



Full Length Article

First Report on the Prevalence of *Colletotrichum scovillei* Associated with Anthracnose on Chili Pepper in Bali, Indonesia

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Abstract

Anthracnose caused by *Colletotrichum* spp. is one of important constrains for chili production in Indonesia. The disease may cause significant yield loses of economic importance. Variation in disease incidence and intensity frequently occur because of the variation in chili cultivar, cultural practices, weather condition, localities and species of *Colletotrichum*. This study was conducted to evaluate the incidence of anthracnose disease on chili pepper in 9 regencies in Bali and species of *Colletotrichum* associated with anthracnose on chili pepper in Bali Island, Indonesia. Survey for disease incidence and intensity was conducted during March to April 2018. Two chili growing areas were surveyed in each regency. Anthracnose disease was found in all the chili growing areas in Bali. The average for anthracnose disease incidence was 63% while the average disease intensity was 68%. Two cultivars of chili pepper that commonly grown by the farmers in Bali namely “Cabe Rawit” with small fruit and hot taste and “Cabe Besar” with relatively bigger fruit and less hot taste showed different tolerance to anthracnose disease. The disease incidence on Cabe Rawit was relatively lower when compared to Cabe Besar in which the average disease incidence and disease intensity for Cabe Rawit were 59% and 64%, respectively, while that of Cabe Besar were 70% and 76%, respectively. Six species of *Colletotrichum* were identified based on the analysis of 18S rRNA gene. Among 18 isolates, 10 isolates (55.55%) were identified as *Colletotrichum scovillei*, 2 isolates (11.11%) were *Colletotrichum acutatum*, 2 isolates (11.11%) were *Colletotrichum gloeosporioides*, 2 isolates were *Colletotrichum nymphaeae* and one isolate each was identified as *Colletotrichum fructicola* and *Colletotrichum truncatum*. Results of present study revealed that *Colletotrichum scovillei* is the most prevalent species that associated with anthracnose disease in Bali, Indonesia. This is the first report on the prevalence of *C. scovillei* associated with anthracnose disease in chili pepper in Bali Indonesia. © 2019 Friends Science Publishers

Keywords: Anthracnose disease; Chili pepper; *Colletotrichumscovillei*; Prevalence

Introduction

Chili pepper is grown in various tropical, sub-tropical and temperate areas of the world including Indonesia (Tong and Bosland, 1999; Bosland and Votava, 2003). The price of chili pepper in Indonesia is sharply fluctuated because of the fluctuation in market supply due to the fluctuation in chili production. Anthracnose disease caused by *Colletotrichum* spp. is one of the most severe and important causes of yield losses in chili pepper ranging from 10 to 80% (Asare-Badiako *et al.*, 2015; Diao *et al.*, 2017).

Anthracnose commonly causes rot on mature fruit, and results in problem both before and after harvest (Bosland and Votara, 2003). Chili pepper can be infected by more than one species of *Colletotrichum* such as *C. acutatum*, *C. capsici*, *C. gloeosporioides* and *C. coccodes* (Pakdeevaporn *et al.*, 2005; Sharma *et al.*, 2005; Than *et al.*, 2008). According to Suryaningsih *et al.* (1996) the most common cause of anthracnose disease in Indonesia is *Colletotrichum capsici*

and *C. gloeosporioides*, while Widodo and Hidayat (2018) reported three species of *Colletotrichum* associated with anthracnose on chili pepper in Indonesia namely *C. acutatum*, *C. capsici* and *C. gloeosporioides*, in which *C. acutatum* was the most prevalent. The anthracnose disease in Bali was reported caused by *Colletotrichum acutatum* (Sudiarta, 2012). Confirmation of the species of *Colletotrichum* is very important step to decide an appropriate control strategy, since different species of *Colletotrichum* may give different responses against control measures. *Colletotrichum acutatum* has been found to possess moderate susceptibility against benzimidazole, while *C. gloeosporioides* is highly susceptible to this fungicide (Peres *et al.*, 2005). One of the obstacles in order to develop control measures against anthracnose is the species complexes of *Colletotrichum*. There is possibility that one type of chili pepper that show anthracnose symptom is infected by more than one species of *Colletotrichum*. This phenomenon may happen in chili pepper grown in Bali Island,

Indonesia. Based on these considerations, it is urgent to know the species diversity of *Colletotrichum* that associated with anthracnose symptom in chili pepper in Bali Island, Indonesia as a reference to develop an appropriate control measure. Purpose of this study was to determine the species of *Colletotrichum* associated with symptomatic tissues of chili pepper grown in Bali, Indonesia.

