Cultural characteristics and pathogenicity analysis of *Phytophthora palmivora*, causal pathogen of black rot in orchids

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Abstract*—Phytophthora palmivora* is the causal pathogen of black rot, one of the major diseases affecting orchids. To evaluate its variability, *P. palmivora* was isolated from infected orchid leaves in Thailand by PARBPH selective medium, was purified again by the modified baiting method, and was cultured on Rose Bengal medium. Single-spore isolates were obtained and 5 representatives were cultured on *potato dextrose agar* (PDA), pea sucrose agar (PSA), cereal meal potato agar (CMPA; a newly developed medium) and V8 juice agar (V8) for cultural characterization. It was found that the different culture media affected growth, appearance, and sporulation. The best culture media for surface mycelium growth were PSA and V8, while CMPA induced sporulation earlier than other media. Pathogenicity analysis using 5 *Dendrobium* 'Earsakul' lines and 5 *P. palmivora* isolates showed that NK-53-9 was the most virulent isolate, which is useful for future screening of black rot resistance. A *Dendrobium* mutant, 'SUT13E18-A', was resistant to all isolates, suggesting its usefulness as a resistance source in future breeding program.

***Index Terms*—** Culture media, *Dendrobium*, resistance, virulence

I. Introduction

Orchid is one of the largest families of flowering plants in the world comprising of ca. 28,000 species and more than 700 different genera [1]. Its wide range of different characteristics in shapes, sizes and colors has made orchid the plant for all types of interests and sought for by collectors. Therefore, they are undoubtedly recognized as an economically important ornamental in the international floriculture industry, both as cut flowers and potted plants [2], [3]. Among various orchid genera, *Dendrobium* orchids have become increasingly popular [3]. And they are also the major cut-flower orchid export for Thailand, however, they usually face with numerous disease problems. Several species of *Phytophthora* have been reported to cause economic damage on orchids worldwide. Black rot (*Phytophthora palmivora*) is an exotic, polyphagous pathogen that has been reported worldwide on different hosts, mainly causing root and crown rot diseases. It has become widespread in Asia and Europe, where it has been reported on several ornamental plants including orchids [4]-[6]. *P. palmivora* is also recorded as a foliar pathogen of cocoa [7] and macadamia [8]. The incidence of these diseases has increased during the past few years due to both polyphagy and means of dissemination. And because it is a thermophilic species, the hot environmental conditions such as in Thailand are favorable for its development [5]. The pathogen can spread throughout the rhizomes, causing rapid plant death [9], [10].

The objectives of this work were to evaluate culture media for *P. palmivora* and to select *P. palmivora* that was the most virulent to preliminarily evaluate resistance levels of the potentially resistant lines in *Dendrobium* 'Earsakul'.

II. Materials and Methods

A. Pathogen Isolation

Isolates of *P. palmivora* from Nakhon Ratchasima Province, Thailand were collected from leaves of susceptible orchid varieties affected by the black rot disease in 2010. They were washed under running tap water, surface-sterilized in 70% ethanol for 5-10 seconds then dried on filter paper. Approximately 2-4 mm-wide tissues were cut from the edge of the lesions and placed on potato dextrose agar (PDA) amended with 10 mg pimaricin, 200 mg ampicilin, 10 mg rifampicin, 10 mg benomyl, 25 mg pentachloronitrobenzene and 50 mg hymexazol (PARBPH) [11]. Inoculated plates were incubated at 25ºC in the dark and examined within 2-3 days. *P. palmivora* isolates were obtained by subculturing the hyphal tips onto PDA, were purified again by the modified baiting method, and were cultured on Rose Bengal medium. Single-spore isolates were obtained from individual fungal colonies.

