *Vanilla (Orchidaceae)* from the North West Amazon in Colombia. *Phytotaxa, 364*(3), 250-258. https://doi.org/10.11646/phytotaxa.364.3.4

Fu, S. & Huang, H, J. (2011). *Orchid Biotechnology II: Molecular Characterization of The Early Response of Orchid Phalaenopsis amabilis to Erwinia chrysantemi* Infection (Chen WH & Chen HH ed. World Sci. https://doi.org/10.1142/9789814327930_0015

Griesbach, R, J. (2002). *Trend in New Crops and New Uses: Development of Phalaenopsis Orchids for The Mass-market* (Janick J & Whipkey A Ed.). ASHS Press.

Jeong, R, D., Jeong, M, A., & Park, M, R. (2017). Gamma irradiation- -induced disease resistance of pear (Pyrus pyrifolia "Niitaka") against *Penicillium expansum*. *Journal of Phytopathology, 165*(9), 626-633. https://doi.org/10.1111/jph.12601

Joko, T., Subandi., Kusumandari., Wibowo, A., Priyatmojo, A. (2014). Activities of plant cell wall-degrading enzymes by bacterial soft rot of orchid. *Archives of Phytopathology and Plant Protection, 47*(10), 1239-1250. http://dx.doi.org/10.1080/03235408.2013

Kraepiel, Y., Pedron, J., Patrit, O., Simond-Cote, E., Herman, V., & Gjsegem, F, V. (2011). Analysis of the plant *bos1* mutant highlights necrosis as an efficient defense mechanism during *D. dadantii/Arabidospis thaliana* interaction. *PLoS ONE, 6*(4), e18991. https://doi.org/10.1371/journal.pone.0018991

Lubis, U, N, Q., Sudarsono, S., & Sukma, D. (2021). The response of *Phalaenopsis amabilis* seedling (in vitro and greenhouse) after salicylic acid treatment to *Dickeya dadantii* infection. *IOP Conf. Series: Earth and Environmental Science, 694*(012041). https://doi.org/10.1088/1755-1315/694/1/012041

Mahfut, Anngreiny, A., Wahyuningsih, S., Handayani, T, T., & Sukimin. (2020). Identification of disease and efforts to protect native orchid plants against bacteria infection in Liwa Botanical Garden. *Journal of Physics: Conference Series, 1641*, 1-8. https://doi.org/10.1088/1742-6596/1641/1/012098

Mohammadi, M., Kaviani, B., & Sedaghathoor, S. (2020). In vivo polyploidy induction of *Phalaenopsis amabilis* in a bubble bioreactor system using colchicine. *Ornamental Horticulture, 27*(2), 204-212. https://doi.org/10.1590/2447-536X.v27i2.2275

Mukherjee, S & Bassler, B, L. (2019). Bacterial quorum sensing in complex and dynamically changing environments. *Nature Review Microbiology, 17*(6), 371-382. https://doi.org/10.1038/s41579-019-0186-5

Nisaq, G, J., Sudarsono, S., & Sukma, D. (2021). Nano silica spray increases *Phalaenopsis pulcherrima* growth and resistance against *Dickeya dadantii* infection. *OP Conf. Series: Earth and Environmental Science, 694*(012040), 788-797. https://doi.org/10.1088/1755-1315/694/1/012040

Nurcahyani, E., Sumardi., Qudus, H, I., Palupi, A., & Sholekhah. (2019). Analysis of chlorophyll *Phalaenopsis amabilis* (L.) results of the resistance to *Fusarium oxysporum* and drought stress. *IOSR Journal of Agriculture and Veterinary Science, 12*(11), 41-46. https://doi.org/10.9790/2380-1211014146

Putri, H, A., Purwito, A., Sudarsono, S., & Sukma, D. (2021). Morphological, molecular, and resistance responses to soft-rot disease variability among plantlets of *Phalaenopsis amabilis* regenerated from irradiated protocorms. *Biodiversitas, 22*(3), 1077-1090. https://doi.org/10.13057/biodiv/d220301

Raihanun, S. (2017). Morphological diversity and the resistance to *Dickeya dadantii* Infection of *Phalaenopsis amabili*s (L.) Blume irradiated with gamma ray[Graduate School Thesis IPB University].

Raynalta, E. (2017). Molecular characterization of *Phalaenopsis* orchid clones, species, and

hybrid, using Pto gene-based SNAP marker[Graduate School Thesis IPB University]
Rigault, M., Citerne, S., Masclaux-Daubresse, C., & Dellagi, A. (2021). Salicylic acid is a key player of *Arabidopsis* autophagy mutant susceptibility to the necrotrophic bacterium *Dickeya dadantii*. *Scientific Reports, 11*(3624), 1-10. https://doi.org/10.1038/s41598-021-83067-6.
Sanjaya, I, P, W., Sudarsono, S., Chan, M-T., & Sukma, D. (2022). Molecular characterization of soft-rot disease pathogen from *Phalaenopsus* and differences in genotype response to its infection. *ISHS Acta Horticulturae 1334: II International Symposium on Tropical and Subtropical Ornamentals*. https://doi.org/10.17660/ActaHortic.2022.1334.14
Sanjaya, IPW, Sukma D, Sudarsono S, & Chan, M-T. (2021). Relationship of resistance-related enzyme activity and salicylic acid content in *Phalaenopsis* species with different levels of resistance to *Dickeya Dadantii*. *Journal of Horticultural Research, 29*(2), 31–44. https://doi.org/10.2478/johr-2021-0018
Singh, S., Singh, Y., & Singh, V. (2019). Divulging the comparing inoculation methods for assessing pathogenicity of *Dickeya dadantii* inciting stalk rot disease of sorghum. *Journal of Pharmacognosy and Phytochemistry, 8*(1), 1409-1413.
Sudarsono, S., Elina, J., Giyanto, & Sukma, D. (2018). Pathogen causing *Phalaenopsis* soft rot disease – 16S rDNA and virulence characterization. *Plant Protect Sci, 54*(1), 1-8. https://doi.org/10.17221/18/2017-PPS
Sukma, D., Elina, J., Giyanto, & Sudarsono. (2017). Disease resistance breeding of *Phalaenopsis* spp. for tropical environment and molecular marker development for plant selection. *Acta Horticulture, 1167*, 237-243. https://doi.org/10.17660/ActaHortic.2017.1167.36
Suputra I, P, W., Wirya G, N, S, S., Sari N, B, K., Temaja I, G, R, M., Innosensia., N, L, P, C. 2022. Identification and Characterization of Soft Rot Bacterial Pathogens on *Phalaenopsis*
Tang, C, Y., & Chen, W, H. (2007). *Orchid Biotechnology: Breeding and Development of New Varieties in Phalaenopsis* (Chen WH & Chen HH, ed.). World Science.
Zahara, M & Win, C, C. (2019). Morphological and Stomatal Characteristics of Two Indonesian Local Orchids. *Journal of Tropical Horticulture, 2*(2),165-69. https://doi.org/10.33089/jthort.v2i2.26