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ASSESSMENT OF LEAF CHARACTERS AND RESISTANCE RESPONSE TO SOFT-ROT PATHOGEN (*DICKEYA DADANTII*) ON INDONESIAN *PHALAENOPSIS* SPECIES

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SUMMARY

Breeding for soft-rot disease (SRD)-resistant *Phalaenopsis* cultivars is crucial for ensuring sustainable orchid production in tropical regions. Indonesia is one of the centers of origin and diversity of *Phalaenopsis*. However, information on potential *Phalaenopsis* donor parents and the mechanisms underlying SRD resistance remains limited, posing challenges for conventional and biotechnological breeding approaches. The concerned study aimed to identify SRD-resistant species among 20 *Phalaenopsis* species native to Indonesia and determine the viable role of pre-existing mechanical structures in post-infection resistance. The SRD screening successfully identified three additional resistant species, namely, *P. corningiana, P. modesta*, and *P. tetraspis*, along with the previously known resistant species, *P. amboinensis*. Leaf anatomy and epidermal traits were remarkably showing significant differences among the species; however, the correlation of leaf anatomy and epidermal traits with SRD resistance was nonsignificant. The staining of cell wall components (cellulose, pectin, and lignin) revealed no considerable differences between the *Phalaenopsis* resistant and very susceptible species. The newly identified SRD-resistant species has yet to reach frequent usage in the *Phalaenopsis* breeding program, recognizing its potential for integration into the *Phalaenopsis* SRD-resistance breeding program. Study results suggested that physical leaf traits exerted less influence on the resistance response.

Keywords: *Phalaenopsis* species**,** cultivars, *Dickeya dadantii*, pre-existing defense, soft-rot disease, SRD-resistance

Key findings: The newly identified SRD-resistant *Phalaenopsis* species, namely, *P. corningiana, P. modesta*, and *P. tetraspis,* are yet to be utilized in a breeding program to authenticate their potential for integration into breeding programs to produce SRD-resistant cultivars.

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INTRODUCTION

The *Phalaenopsis* genus is one of the remarkable commodities in the floral industry, with more than 40,000 *Phalaenopsis* hybrids succeeding in their registration at the Royal Horticultural Society, UK (Chen and Chin, 2021; Han *et al*., 2025). Although renowned for their long-lasting blooms and unique flower characteristics, *Phalaenopsis* is also prone to pathogens because of the high temperatures and relative humidity required for its optimal vegetative growth (Shen *et al*., 2018). Soft-rot disease (SRD), caused by necrotrophic bacteria such as *Pectobacterium* and *Dickeya* species, is one of the primary diseases of *Phalaenopsis* (Charkowski, 2018; Gijsegem *et al*., 2021). In *Phalaenopsis*, the SRD symptoms appear as water-soaked lesions that rapidly expand (24–48 hours post-infection) and have no yellow margins at the surrounding tissues (Abdullah and Kadzimin, 1993). These disease symptoms resulted from the plant-cell-wall-degrading enzyme (PCWDE), which leads to cell wall degradation and, subsequently, cell lysis (Gijsegem *et al*., 2021). In *Phalaenopsis* species, the SRD has had reports in various countries, including Malaysia (Abdullah and Kadzimin, 1993), Korea (Lee *et al.*, 1999), the USA (McMillan *et al.*, 2007), and Indonesia (Sanjaya *et al*., 2020).

During *Phalaenopsis* growth stages, the SRD infection can result in robust losses due to a rapid disease expansion, and the inoculum can easily spread to adjacent healthy plants (McMillan *et al*., 2007). In orchid cultivation, the SRD disease control is typically successful through chemical pesticides and manual removal of infected plants to prevent further disease spread (McMillan *et al.*, 2007). However, copper-based bactericides can pollute the surrounding environment, generating environmental concerns, while manual rouging has also not shown satisfactory disease control (McMillan *et al*., 2007; Pesce *et al*., 2025). Plant disease progression requires a susceptible host, suitable environmental conditions, and a virulent pathogen (Roussin-Leveillee *et al*., 2024). Hence, a genetic approach through breeding of *Phalaenopsis* resistant genotypes to SRD is a potential strategy to mitigate losses caused by SRD.

Breeding for SRD-resistant *Phalaenopsis* requires an SRD-resistant parental genotype as a donor for this trait. The *Phalaenopsis* orchid comprises 92 species, categorized into five subgenera: *Phalaenopsis, Polychilos, Aphyllae, Parishianae,* and *Proboscidioides* (Hsu *et al*., 2018). Among these 92 species, more than 20 species were existing in Indonesia. Therefore, the screening of *Phalaenopsis* species for SRD resistance has progressed on several species, such as *P. amabilis, P. amboinensis, P. bellina, P. cornu-cervi, P. fimbriata, P. pantherina, P. mentawaiensis, P. schilleriana*, and *P. pulcherrima* var. 'Aceh'; however, only the single species *P. amboinensis* appeared *P. pantherina, P. mentawaiensis, P. schilleriana*, resistant to SRD caused by *Dickeya dadantii* (Sukma *et al*., 2017; Sanjaya *et al*., 2020). Hence, evaluating more *Phalaenopsis* species resistant to SRD is vital to enrich the *Phalaenopsis* breeding program.