Materials and Methods

Survey for Anthracnose Disease Incidence

Survey of anthracnose disease incidence was done in nine regencies in Bali where chili pepper is grown. Observation of disease incidence was carried out on 500 chili plants in three sampling sites per location and two location per regency. Number of plants with anthracnose symptom was recorded to determine disease incidence. In addition to disease incidence, disease intensity was also determined by observing 10 plants per sampling sites. The disease intensity was calculated based on method developed by Sinaga (2006).

Isolation and Identification of *Colletotrichum* spp.

A total of 18 samples of symptomatic fruits (two samples per regency) were taken and used for isolation of *Colletotrichum* spp. The infected fruits were washed with tap water to remove the surface contamination and washed with sterile distilled water. The fruit was cut off between symptomatic and healthy areas into pieces (1 cm × 1 cm) and cultured on potato dextrose agar (PDA) in a Petri dish. This culture was incubated in the dark until obvious fungal hyphae appear from the edge of the fruit cuttings. The fungus was then purified and a single spore isolation was done prior to further use. Koch postulate procedure was done to ensure that the isolated fungi were the cause of anthracnose disease on chili pepper. All isolates were inoculated onto chili pepper fruits and incubated in the dark at 25°C. The development of symptom was observed three days after incubation. Reisolation was done from infected chili fruit to get pure isolate of *Colletotrichum*. These isolates were then maintained on PDA slant medium before used.

All 18 pure cultures of *Colletotrichum* spp. were identified based on morphological characteristics using the keys for fungal identification developed by Pitt and Hocking (1997) such as shape and color of colony on PDA medium, shape and size of conidia, and presences of acervuli and setae. Molecular identification was also done through analysis of 18S rRNA genes based on method developed by Photita *et al.* (2005).

Molecular Identification

All 18 isolates of *Colletotrichum* were cultured on PDA medium for three days under room temperature (28±2°C). DNA genomes of *Colletotrichum* spp. Were extracted by taking the hyphae from the edge of colony and put into

centrifuge tube and was suspended with 100 mL PrepMan Ultra reagent (PrepMan Ultra Protocol, Applied Biosystem, USA). Samples were then vortexed for 30 sec and put on heat block at 95°C to 100°C for 10 min and the was put under room temperature for 2 min. Samples were then centrifuged at 10,000 rpm for 2 min and pellet containing DNA was taken and was used for further test (Cano *et al.*, 2004).

Amplification of 18S rRNA gene was done using PCR with two primers *Internal Transkript Spacer* (ITS) 1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS 4 (5-TCCTCCGCTTATTGATATGC-3). Reaction was done using *Takara PCR thermal cycler Personal* tool (Takara Bio, Otsu, Japan) with Ex Tag (Takara Bio, Otsu, Japan) under condition : *pre-denaturation* 94°C (4 min) followed by 35 *cycles* denaturation at 94°C (35 sec), *annealing* 52°C (55 sec), *elongation* 72°C (2 min) and *post elongation* 72°C for 10 min (Nishizawa *et al.*, 2010).

Nucleotide sequence was determined using *Big Dye Terminator Cycle Sequencing Ready Reaction Kit* (Applied Biosystems, Foster City, CA, USA) according to the manual of the equipment with *PE Applied Biosystems Automated DNA Sequencer* (model 3130xl, Applied Biosystems). Sequence of DNA *double helix* was assembled and analyzed using Genetyx (version 11.0) and Genetyx-ATSQ (version 4.0) software (Genetyx, Tokyo, Japan), in a sequential order and compared with the same DNA sequence that was accessed from DDBJ/EMBL/GenBank through NCBI BLAST program (Thompson *et al.*, 1997).