B. Cultural Characterization

Five single-spore *P. palmivora* isolates (NK-53-5, NK-53-6, NK-53-7, NK-53-9 and NK-59-11) were characterized. A 4-mm-diameter agar disk of each isolate was obtained by cutting with a sterile cork borer, and placed onto 4 different culture media: (1) PDA (20% (w/v) potato, 2% (w/v) D-glucose, 2% (w/v) agar); (2) pea sucrose agar (PSA; 12.5% (w/v) pea, 1% (w/v) sucrose, 1.5% (w/v) agar); (3) corn meal potato agar (CMPA; 5% (w/v) oat, 5% (w/v) corn grit, 5% (w/v) rice bran, 5% (w/v) potato, 1% (w/v) D-glucose, 4% (w/v) potato dextrose agar and 0.3% agar) and (4) V8 juice agar (V8; (20% (v/v) V8 juice, 0.3% (w/v) calcium carbonate (CaCO3) and 1.5% (w/v) agar) in 9-cm-diameter Petri dishes and incubated at 25ºC in the dark. Five replicates per isolate

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were made, and the following observations were taken daily to a maximum of 3 weeks after plating on the medium: (1) colony size (area = ¶ (width/2) (length/2)) and colony appearance (colonies were characterized based on surface characteristics [rosette, radiate, stellate and irregular]) [12]-[14].

C. Pathogenicity Tests

Agar pieces containing mycelium of 5 single-spore *P. palmivora* isolates were collected from 5-day-old colonies grown on CMPA, were transferred to sterile bottles, were covered with sterile reverse osmosis water (ROW), and were incubated overnight at 25ºC in the dark [15]. Inoculums were adjusted to 106 zoospores/ml, and 3µl drops of each suspension were inoculated on leaves of 5 *Dendrobium* lines (three controls; SUT13C0-1, SUT13C0-2, SUT13C0-3 and two mutants; SUT13E18-A and SUT13E18-E) after pin wounding in a detached leaf assay [16]. While control (no inoculum) was inoculated with ROW. All inoculated leaves were incubated under 25ºC in the dark. Four replicates per *Dendrobium* line were made, and symptom development was observed at 3 and 5 days after inoculation; scores were attributed according to the following scale: 0, no symptom; 1, very small localized lesions; 2, yellow around lesions; 3, yellow around lesions, hyphae was revealed; 4, brown lesions, hyphae was expanded; 5, brown lesions, hyphae was expanded outside the area covered [17].

D. Statistical Analysis

Data was analyzed using analysis of variance (ANOVA) of colony size and (X+1)1/2 transformed severity of symptom score. Mean comparison was performed by Duncan's multiple range test (DMRT) to evaluate the differences in the ability of culture media to promote mycelial growth and severity of symptoms using SPSS version 14.0 [18].

III. Results

Five single-spore isolates of *P. palmivora* (NK-53-5, NK-53-6, NK-53-7, NK-53-9 and NK-59-11) which were grown on various media (PDA, PSA, CMPA, and V8) were characterized after 3 weeks. It was shown that the effects of isolates were highly significant (p<0.01) on colony size. The most rapidly grown isolate was NK-53-6 with a colony size of 19.27 cm^2, which was significantly higher than other isolates. Whereas NK-59-11 and NK-53-9 had the smallest colony size at 3 and 7 days after culture, respectively. But after cultured for 14 days, all isolates reached similar colony size except for NK-53-7. And colonies of all isolates grew to edges of Petri dishes at 21 days after culture (Table I).

When these isolates were grown on PDA, PSA, CMPA, and V8, it was found that different culture media affected colony size of all *P. palmivora* isolates significantly (p<0.01). Overall, the best media for promoting colony size were PSA and V8 for all culture periods. At 3 and 7 days after culture, the colony sizes on PSA and V8 were 21.68, 20.38 and 60.00 and 61.81 cm^2, respectively, which were significantly higher than those of other media, particularly PDA. Although the colony sizes on PSA and V8 were not significantly different from that on CMPA at 14 days after culture, their colony size was still 1.2-fold significantly larger than that on PDA (Table II).