Phalaenopsis's resistance mechanism to soft-rot disease remains unclear. In general, plants prevent pathogen infection with pre-existing defense, as constitutively expressed before pathogen infection (Boots and Best, 2018). Several studies have enunciated a relationship between pre-existing defense mechanisms, such as leaf anatomy and morphology characteristics like stomata, epidermal traits, leaf air space, and cell wall constituents, and SRD resistance in various crops (Dutton *et al*., 2019; Guttman *et al*., 2021). Considerably correlated anatomical characters to SRD resistance may also help in selecting SRD-resistant cultivars; however, no such study has explored this possibility in *Phalaenopsis*. Hence, this research analyzed the relationship of leaf anatomy and epidermal characters with SRD resistance in *Phalaenopsis.* The presented study aimed to evaluate the SRD resistance in other *Phalaenopsis* species found in Indonesia to enrich the genetic resources available for SRD-resistance breeding. It also aimed to assess the contribution of pre-existing physical leaf structure on SRD resistance post-inoculation through correlation analysis between leaf

anatomical and epidermal traits and SRD resistance in *Phalaenopsis.*

MATERIALS AND METHODS

Plant material and procedure

These experiments proceeded using 20 *Phalaenopsis* species. This included *P. amabilis* var. 'Jawa Barat,' *P. amabilis* var. 'Sulawesi,' *P. amboinensis*, *P. bellina*, *P. corningiana*, *P. cornu cervi* f. sanguinea 'Red Devil,' *P. deliciosa*, *P. fimbriata*, *P. floresensis*, and *P. inscriptiosinensis*. Others were *P. javanica*, *P. kapuasensis*, *P. mariae*, *P. modesta*, *P. pulcherrima* var. 'Aceh,' *P. pulcherrima* (formerly *Doritis pulcherrima*), *P. speciosa*, *P. tetraspis*, *P. venosa*, and *P. zebrine.* The collected species came from the jungle with no parentage information. These *Phalaenopsis* plants entailed growing on a fern slab with sphagnum moss for at least six months before inoculation with the pathogen *Dickeya dadantii.* All plants remained in a greenhouse with a temperature of 30 °C–32 °C during the day and 25 °C–28 °C at night, with a relative humidity of 50%. These orchid species received a weekly spray of foliar fertilizer NPK (20:20:20) at the concentration of 1.5 g L^{-1}.

Screening for SRD resistance

The SRD resistance screening in *Phalaenopsis* species succeeded in using a single-factor complete randomized design with three biological and three technical replications. The *D. dadantii* isolate used in these experiments was a pure culture in a frozen glycerol stock, previously molecularly identified by Sanjaya *et al.* (2022). The *D. dadantii* isolate for inoculation received culturing in a nutrient agar (NA) medium according to Sanjaya *et al.* (2020) with some modifications. A single colony of bacteria from the NA medium, upon selection, sustained culturing in 15 mL of Luria-Bertani (LB) medium, then agitated at 100 RPM overnight at room temperature. Bacterial cultures then entailed moving to 2 mL Eppendorf tubes before centrifuging at 8000 RPM for six minutes. Pellets' dilution used sterile distilled water to a concentration of 10^7 CFU mL^{-1}, which was then used for the inoculation. Inoculation of *D. dadantii* continued using the detached leaf inoculation protocol of Sudarsono *et al.* (2018), modified by Sanjaya *et al.* (2020). The second mature leaf from the apex of each plant, as cut and wiped, utilized sterile distilled water to remove surface contaminants. Each leaf, cut into segments of approximately 2 cm length, had the segment width varying among species. Before bacterial inoculation, each segment, pricked twice on the abaxial surface, had the left side serving as a mock (the control), with the right side inoculated. The leaf segments got placed in a closed plastic box lined with sterile tissue paper moistened with sterile distilled water. Mock treatment received 10 μL of sterile distilled water, while inoculated treatment obtained drops with 10 μL of *D. dadantii* inoculum. Placing the plastic box in the incubation room at 27 °C had an ambient lighting and relative humidity of 75%.