Phylogeny analysis was done using MEGA 4.0 program (Kumar *et al.*, 2001). Method of Neighbor Joining (NJ) was done with 1000x bootstrap with the following steps: (1). Seaching for similarities among sequences. Data was stored in otepad in a FASTA format, analyzed using Blast-WU facility available online in site of www.ebi.ac.uk/Clustalw. Based on similarity analysis can be identified if the level of similarity of 18S rRNA gene than 97% can be considered as a new species (Pangastuti, 2006). (2). Drawing up phylogenetic tree using MEGA program.

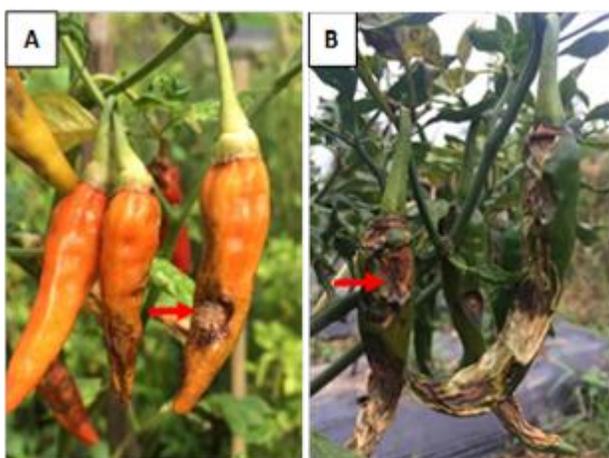
Results

Disease Incidence and Intensity of Anthracnose on Chili Pepper

Anthracnose disease was observed in all the 9 regencies in Bali where chili pepper is grown. The disease incidence varied from 30% to 98% with the average 63%, while the disease intensity varied from 20% to 95% with the average 68%. There are two cultivars of chili pepper are comonly being cultivated in Bali *i.e.*, cultivar Cabe Rawit (small fruit with hot taste) and cultivar Cabe Besar (big fruit and less hot taste). Both of these cultivars were susceptible to anthracnose disease, however to some extent the cultivar Cabe Rawit is more tolerant to anthracnose disease when compared to cultivar Cabe Besar. This is indicated by the average of disease incidence and intensity on cultivar Cabe Rawit was 59% and 64%, while on cultivar Cabe Besar was 70% and 76%, respectively. (Table 1).

Table 1: Disease incidence and intensity of anthracnose on chilli pepper in 9 regencies of Bali