TABLE I: EFFECTS OF SINGLE-SPORE ISOLATES ON COLONY SIZE OF *PHYTOPHTHORA PALMIVOLA* AT 3, 7, 14 AND 21 DAYS AFTER CULTURE

Isolates	Colony size (cm^2)			
	3 days	7 days	14 days	21 days
NK-53-5	14.02 ± 1.99 b [1]	49.15 ± 3.79 ab	63.64 ± 3.26 a	63.64 ± 3.26
NK-53-6	19.27 ± 1.72 a	53.06 ± 3.94 a	62.97 ± 0.67 a	63.64 ± 3.26
NK-53-7	15.23 ± 1.87 b	44.58 ± 4.89 bc	55.37 ± 3.62 b	63.64 ± 3.26
NK-53-9	12.64 ± 1.73 c	40.81 ± 4.07 c	62.48 ± 1.07 a	63.64 ± 3.35
NK-59-11	10.24 ± 1.44 d	46.54 ± 4.54 b	61.30 ± 1.46 a	63.64 ± 3.26

[1] Data are presented as means ± SE. Means in the same column with different letters are significantly different (p<0.05) based on Duncan's multiple range test (DMRT).

TABLE II: EFFECTS OF CULTURE MEDIA ON COLONY SIZE OF *PHYTOPHTHORA PALMIVOLA* AT 3, 7, 14 AND 21 DAYS AFTER CULTURE

Media	Colony size (cm^2)			
	3 days	7 days	14 days	21 days
PDA	5.74 ± 0.52 c [1]	21.74 ± 1.82 c	54.40 ± 3.02 b	63.64 ± 4.35
PSA	21.68 ± 0.86 a	60.00 ± 1.60 a	63.64 ± 4.35 a	63.64 ± 4.35
CMPA	9.18 ± 1.26 b	43.88 ± 3.03 b	62.95 ± 0.70 a	63.64 ± 4.44
V8	20.38 ± 0.84 a	61.81 ± 1.29 a	63.64 ± 4.35 a	63.64 ± 4.35

[1] Data are presented as means ± SE. Means in the same column with different letters are significantly different (p<0.05) based on Duncan's multiple range test (DMRT).

A highly significant interaction (p<0.01) on colony size was observed among single-spore isolates of *P. palmivora* grown on different culture media. When both factors were evaluated at 3 days after culture, a maximum colony size was obtained in NK-53-7 when cultured on PSA (25.03 cm^2), which was not significantly different from those of NK-53-6 cultured on V8 and PSA, NK-53-5 cultured on V8 and NK-53-9 cultured on PSA. And after cultured for 7 days, most isolates which were cultured on PSA and V8, and some isolates which were cultured on CMPA had grown to significantly larger colony size than those cultured on PDA. This tendency was observed until 14 days after culture (Table III). Growth characteristics of mycelium depended on the media used. Among the 4 media, it was found that a radiate morphology was observed when all isolates were cultured on PSA, a rosette morphology was found on PDA and CMPA. By contrast, V8 showed irregular colony morphology. All isolates were white and aerial mycelium was found on most media. The highest aerial mycelium formation was found on PDA and CMPA, while PSA and V8 induced poor aerial mycelium formation (Fig. I).

Pathogenicity of five single-spore *P. palmivora* isolates (NK-53-5, NK-53-6, NK-53-7, NK-53-9 and NK-59-11) were evaluated on five *Dendrobium* lines (SUT13C0-1, S$UT13C0-2, SUT13C0-3, SUT13E18-A and SUT13E18-E) by a detached leaf assay. *Dendrobium* leaves started to show necrotic lesions