The observation of SRD symptoms occurred every 12 hours (0, 12, 24, 36, and 48) post inoculation (HPI) by taking a photograph of the treated leaf, with the symptom represented by water-soaked lesion diameter measurements conducted manually using ImageJ software. Disease severity and resistance classes reached calculations according to the formula by Sanjaya *et al.* (2020). Symptom scoring was from 0 to 10, with criteria as follows: 0 (0 mm), 1 (0.1–1 mm), 2 (1.1–2 mm), 3 (2.1–3 mm), 4 (3.1–4 mm), 5 (4.1–5 mm), 6 (5.1–6 mm), 7 (6.1–7 mm), 8 (7.1–8 mm), 9 (8.1–9 mm), and 10 (>9 mm). Disease severity (DS) computation used the following formula:

$$\mathbf{DS}\ (\%) = \frac{\Sigma\,(n_i \times v_i)}{Z \times N}$$

Where n_i = the number of samples in the score group, v_i = disease score of group n_i, Z = maximum score value, and N = total number of samples observed.

Disease resistance class determination was from the disease severity score according to Sanjaya *et al.* (2020): < 20% = resistant

(R), 20%–40% = moderately resistant (MR), 41%–60% = moderately susceptible (MS), 61%–80% = susceptible (S), and > 80% = very susceptible (VS). Progression of SRD in the observed *Phalaenopsis* has a representation from the area under the disease progression curve (AUDPC) according to a formula by Sanjaya *et al.* (2020):

$$\mathbf{AUDPC} = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

Where y_i is the soft rot diameter at the i^{th} observation, t_i is the time at the i^{th} observation, and n is the total number of observations.

Phalaenopsis leaf and epidermal traits evaluation

Although the study initially included 12 species, researchers selected eight for detailed experimentation based on SRD screening results. These comprised eight species, four representing the resistant ones (*P. amboinensis, P. corningiana, P. modesta,* and *P. tetraspis*) and four representing the very susceptible class (*P. bellina, P. fimbriata, P. inscriptiosinensis,* and *P. zebrina*), as determined by SRD screening results. Leaf water content entailed determination as a percentage of the oven-dry mass. Leaf anatomy, observed quantitatively, included adaxial and abaxial epidermis, mesophyll, and total leaf thickness. The leaf sample slide preceded preparation for leaf anatomy observation using the midsection of a mature second leaf (Metusala, 2017; Baishnab *et al.*, 2020). Cell wall components distribution (lignin, pectin, and cellulose) underwent assessment visually through staining with a specific binding dye (Mitra and Loque, 2014). Transverse leaf sections staining used toluidine blue O (0.02% w/v) to differentiate between the pectin and lignin, which detects the presence of cellulose (Mitra and Loque, 2014).

Preparations of epidermis specimens used the replica method, as outlined by Windarsih *et al.* (2022), with some modifications. Mature second leaves from each plant sustained cleaning on both surfaces. Clear nail polish application to the midregion of both leaf surfaces ensued, allowing to air dry after. Once dried, peeling the nail polish off used a clear tape before mounting on a microscope slide. The epidermal traits observed included the number of epidermal cells, the number of stomata, stomatal density, and the stomatal index. Stomatal density and the stomatal index entailed calculation according to Baishnab *et al.* (2020). Counting the number of stomata and epidermal cells proceeded using an Olympus CX23 microscope under 40× magnification. Stomatal density and the stomatal index evaluation came from three random views of each slide.

Data analysis

All the recorded quantitative data underwent analysis of variance (ANOVA) at α = 0.05, followed by Tukey HSD test with significant differences. Correlation analysis between leaf physical characters and SRD symptom diameter succeeded in using Pearson correlation at α = 0.05. Data analysis and visualization continued using the SAS program (https://welcome.oda.sas.com) and R Studio.

RESULTS

Phalaenopsis screening for SRD resistance

Twenty Indonesian *Phalaenopsis* species screening for SRD resistance revealed only 16 species showed a consistent response across the biological and technical replicates to SRD. Meanwhile, four species, namely, *P. amabilis* var. 'Jawa Barat,' *P. amabilis* var. 'Sulawesi,' *P. floresensis*, and *P. javanica*, showed the highest variability between biological replicates. This revealed possible intraspecific variations within these species. The exclusion of these *Phalaenopsis* species from further investigations ensued.

The SRD-resistance response varied between screened *Phalaenopsis* species genotypes (Table 1, Figure 1). The species *P. corningiana, P. modesta,* and *P. tetraspis* exhibited at par resistance responses to SRD like the previously identified SRD-resistant

Table 1. Soft rot symptom diameter, disease severity, and resistance class in 16 *Phalaenopsis* species against *Dickeya dadantii* infection.