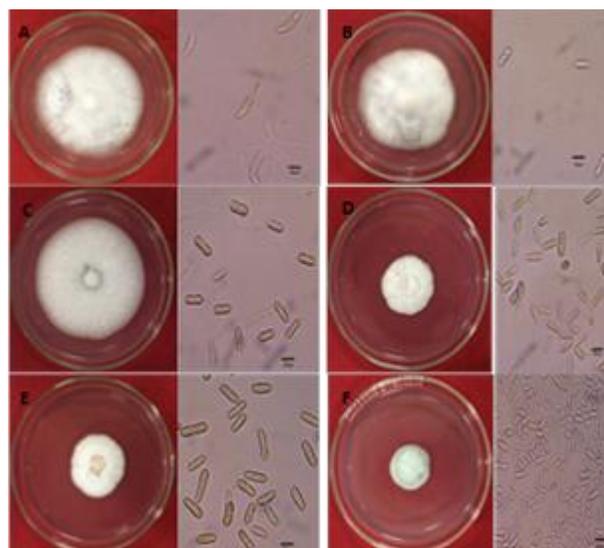
No	Survey site	Disease incidence (%)	Disease intensity (%)	Cultivar of chilli pepper
1	District of Mengwi, Badung regency	91	85	Cabe Rawit
2	District of Petang, Badung regency	30	36	Cabe Besar
3	District of Baturiti, Tabanan regency	92	80	Cabe Besar
4	District of Marga, Tabanan regency	98	90	Cabe Rawit
5	District of Sukasada, Buleleng regency	60	51	Cabe Rawit
6	District of Sukasada, Buleleng regency	95	87	Cabe Besar
7	District of Payangan, Gianyar regency	71	78	Cabe Besar
8	District of Payangan, Gianyar regency	90	94	Cabe Rawit
9	District of Klungkung, Klungkung regency	30	20	Cabe Rawit
10	District of Banjarangkan, Klungkung regency	36	30	Cabe Rawit
11	District of Rendang, Karangasem regency	90	83	Cabe Rawit
12	District of Rendang, Karangasem regency	41	81	Cabe Besar
13	District of Kintamani, Bangli regency	97	91	Cabe Rawit
14	District of Kintamani, Bangli regency	91	95	Cabe Besar
15	District of Mendoyo, Jembrana regency	40	75	Cabe Rawit
16	District of Jembrana, Jembrana regency	36	77	Cabe Rawit
17	District of Denpasar Utara, Denpasar regency	21	26	Cabe Rawit
18	District of Denpasar Selatan, Denpasar regency	23	51	Cabe Rawit
Average		63	68	
Average for Cabe Rawit		59	64	
Average for Cabe Besar		70	76	

**Fig. 1:** Field symptoms of anthracnose on chilli pepper cv. Cabe Rawit (A) and cv. Cabe Besar (B). Arrows indicate the light brown sunken lesions (A) on cv. Cabe Rawit, and brown sunken lesions (B) on cv. Cabe Besar

Symptoms of anthracnose on chili pepper in the field was characterized by dark brown sunken lesions on the fruits of chili of cv. Cabe Besar and brown sunken lesions on the fruit of chili of cv. Cabe Rawit as shown in Fig. 1.

Morphological Characteristics of *Colletotrichum* spp.

Most of *Colletotrichum* species formed cottony white colony on PDA medium incubated at room temperature ($28 \pm 2^\circ\text{C}$), except for isolate BLCB formed gray cottony colony as presented in Fig. 2. Among 6 species of *Colletotrichum* identified in this study, three species namely *C. gloeosporioides* TBCB, *C. fructicola* KRKR and *C. truncatum* DPCR2 showed relatively fast growth rate with range between 8.50 mm to 9.60 mm/day. While other three

**Fig. 2:** Colonies appearance of *Colletotrichum* isolates on PDA medium (left) and conidia observed under light microscope (right). A. *Colletotrichum truncatum* DPCR2, B. *Colletotrichum fructicola* KRKR, C. *Colletotrichum gloeosporioides* TBCB, D. *Colletotrichum scovillei* BDCR, E. *Colletotrichum nymphaeae* BLCR and F. *Colletotrichum acutatum* BLCB. Bars represent $20 \mu\text{m}$

species namely *C. acutatum* BLCB, *C. nymphaeae* BLCR and *C. scovillei* BDCR showed relatively slow growth rate ranged between 3.35 mm to 4.35 mm/day (Table 2).

Five species of *Colletotrichum* namely *C. gloeosporioides* TBCB, *C. fructicola* KRKR, *C. acutatum* BLCB, *C. nymphaeae* BLCR and *C. scovillei* BDCR produced conidia with cylindrical shape and rounded ends, while *C. truncatum* DPCR2 produced falcate conidia with pointed ends (Fig. 2). Size of conidia varied among isolates in which the length of conidia ranged from $16.30 \pm 3.89 \mu\text{m}$

Table 2: Growth rate of *Colletotrichum* isolates on PDA medium incubated at 28 ± 2°C

No	Species	Growth rate (mm/day ± ds)
1	<i>Colletotrichum truncatum</i> DPCR2	8.71 ± 0.10
2	<i>C. fruticola</i> KRCR	8.50 ± 0.37
3	<i>C. gloeosporioides</i> TBCB	9.60 ± 0.26
4	<i>C. scovillei</i> BDCR	4.35 ± 0.07
5	<i>C. nymphaeae</i> BLCR	4.25 ± 0.12
6	<i>C. acutatum</i> BLCB	3.35 ± 0.07

ds: deviation standard

Table 3: Size of conidia of *Colletotrichum* spp.