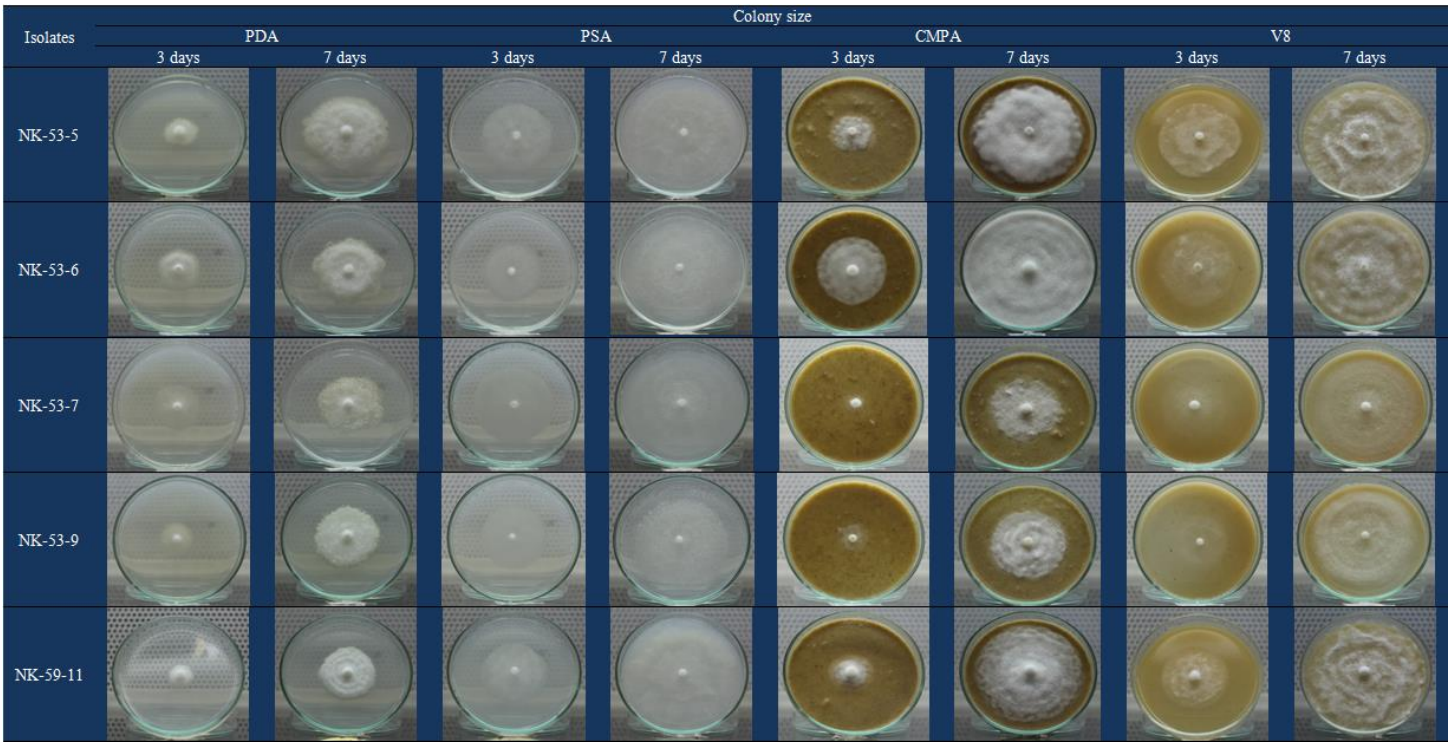

Fig. 1. Colony morphology of single-spore *Phytophthora palmivora* isolates on PDA, PSA, CMPA and V8 after 3 and 7 days of growth at 25°C in the dark.

with the hyphae covering them at 3 days after inoculation. When all isolates were inoculated on *Dendrobium* leaves with a 10^6 zoospores/ml suspension, necrotic lesions and hyphae were formed differently among isolates (p<0.01). After inoculation for 3 days, NK-53-9 was the most virulent isolate with a severity score of 1.20, which was not significantly different from those of NK-53-6 and NK-59-11. Control did not show any symptoms for the entire period of experiment (Table IV).

TABLE II: COLONY SIZE OF SINGLE-SPORE *PHYTOPHTHORA PALMIVOLA* ISOLATES CULTURED ON DIFFERENT CULTURE MEDIA AT DIFFERENT CULTURE PERIODS