Genotype	12 HPI			24 HPI			36 HPI			48 HPI		
	SD (mm)	DS (%)	RC	SD (mm)	DS (%)	RC	SD (mm)	DS (%)	RC	SD (mm)	DS (%)	RC
AMB	0.50	10.0	(R)	0.50	10.0	(R)	0.50	10.0	(R)	0.90	13.3	(R)
BEL	6.32	70.0	(S)	4.20	100.0	(VS)	21.61	100.0	(VS)	28.01	100.0	(VS)
COR	0.70	10.0	(R)	0.80	10.0	(R)	0.90	10.0	(R)	0.90	13.3	(R)
PCC	1.40	20.0	(MR)	1.90	26.7	(MR)	2.20	26.7	(MR)	4.90	56.7	(MS)
DEL	1.90	23.3	(MR)	3.20	33.3	(MR)	6.10	66.7	(S)	8.60	90.0	(VS)
FIM	4.20	46.7	(MS)	0.70	93.3	(VS)	14.10	100.0	(VS)	25.80	100.0	(VS)
INS	3.60	40.0	(MR)	9.00	86.7	(VS)	15.20	100.0	(VS)	17.30	100.0	(VS)
KAP	1.02	16.7	(R)	1.24	20.0	(MR)	1.69	23.3	(MR)	2.13	23.3	(MR)
MAR	0.79	10.0	(R)	2.49	30.0	(MR)	3.22	40.0	(MR)	3.87	43.3	(MS)
MOD	0.72	10.0	(R)	0.78	10.0	(R)	0.80	10.0	(R)	0.99	16.7	(R)
DOR	2.61	30.0	(MR)	4.54	50.0	(MS)	8.57	90.0	(VS)	10.25	96.7	(VS)
PPU	1.12	20.0	(R)	2.48	30.0	(MR)	4.15	46.7	(MS)	6.73	73.3	(S)
SPE	0.93	10.0	(R)	1.18	16.7	(R)	1.19	16.7	(R)	1.35	20.0	(MR)
TET	0.80	10.0	(R)	0.80	10.0	(R)	1.10	16.7	(R)	1.20	16.7	(R)
VEN	2.50	26.7	(MR)	4.00	43.3	(MS)	4.90	53.3	(MS)	5.50	56.7	(MS)
ZEB	3.27	36.7	(MR)	6.96	73.3	(S)	13.24	93.3	(VS)	18.12	100.0	(VS)
Tukey HSD	0.232	23.5	-	0.544	26.6	-	0.885	19.5	-	1.094	19.0	-

Note: means followed by different superscript small letters indicate significant difference within the column (Tukey: $p < 0.05$), SD = symptom diameter, DS = disease severity, RC = resistance class, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, VS = very susceptible, AMB = *P. amboinensis*, BEL = *P. bellina*, COR = *P. corningiana*, PCC = *P. cornu-cervi* f. sanguinea 'Red Devil,' DEL = *P. deliciosa*, FIM = *P. fimbriata*, INS = *P. inscriptiosinensis*, KAP = *P. kapuasensis*, MAR = *P. mariae*, MOD = *P. modesta*, DOR = *P. pulcherrima* (formerly *Doritis pulcherrima*), PPU = *P. pulcherrima* var. 'Aceh,' SPE = *P. speciosa*, TET = *P. tetraspis*, VEN = *P. venosa*, and ZEB = *P. zebrina.*