No. Species	Conidial length (µm) ± ds	Conidial width (µm) ± ds
1 <i>Colletotrichum truncatum</i> DPCR2	35.95 ± 6.85	9.53 ± 1.11
2 <i>C. fruticola</i> KRCR	25.67 ± 3.56	9.13 ± 1.67
3 <i>C. gloeosporioides</i> TBCB	29.20 ± 1.83	9.85 ± 1.04
4 <i>C. scovillei</i> BDCR	27.90 ± 3.95	9.49 ± 1.43
5 <i>C. nymphaeae</i> BLCR	33.07 ± 11.41	10.08 ± 1.36
6 <i>C. acutatum</i> BLCB	16.30 ± 3.89	6.65 ± 0.92

ds = deviation standard

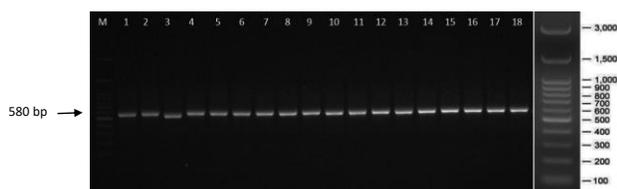


Fig. 3: PCR amplification of 18S rRNA genes of *Colletotrichum* isolates on agarose gel (M = marker 1 Kb ladder (fermentas), 1 to 18 = isolates of *Colletotrichum*.; arrow indicates PCR products of isolates of *Colletotrichum*. 1. Isolate BDCB, 2. Isolate BDCR, 3. Isolate DPCR1, 4. Isolate DPCR2, 5. Isolate GRCB, 6. Isolate GRCR, 7. Isolate KLCR1, 8. Isolate KLCR2, 9. Isolate KRCB, 10. Isolate KRCR, 11. Isolate BLCB, 12. Isolate BLCR, 13. Isolate TBCB, 14. Isolate TBCR, 15. Isolate SGCB, 16. Isolate SGCR, 17. Isolate JBCR1, 18. Isolate JBCR2

to 35.95 + 6.85 mm, while the width ranged from 6.65 + 0.92 µm to 10.08 + 1.36 µm (Table 3). The smallest size of conidia was produced by *C. acutatum* BLCB, while the biggest conidia was produced by *C. truncatum* DPCR2.

Molecular Identities of *Colletotrichum* spp.

Amplification of PCR products of sequences 18S rRNA gene 18 isolates of *Colletotrichum* by using two primers viz., *Internal Transkript Spacer* (ITS) 1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS 4 (5-TCCTCCGCTTATTGATATGC-3) resulted in DNA fragments of ± 580 bp (Fig. 3). The DNA fragments were then purified and subjected to sequencing to determine the species of *Colletotrichum* based on their similarities with other species of fungi that previously have been identified and deposited in GenBank.

Based on sequence comparison with database of GenBank using BLAST program, 10 isolates namely isolates

Table 4: Summary of the results of molecular identification of *Colletotrichum* spp. associated with anthracnose on chilli pepper grown in Bali Indonesia

No	Isolates	Species
1	BDCR*	<i>Colletotrichum scovillei</i>
2	BDCB	<i>Colletotrichum scovillei</i>
3	JBCR1	<i>Colletotrichum scovillei</i>
4	JBCR2	<i>Colletotrichum scovillei</i>
5	KLCR1	<i>Colletotrichum scovillei</i>
6	GRCR	<i>Colletotrichum scovillei</i>
7	KRCB	<i>Colletotrichum scovillei</i>
8	SGCR	<i>Colletotrichum scovillei</i>
9	SGCB	<i>Colletotrichum scovillei</i>
10	TBCR	<i>Colletotrichum scovillei</i>
11	BLCB	<i>Colletotrichum acutatum</i>
12	DPCR1	<i>Colletotrichum acutatum</i>
13	KLCR2	<i>Colletotrichum gloeosporioides</i>
14	TBCB	<i>Colletotrichum gloeosporioides</i>
15	BLCR	<i>Colletotrichum nymphaeae</i>
16	GRCB	<i>Colletotrichum nymphaeae</i>
17	KRCR	<i>Colletotrichum fruticola</i>
18	DPCR2	<i>Colletotrichum truncatum</i>