Isolates	Media	Colony size (cm^2)		
		3 days	7 days	14 days
NK-53-5	PDA	4.39 ± 0.25 gh [1]	24.03 ± 1.56 efg	63.64 ± 3.55 a
	PSA	20.76 ± 1.11 bc	63.64 ± 3.55 a	63.64 ± 3.55 a
	CMPA	6.92 ± 0.45 fg	45.29 ± 2.03 cd	63.64 ± 3.55 a
	V8	24.01 ± 1.13 ab	63.64 ± 3.55 a	63.64 ± 3.55 a
NK-53-6	PDA	8.25 ± 0.29 f	25.17 ± 4.38 efg	60.97 ± 2.68 ab
	PSA	24.43 ± 2.77 a	59.80 ± 3.67 ab	63.64 ± 3.55 a
	CMPA	19.65 ± 1.08 cd	63.64 ± 3.55 a	63.64 ± 3.55 a
	V8	24.75 ± 1.59 a	63.64 ± 3.55 a	63.64 ± 3.55 a
NK-53-7	PDA	8.37 ± 1.37 f	22.57 ± 7.70 fg	33.91 ± 9.08 c
	PSA	25.03 ± 0.87 a	63.08 ± 0.57 a	63.64 ± 3.55 a
	CMPA	7.52 ± 2.56 fg	34.48 ± 9.03 de	60.30 ± 3.34 ab
	V8	20.00 ± 0.86 cd	58.20 ± 5.44 ab	63.64 ± 3.55 a
NK-53-9	PDA	4.87 ± 0.26 fgh	21.38 ± 1.61 fg	59.23 ± 4.02 ab
	PSA	21.39 ± 1.05 abc	49.82 ± 5.33 bc	63.64 ± 3.55 a
	CMPA	5.08 ± 0.97 fgh	29.93 ± 3.73 ef	63.64 ± 3.55 a
	V8	17.14 ± 0.79 de	59.93 ± 3.71 ab	63.64 ± 3.55 a
NK-59-11	PDA	2.83 ± 0.17 h	15.57 ± 1.53 h	54.26 ± 4.91 b
	PSA	16.77 ± 0.50 de	63.64 ± 3.55 a	63.64 ± 3.55 a
	CMPA	5.36 ± 0.29 fgh	43.30 ± 0.97 cd	63.64 ± 3.55 a
	V8	16.00 ± 0.64 e	63.64 ± 3.55 a	63.64 ± 3.55 a

[1] Data are presented as means ± SE. Means in the same column with different letters are significantly different (p<0.05) based on Duncan's multiple range test (DMRT).

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TABLE IV: EFFECTS OF SINGLE-SPORE ISOLATES OF *PHYTOPHTHORA PALMIVOLA* ON SEVERITY OF SYMPTOMS OF *DENDROBIUM* LEAVES BY A DE-TATCHED LEAF ASSAY AT 3 AND 5 DAYS AFTER INOCULATION

Isolates	Severity of symptoms [1]	
	3 days	5 days
Control (ROW) [2]	0.00 ± 0.00 c [3]	0.00 ± 0.00 c
NK-53-5	0.70 ± 0.35 b	0.80 ± 0.35 b
NK-53-6	0.90 ± 0.28 ab	1.40 ± 0.39 a
NK-53-7	0.45 ± 0.17 b	1.25 ± 0.40 a
NK-53-9	1.20 ± 0.44 a	1.55 ± 0.44 a
NK-59-11	0.85 ± 0.22 ab	1.35 ± 0.39 ab

[1] Severity scale: 0, no symptom; 1, very small localized lesions; 2, yellow around lesions; 3, yellow around lesions, hyphae was revealed; 4, brown lesions, hyphae was expanded; 5, brown lesions, hyphae was expanded outside the area covered [17].

[2] ROW: reverse osmosis water

[3] Data are presented as means ± SE. Means in the same column with different letters are significantly different (p<0.05) based on Duncan's multiple range test (DMRT).

The effects of *Dendrobium* lines on severity of symptoms were highly significant (p<0.01). After inoculation for 3 days, SUT13C0-3 was the most susceptible with severity score of 1.75, which was significantly higher than those of other lines. Similar results were observed at 5 days after inoculation, but with increasing severity of symptoms. In addition, we found that one of the mutant, SUT13E18-A, had no symptom on its leaves throughout the experiment (Table V).