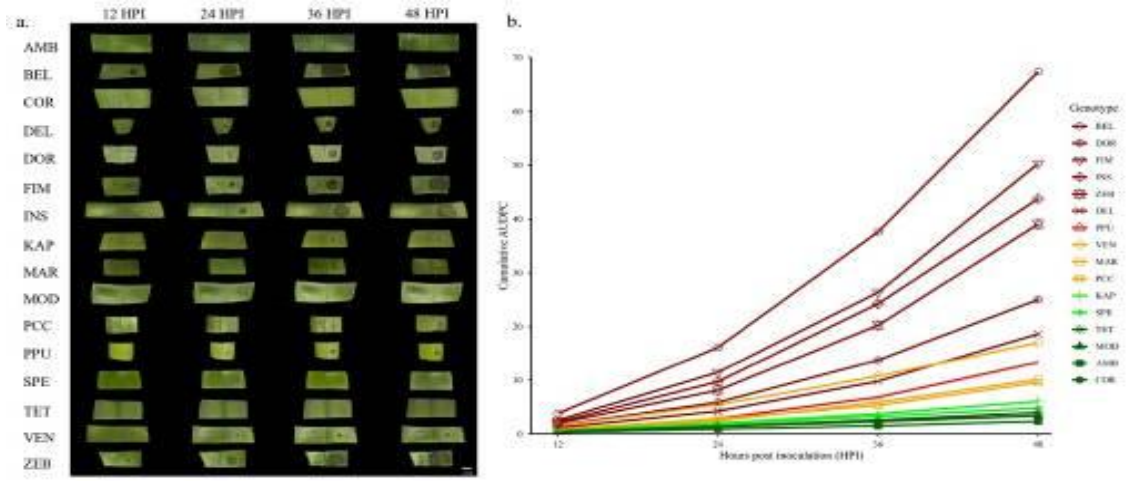

Figure 1. Soft-rot symptom development (a) and cumulative AUDPC (b) of soft-rot pathogen *Dickeya dadantii* on 16 *Phalaenopsis* species at 12-, 24-, 36-, and 48-hour post inoculation (HPI). AMB = *P. amboinensis*, BEL = *P. bellina*, COR = *P. corningiana*, PCC = *P. cornu-cervi* f. sanguinea 'Red Devil,' DEL = *P. deliciosa*, FIM = *P. fimbriata*, INS = *P. inscriptiosinensis*, KAP = *P. kapuasensis*, MAR = *P. mariae*, MOD = *P. modesta*, DOR = *P. pulcherrima* (formerly *Doritis pulcherrima*), PPU = *P. pulcherrima* var. 'Aceh,' SPE = *P. speciosa*, TET = *P. tetraspis*, VEN = *P. venosa*, and ZEB = *P. zebrina;* scale bar indicates 1 cm.

species, *P. amboinensis* (Sukma *et al.,* 2017; Sanjaya *et al*., 2020). *P. amboinensis, P. corningiana, P. modesta, P. tetraspis, P. kapuasensis*, and *P. speciosa* revealed the smallest SRD symptom at 48 HPI. However, the most prominent SRD symptoms resulted in *P. bellina, P. fimbriata, P. inscriptiosinensis*, and *P. zebrina*. The SRD-symptom diameter of *P. kapuasensis* and *P. speciosa* was nonsignificantly higher than that of *P. amboinensis, P. corningiana, P. modesta,* and *P. tetraspis*. Classification of the SRD resistance class based on disease severity showed only the four species, *P. amboinensis, P. corningiana, P. modesta,* and *P. tetraspis,* received a resistant classification. Meanwhile, the two species, *P. kapuasensis* and *P. speciosa,* attained a moderately resistant classification.

Out of the 16 studied *Phalaenopsis* species with a consistent resistance response, four species genotypes gained the category as a resistant class (*P. amboinensis, P. corningiana, P. modesta*, and *P. tetraspis*). Others received the following: two were in the moderate resistant class (*P. speciosa* and *P. kapuasensis*), three were in the moderate susceptible class (*P. cornu cervi* f. sanguinea 'Red Devil,' *P. mariae*, and *P. venosa*), and one in the susceptible class (*P. pulcherrima* var. 'Aceh'). Six species emerged under the very susceptible class (*P. bellina, P. deliciosa, P. fimbriata, P. inscriptiosinensis, P. pulcherrima,* and *P. zebrina*). The SRD-resistant and moderately resistant genotypes, such as *P. amboinensis, P. corningiana, P. modesta, P. tetraspis, P. kapuasensis*, and *P. speciosa,* also showed a slower disease progression than the very susceptible, susceptible, and moderately susceptible *Phalaenopsis* genotypes. They showed a resistance mechanism (Figure 1b). Hence, future studies on the SRD-resistance mechanism and breeding of SRD-resistant *Phalaenopsis* cultivars may utilize the *P. amboinensis, P. corningiana, P. modesta,* and *P. tetraspis* as donor parents for this trait.

Leaf and epidermal traits of resistant vs. very susceptible species

Among *Phalaenopsis* species representing distinct resistance responses to *D. dadantii*, significant differences were evident for water content, leaf anatomy, and epidermal characteristics (Table 2, Figure 2). The species *P. amboinensis* had the highest water content (92.41%), while the lowest water content appeared in the species *P. modesta* (86.86%). The highest leaf, mesophyll, and epidermis thicknesses resulted in *P. bellina*, while the lowest occurred in *P. modesta*. Observations of the adaxial surface showed stomata emerged only in two species, *P. zebrina* and *P. inscriptiosinensis*. The topmost epidermis number was notable in *P. corningiana*, while the lowest was in *P. inscriptiosinensis*. The maximum number of stomata density and stomata index were noteworthy in the species *P. zebrina* (5.33 mm^{-2} and 1.05%) and *P. inscriptiosinensis* (0.9 mm^{-2} and 0.18%), which significantly differed from other genotypes (0 mm^{-2} and 0%). Observation of the abaxial surface revealed the highest epidermal cell number existed in the species *P. modesta*, while the fewest was notable in *P. amboinensis*. The ultimate stomatal density manifested in the species *P. modesta* (34.51 mm^{-2}), while *P. tetraspis* displayed the lowest stomatal density (18.1 mm^{-2}). The highest stomata index resulted in the species *P. amboinensis* (6.17%), while *P. modesta* showed the lowest stomata index (3.81%).