* Name of isolate was given based on the name of survey site (regency) and cultivar of chili pepper. BDCR refers to as Badung regency (BD) and cultivar Cabe Rawit (CR)

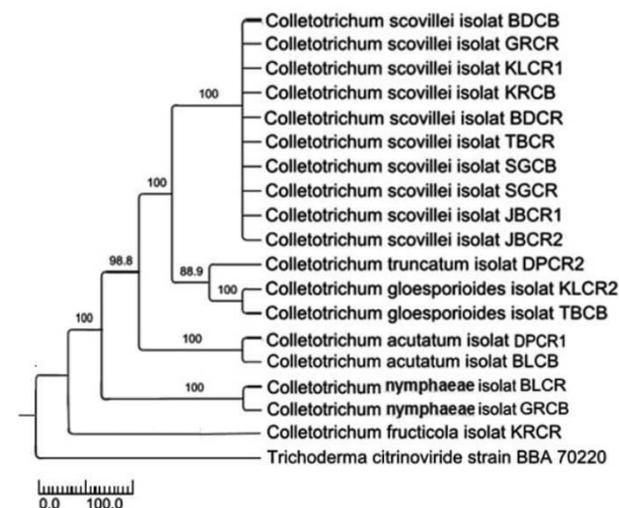


Fig. 4: Phylogenetic relationship tree of 18 isolates of *Colletotrichum* with isolate of *Trichoderma citrinoviride* strain BBA 90220 based on 18S rRNA gene using Maximum Parsimony method

BDCR, BDCB, GRCR, JBCR1, JBCR2, KLCR1, KRCB, SGCR, SGCB and TBCR have close relationship with several isolates of *Colletotrichum scovillei* such as *C. scovillei* isolate LJ22, *C. scovillei* isolate PHPG6, *C. scovillei* isolate PHPG9, *C. scovillei* isolate PHL6, *C. scovillei* isolate PHL7, *C. scovillei* isolat STCS20151112 and *C. scovillei* isolate CBS with similarities ranged from 99 to 100% (Table 4 and Fig. 4).

Two isolates namely BLCB and DPCR1 have close relationship and homolog with several isolates of *Colletotrichum acutatum* such as *Colletotrichum acutatum*

ACUBP1 (KJ627843.1), *Colletotrichum acutatum* DY16011401 (KX436091.1), *Colletotrichum acutatum* ANU-APEC0015F (KX344998.1), *Colletotrichum acutatum* DY16011401 18S (KX436092.1), *Colletotrichum acutatum* gene ITS2 (LC104999.1), *Colletotrichum acutatum* fa70NT (KF732910.1), *Colletotrichum acutatum* strain BBA71292 (AJ301964.1), *Colletotrichum acutatum* strain BBA67859 (AJ301932.1), *Colletotrichum acutatum* Ya543 (AB444085.1), *Colletotrichum acutatum* 16-418 (LC194225.1) with maximum similarities ranged between 99 to 100% (Table 4 and Fig. 4).