TABLE V: EFFECTS OF *DENDROBIUM* LINES ON SEVERITY OF SYMPTOMS AT 3 AND 5 DAYS AFTER INOCULA TION WITH *PHYTOPHTHORA PALMIVOLA*

Lines	Severity of symptoms[1]	
	3 days	5 days
SUT13C0-1	0.33 ± 0.13 c [2]	0.75 ± 0.28 c
SUT13C0-2	1.00 ± 0.17 b	1.38 ± 0.22 ab
SUT13C0-3	1.75 ± 0.45 a	2.00 ± 0.50 a
SUT13E18-A	0.00 ± 0.00 c	0.00 ± 0.00 d
SUT13E18-E	0.33 ± 0.16 c	1.17 ± 0.36 bc

[1] Severity scale: 0, no symptom; 1, very small localized lesions; 2, yellow around lesions; 3, yellow around lesions, hyphae was revealed; 4, brown lesions, hyphae was expanded; 5, brown lesions, hyphae was expanded outside the area covered [17].

[2] Data are presented as means ± SE. Means in the same column with different letters are significantly different (p<0.05) based on Duncan's multiple range test (DMRT).

The interactions between single-spore isolates and culture media were highly significant (p<0.01). Severity of symptoms on *Dendrobium* leaves were the highest when SUT13C0-3 was inoculated with NK-53-9 and NK-53-6, and when SUT13E18-E was inoculated with NK-53-7 for 5 days after inoculation (Table VI). By contrast, all *Dendrobium* leaves that were inoculated with control (ROW), remained unchanged throughout the experiment. In addition, it was found that SUT13E18-A was potentially resistant to all isolates of *P. palmivora* with no symptom observed (Table VI).

IV. DISCUSSION

Cultural characteristics of single-spore *P. palmivora* isolates from Nakhon Ratchasima, Thailand were affected by different media used. All isolates exhibited maximum mycelial growth when they were cultured on PSA and V8, while PDA induced the lowest growth. Therefore, optimal culture media for mycelia growth of all *P. palmivora* isolates were PSA and V8. CMPA, a newly developed medium, however, produced the earliest sporulation. Similarly, V8 agar and broth have been reported as excellent growth media for species of *Phytophthora* and *Pythium* [19]. V8 juice agar was also the best medium for growth and reproduction in *P. colocasiae* giving 83.47 mm of mycelial growth while PDA gave a mycelial growth of only 51.46 mm [20]. In addition, carrot agar also promoted a maximum radial growth of *P. colocasiae* [21]. PSA, and PSB (agar-free) have been used for culturing *P. parasitica*, and PSB was successfully used to obtain CF for in vitro selection of *Citrus jambhiri* for resistance to *Phytophthora* species [22]. Likewise, supplementing other legumes in soybean agar medium was also superior to VJ (V8) agar for supporting growth rates and reproduction of sporangia of *Phytophthora* and *Pythium* species [23]. The colony characteristics of *Phytophthora* species; appearance, rates and manner of growth, amount of sporulation, and sporangia sizes appear to depend on media types [13]. They usually grow best on media that contain thiamine, a suitable carbohydrate source (sucrose) organic additives (potato, oat, pea etc.), nitrogen sources, inorganic salts and minor elements [24]. In addition, growth characteristics of mycelium also depended on the types of media used. While our results showed rosette, radiate and irregular morphology when isolates of *P. palmivora* were cultured on PDA and CMPA, PSA, and V8, respectively, *Phytophthora* species from cocoa (*Theobroma cacao*, L.) exhibited stellate, cottony and cottony and rosette with slight stellate morphology for *P. palmivora*, *P. megakarya* and *P. capsici* on V8, respectively [13]. Moreover, *P. arenaria* isolates produced colonies with a radiate morphology on V8A and CA and radiate patterns on MEA and PDA, while some isolates also produced irregular colony morphologies [14]. The variation in colony patterns depended on the frequency, angle and extent of hyphal branching, and emphasized that certain *Phytophthora* species had distinctive colony patterns that persisted under a variety of cultural conditions [13].