Pearson's correlation analysis between leaf anatomy and epidermal characteristics revealed a significant correlation between leaf characters; however, a nonsignificant correlation was noticeable with SRD resistance post-inoculation based on symptom diameter (Figure 3). The results showed pre-existing leaf physical barriers were not the primary mechanism in resistance to SRD post-infection. A robust and significant negative correlation existed between leaf water content and abaxial epidermis number, adaxial epidermis number, and abaxial stomata density. In contrast, a recorded significant positive association emerged between the leaf water content and abaxial stomata index.

Table 2. *Phalaenopsis* leaf water content, anatomy, and epidermal characteristics.

Character	Resistant species				Very susceptible species				Tukey HSD
	MOD	AMB	TET	COR	INS	ZEB	BEL	FIM	
Water content (%)	86.86	92.41	90.39	88.63	91.4	89.29	91.92	90.09	3.76
Adaxial stomata number (/FV)	0	0	0	0	0.11	0.78	0	0	0.53
Adaxial epidermis number(/FV)	133.8	64.4	90.6	158.7	63.2	89.1	85.8	105.6	54.15
Adaxial stomata density (/mm^2)	0	0	0	0	0.9	5.33	0	0	4.61
Adaxial stomata index (%)	0	0	0	0	0.18	1.05	0	0	0.90
Abaxial stomata number (/FV)	6.78	4.67	3.56	6.11	3.78	5.33	5.11	5.56	2.78
Abaxial epidermis number (/FV)	169.7	71.6	73	116.1	69.4	97.2	77.9	115.7	57.50
Abaxial stomata density (/mm^2)	34.51	23.76	18.1	31.11	19.23	27.15	26.02	28.28	14.22
Abaxial stomata index (%)	3.81	6.17	4.64	5.1	5.17	5.5	6.12	4.55	1.98
Leaf thickness (μm)	405.52	933.80	750.72	1093.21	635.81	798.02	1000.62	636.9	342.36
Epidermis thickness (μm)	15.08	23.16	24.38	26.9	24.51	18.23	49.42	22.63	6.63
Mesophyll thickness (μm)	363.62	886.81	694.23	1021.54	568.99	742.11	979.41	586.31	218.27

Note: means followed by different superscript small letters indicate a significant difference within row (Tukey: $p < 0.05$), FV = field of view at 40× magnification (r = 0.5 mm); AMB = *P. amboinensis*, BEL = *P. bellina*, COR = *P. corningiana*, FIM = *P. fimbriata*, INS = *P. inscriptiosinensis*, MOD = *P. modesta*, TET = *P. tetraspis*, and ZEB = *P. zebrina.*

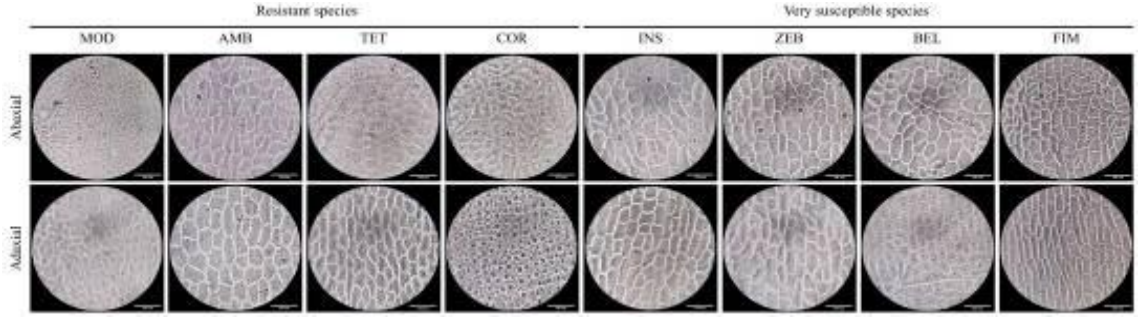

Figure 2. Leaf epidermal anatomy of representative SRD-resistant and very susceptible *Phalaenopsis* species caused by *Dickeya dadantii* at 40× magnification. MOD = *P. modesta*, AMB = *P. amboinensis*, TET = *P. tetraspis,* COR = *P. corningiana*, INS = *P. inscriptiosinensis*, ZEB = *P. zebrina*, BEL = *P. bellina,* and FIM = *P. fimbriata;* scale bar indicates 100 μm.