Isolates TBCB and KLCR2 showed close relationship and homolog several isolates of *Colletotrichum gloeosporioides* such as *Colletotrichum gloeosporioides* strain bpf-2 (KX960784.1), *Colletotrichum gloeosporioides* strain F210004 (KX197385.1), *Colletotrichum gloeosporioides* isolate CgloTIN09 (KJ676454.1), *Colletotrichum gloeosporioides* isolate CgloTIN10 (KJ676455.1), *Colletotrichum gloeosporioides* strain EGJMP6 (KF192821.1), *Colletotrichum gloeosporioides* strain EGJMP7 (KF177685.1), *Colletotrichum gloeosporioides* strain EGJMP5 (KF177684.1), *Colletotrichum gloeosporioides* isolate 104AM/T (GU066671.1), *Colletotrichum gloeosporioides* strain MAFF239933 (AB439815.1), *Colletotrichum gloeosporioides* strain MAFF239930 (AB439814.1) with similarities ranged between 99 to 100% (Table 4 and Fig. 4).

Based on sequence comparison with database of GenBank isolates BLCR and GRCB homolog with several isolates of *Colletotrichum nymphaeae* such as *Colletotrichum nymphaeae* CDMF-1 (MF187553.1), *Colletotrichum nymphaeae* isolate A48 (LT717067.1), *Colletotrichum nymphaeae* isolate A47 (LT717066.1), *Colletotrichum nymphaeae* isolate A41 (LT717060.1), *Colletotrichum nymphaeae* isolate A20 (LT717042.1), *Colletotrichum nymphaeae* isolate A15 (LT717039.1), *Colletotrichum nymphaeae* strain CBS129937 (MH865686.1), *Colletotrichum nymphaeae* strain CBS129936 (MH865685.1), *Colletotrichum nymphaeae* strain CBS129935 (MH865684.1), *Colletotrichum nymphaeae* strain CBS129933 (MH865682.1) with similarities of 99% (Table 4 and Fig. 4).

Sequence of 18S rRNA gene of isolate KRCR homolog with several isolates of *Colletotrichum fructicola* karena such as *Colletotrichum fructicola* strain HNCS-23 (MK041526.1), *Colletotrichum fructicola* strain HNCS-22 (MK041525.1), *Colletotrichum fructicola* strain HNLY-18 (MK041524.1), *Colletotrichum fructicola* strain GXNN-11 (MK041522.1), *Colletotrichum fructicola* strain JXYX-32 (MK041521.1), *Colletotrichum fructicola* strain JXYX-31 (MK041520.1), *Colletotrichum fructicola* strain JXNC-7 (MK041519.1), *Colletotrichum fructicola* strain HBMC-213 (MK041517.1), *Colletotrichum fructicola* strain HBXZ-2 (MK041505.1), *Colletotrichum fructicola* strain HBJF-6 (MK041498.1) with maximum similarity 99%. While isolate DPCR2 homolog with several isolates of *Colletotrichum truncatum* such as

Colletotrichum truncatum PHT2 (KY799045.1), *Colletotrichum truncatum* PHPG14 (KY799043.1), *Colletotrichum truncatum* PHPG8 (KY799042.1), *Colletotrichum truncatum* PHL10 (KY799041.1), *Colletotrichum truncatum* PHL3 (KY799040.1), *Colletotrichum truncatum* PHK2 (KY799039.1), *Colletotrichum truncatum* PHK1 (KY799038.1), *Colletotrichum truncatum* CMP11 (KY799031.1), *Colletotrichum truncatum* CMP9 (KY799029.1), *Colletotrichum truncatum* CMP7 (KY799028.1) with maximum similarity 100% (Table 4 and Fig. 4).

Discussion

Result of our study showed that anthracnose disease occurred in all 9 (nine) regencies in Bali where chili pepper are grown. The average disease incidence was more than 60% with average disease intensity more than 65%. This result indicated that anthracnose is a serious constraint for chili pepper production in Bali Indonesia. Other researchers also reported that anthracnose occurred in other part of the world where cilli pepper are grwon (Isaac, 1992; Suryaningsih *et al.*, 1996; Poonpolgul and Kumphai, 2007; Asare-Badiako *et al.*, 2015; Diao *et al.*, 2017).