When the five *Dendrobium* lines were inoculated with virulent isolates of *P. palmivora* in a detached leaf assay (10^6 zoospores/ml), NK-53-9, NK-53-6 and NK-53-7 were found to elicit the highest severity of symptoms on *Dendrobium* leaves. Similarly, when curcuma was inoculated with 10^6 zoospores/ml of *P. palmivora*, disease symptoms similar to those appear in natural conditions were reproduced [25]. The severity of symptoms also depended on plant genotypes. When rubber (*Hevea brasiliensis*) leaves were inoculated with $5x10^6$ spores/ml of *P. palmivora*, the BPM-24 (resistant) line was found to be the most resistant with small lesion size while the RRIM600 (susceptible) line showed opposite observation [26]. We also found differential symptom severity among various *Dendrobium* lines. SUT13E18-A (a *Dendrobium* mutant) was resistant to all isolates of *P. palmivora* evaluated in this study. Similarly, transgenic orange that contained PR-5-type proteins with antifungal activity against several classes of fungi and oomycetes showed higher tolerant to *Phytophthora* than the control [27].

TABLE VI: SEVERITY OF SYMPTOMS ON DIFFERENT *DENDROBIUM* LINES AFTER INOCULATION WITH DIFFERENT SINGLE-SPORE ISOLATES OF *PHYTOPHTHORA PALMIVOLA*

Lines	Isolates	Severity of symptoms[1]	
		3 days	5 days
SUT13C0-1	ROW (control)[2]	0.00 ± 0.00 f[3]	0.00 ± 0.00 g
	NK-53-5	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-6	0.00 ± 0.00 f	0.75 ± 0.25 efg
	NK-53-7	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-9	0.75 ± 0.25 def	1.00 ± 0.00 defg
	NK-59-11	1.25 ± 0.48 cd	2.75 ± 1.31 bcd
SUT13C0-2	ROW (control)	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-5	1.00 ± 0.41 cde	1.50 ± 0.29 cde
	NK-53-6	1.50 ± 0.29 cd	1.75 ± 0.25 cde
	NK-53-7	1.25 ± 0.25 cd	2.25 ± 0.48 cd
	NK-53-9	0.25 ± 0.25 ef	0.25 ± 0.25 fg
	NK-59-11	2.00 ± 0.00 bc	2.50 ± 0.29 bc
SUT13C0-3	ROW (control)	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-5	2.50 ± 1.44 bc	2.50 ± 1.44 cde
	NK-53-6	3.00 ± 0.00 b	4.50 ± 0.29 a
	NK-53-7	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-9	5.00 ± 0.00 a	5.00 ± 0.00 a
	NK-59-11	0.00 ± 0.00 f	0.00 ± 0.00 g
SUT13E18-A	ROW (control)	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-5	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-6	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-7	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-9	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-59-11	0.00 ± 0.00 f	0.00 ± 0.00 g
SUT13E18-E	ROW (control)	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-5	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-6	0.00 ± 0.00 f	0.00 ± 0.00 g
	NK-53-7	1.00 ± 0.58 def	4.00 ± 0.87 ab
	NK-53-9	0.00 ± 0.00 f	1.50 ± 0.87 cdef
	NK-59-11	1.00 ± 0.58 def	1.50 ± 0.87 cdef

[1] Severity scale: 0, no symptoms; 1, very small localized lesions; 2, yellow around lesions; 3, yellow around lesions, hyphae was revealed; 4,brown lesions, hyphae was expanded; 5,brown lesions, hyphae was expanded outside the area covered [17].

[2] ROW: reverse osmosis water

[3] Data are presented as means ± SE. Means in the same column with different letters are significantly different (p<0.05) based on Duncan's multiple range test (DMRT).

V. CONCLUSION

These results suggest that PSA and V8 agar are the optimal media for promoting mycelial growth and CMPA, a newly developed medium, can induce the earliest sporulation in *P. palmivora*. We obtained the most virulent isolate (NK-53-9) to be used as an efficient screening agent for black rot resistance in *Dendrobium*. In addition, a *Dendrobium* 'Earsakul' mutant, SUT13E18-A, was found to be resistant to all isolates. These results are useful for the development of black rot resistant *Dendrobium* cultivars in the future.

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