Observations of leaf transverse sections showed no considerable difference in leaf air space between resistant and susceptible *Phalaenopsis* species (Figure 4). All the studied *Phalaenopsis* genotypes have packed mesophyll cells with minimal airspace between cells. Visual observation of cell wall components, namely, pectin, lignin, and cellulose, exhibited no observable difference in the distribution between the resistant and very susceptible genotypes. The toluidine blue-stained section indicated pectin and lignin distribution in purple and blue colors, respectively (Figure 4) (Mitra and Loque, 2014). In most genotypes, leaf cell walls comprised pectin, while lignin primarily occurred in vascular bundles. However, lignified mesophyll cell walls were also evident

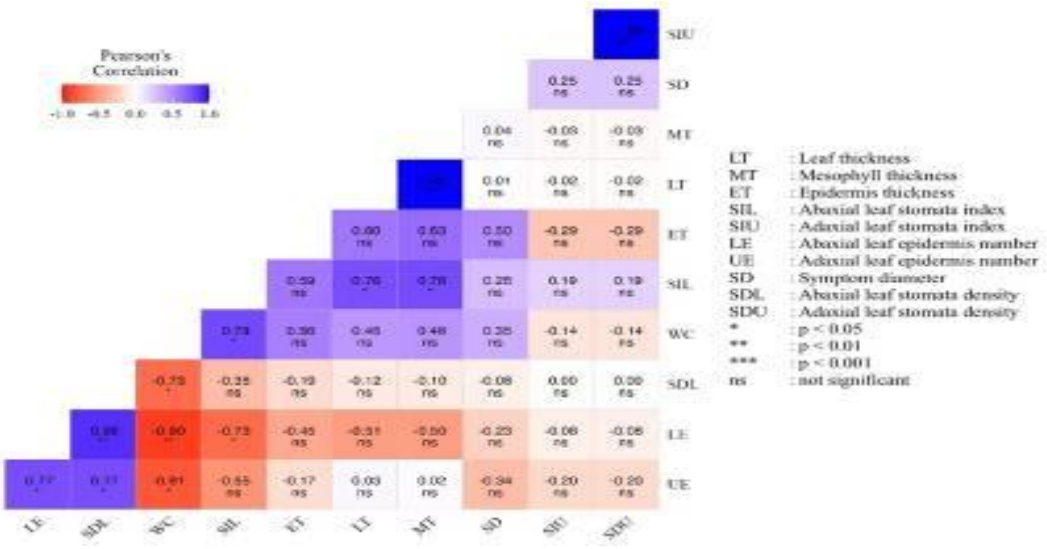

Figure 3. Correlation analysis between leaf morphology and anatomy characteristic showed nonsignificant correlation with soft rot tolerance based on symptom diameter.

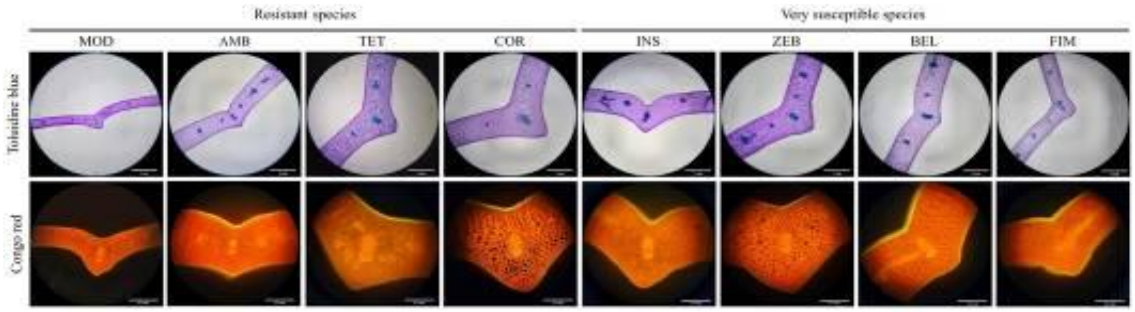

Figure 4. Transversal leaf section anatomy of representative SRD-resistant and very susceptible *Phalaenopsis* species caused by *Dickeya dadantii* at 4× magnification (Toluidine blue) and 10× magnification (Congo red). MOD = *P. modesta*, AMB = *P. amboinensis*, TET = *P. tetraspis,* COR = *P. corningiana*, INS = *P. inscriptiosinensis*, ZEB = *P. zebrina*, BEL = *P. bellina,* and FIM = *P. fimbriata;* scale bar on Toluidine blue stained sample indicates 1 mm; scale bar on Congo red stained sample indicates 0.5 mm.

in the resistant genotypes, namely, *P. amboinensis, P. corningiana,* and *P. tetraspis*, as well as in the very susceptible genotype, *P. zebrina*. Congo red-stained section showed a cellulose presence, indicated with a bright red under a blue light excitation (Mitra and Loque, 2014). An even distribution of cellulose throughout the leaf gave no considerable difference between the resistant and very susceptible *Phalaenopsis* species (Figure 4).