Among 18 isolates of *Colletotrichum* identified in this study, 10 isolates (55.55%) were identified as *Colletotrichum scovillei*, two isolates (11.11%) were *Colletotrichum acutatum*, two isolates (11.11%) were *Colletotrichum gloeosporioides*, two isolates were *Colletotrichum nymphaeae* and one isolate each was identified as *Colletotrichum fructicola* and *Colletotrichum truncatum* respectively. Results of present study revealed that *Colletotrichum scovillei* is the most prevalent species that associated with anthracnose on chili pepper in Bali, Indonesia.

Anthracnose disease on chili pepper has been known to cause significant yield losses in several contries in the world where chili pepper is grown. The cause of the disease is *Colletotrichum* spp. Several species of *Colletotrichum* have been identified as the cause of anthracnose disease on chili pepper namely *Colletotrichum acutatum*, *C. Gloeosporioides*, *C. truncatum*, *C. scovillei* and *C. fructicola*. Prevalence of species of *Colletotrichum* has been known to vary depending on the geographical condition. *Colletotrichum acutatum* was reported as the most predominat species causing anthracnose disease in chili pepper in several areas in Indonesia (Widodo and Hidayat, 2018). They found three species of *Colletotrichum* from 97 isolates of *Colletotrichum* identified. Fiftyseven isolates (58.76%) was identified as *C. acutatum*, 28 isolates (28.87%) was identified as *C. capsici* and 12 isolates (12.37%) was identified as *C. gloeosporioides*. The results of this study is different from our present study, in which we found six species of *Colletotrichum* and *C. scovillei* is the most prevalent species. This is the first report on the prevalence of *C. scovillei* associated with anthracnose disease in chili

pepper in Bali, Indonesia. Previous researchers with limited number of isolates reported that anthracnose on chili pepper grown in Bali Island is *C. acutatum* (Sudiarta, 2012; Widodo and Hidayat, 2018). *Colletotrichum scovillei* was reported as the cause of anthracnose disease on chili pepper in other countries such as Japan (Kanto et al., 2014), China (Zhao et al., 2016) and Korea (Oo et al., 2017).

Four species of *Colletotrichum* is reported for the first time associated with chili pepper anthracnose in Bali Island Indonesia namely *C. scovillei*, *C. fructicola*, *C. nymphaeae* and *C. truncatum*. *Colletotrichum scovillei* was reported as prevalent species associated with anthracnose on chili pepper in several countries (Kanto et al., 2014; Zhao et al., 2016; Oo et al., 2017) but it was not detected in Australia (De Silva et al., 2017). *Colletotrichum fructicola* was reported involved in chili anthracnose in India (Sharma and Shenoy, 2014; Katoch et al., 2016), while other report stated that *C. fructicola* caused anthracnose in cassava (*Manihot esculenta*) in Brazil (Braganca et al., 2016) indicated that *C. fructicola* may cause disease in plant other than chili. *Colletotrichum nymphaeae* was reported as one of *Colletotrichum* species associated with fruit rot on chili in India (Katoch et al., 2016), while *C. truncatum* was reported as the cause of anthracnose on chili pepper in Pakistan (Tariq et al., 2017).

Results of this study proved that species of *Colletotrichum* associated with anthracnose on chili pepper is dynamic which might be influenced by geographical condition. This information is important as consideration to develop control strategy for anthracnose management on chili pepper, in particular in Bali, Indonesia.

Conclusion

Six species of *Colletotrichum* were identified associated with anthracnose on chili pepper in Bali, Indonesia namely *Colletotrichum scovillei*, *C. acutatum*, *C. gloeosporioides*, *C. fructicola*, *C. truncatum* and *C. nymphaeae*. Among them, *C. scovillei* is the most prevalent. This is the first report on the prevalence of *C. scovillei* associated with anthracnose on chili pepper in Bali, Indonesia. Four species of *Colletotrichum* are being reported for the first time associated with anthracnose on chili in Bali Island namely *C. scovillei*, *C. fructicola*, *C. truncatum* and *C. nymphaeae*.

Acknowledgement

We highly appreciate the Research Institute of Udayana University of Bali, Indonesia that kindly provided the research grant for the accomplishment of this study in the fiscal year 2018.

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(Received 14 January 2019; Accepted 09 March 2019)