DISCUSSION

The screening results were in accordance with previous investigations carried out by Sukma *et al.* (2017) and Sanjaya *et al.* (2020), where reports on the species *P. amboinensis* as SRD-resistant were consistent. Meanwhile, the species *P. bellina* emerged as very susceptible to SRD caused by *D. dadantii*. The variability of response between biological replicates on *P.*

amabilis var. 'Jawa Barat,' *P. amabilis* var. 'Sulawesi,' *P. floresensis*, and *P. javanica* in this study were attributable to the genetic variability between individual plants in the wild population. Sanjaya *et al.* (2022) also reported intraspecific variations of SRD resistance in the wild population of *P. amabilis* Jawa Barat. The resistance class of screened species was also consistent with the previous study by Sukma *et al.* (2017) and Sanjaya *et al*. (2020), except for the species *P. modesta,* with previous reports as moderately susceptible. Out of 92 identified species of *Phalaenopsis*, only 18 species from the *Phalaenopsis* and *Polychilos* have reached frequent usage in *Phalaenopsis* breeding programs (Hsu *et al*., 2018). The newly identified SRD-resistant three species, namely, *P. corningiana, P. modesta,* and *P. tetraspis*, have yet to be utilized in the *Phalaenopsis* breeding program, recognizing their potential for integration into breeding programs to produce SRD-resistant cultivars.

The leaf anatomical and epidermal traits of the observed *Phalaenopsis* species differed from one another could help to delimit species within orchid genera, as reported by Liu *et al*. (2025) in *Phalaenopsis* and Hu *et al*. (2025) in *Cymbidium*. Most *Phalaenopsis* species exhibited very sparse stomata on the leaf adaxial surface, and most stomata appeared on the abaxial leaf surface (Liu *et al.,* 2025). The correlation analysis of leaf anatomy, epidermal characteristics, and water content revealed a nonsignificant correlation with SRD resistance.

Although stomatal density and index in mature leaves expressed non-correlation with SRD resistance, the potential role of stomatal dynamics and systemic suppression of stomata development on newly emerging tissues cannot be excluded. Therefore, whole-plant assays without wounding are necessary to evaluate stomata as natural entry points for pathogens (Arnaud *et al.,* 2012; Dutton *et al*., 2019). *P. amboinensis* and *P. modesta* belong to resistant species; however, exhibited different level of water content in the leaf. Among the species, variation in leaf water content may show a correlation to stomatal density, as higher abaxial stomatal density has an association with increased transpiration and reduced water content (Daningsih *et al.,* 2022).

The leaf transverse section observations of the *Phalaenopsis* species showed no visible airspace existed between the cells of the mesophyll tissue. In contrast, Guttman *et al.* (2021) reported that more leaf air space appeared in SRD-resistant calla lily repressed anaerobic conditions favored for SRD infection. The SRD pathogens' descriptions were often the brute force pathogen that relies on an arsenal of PCWDE to disrupt and macerate the cell wall and eventually cause the cell's death (Charkowski, 2018). Cell walls are one of the pre-existing structural defenses that provide a physical barrier to inhibit pathogens from spreading in the plants. Qualitative observations of lignin, pectin, and cellulose distribution did not show a specific distribution that differentiates the resistant and very susceptible species (Figure 3). It suggests pre-existing pectin, lignin, and cellulose distribution in the cell wall did not show the primary SRD resistance mechanism post-infection in *Phalaenopsis*. However, cell wall components are the potent elicitors or signal molecules to trigger plant immune response (Wan *et al*. 2021). We concede the limitations of the histochemical procedure, which presents only qualitative information and lacks the resolution needed to draw definitive conclusions.

Enhanced lignin biosynthesis post-infection could play a crucial role in SRD resistance in various crops (Ma *et al*., 2018; Ge *et al*., 2023). Accumulation of defense lignin composed of type H, G, and S monolignin observed in leaves of Chinese cabbage upon infection of *Erwinia carotovora* (Zhang *et al*., 2007). The higher lignin content and S monolignol revealed the negative correlation with bacterial wilt severity in tobacco (Ma *et al*., 2018). The higher lignin accumulation resulted in the resistant Chinese cabbage by inoculating with *Pcc* (Ge *et al.,* 2023). The pectinolytic enzyme of the SRD pathogen exhibited a lower activity on pectin substrates with a higher degree of esterification (Sason *et al.*, 2023).

CONCLUSIONS

This study successfully identified three SRD-resistant *Phalaenopsis* species, namely, *P. corningiana*, *P. tetraspis*, and *P. modesta*, enriching the breeding pipeline for SRD-resistant cultivar development. Observations on leaf anatomical and epidermal characteristics showed a significant difference between *Phalaenopsis* species, signifying the role of these characters in delimiting species within the orchid genus; however, a nonsignificant correlation was evident between these characters and SRD resistance in *Phalaenopsis*. The detached leaf method with wounding in this study can represent the resistance response to SRD; however, fails to capture the systemic defense responses and stomata dynamics as a natural entry point for bacterial pathogens. The results suggested a pre-existing defense structure was not the primary resistance mechanism against SRD post-infection in *Phalaenopsis.*

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