Available online at www.sciencedirect.com

MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc

Full paper

Colletotrichum species on grape in Guizhou and Yunnan provinces, China

Li-Juan Peng [a,b], *Tao Sun* [c], *You-Lian Yang* [d], *Lei Cai* [e], *Kevin D. Hyde* [f], *Ali H. Bahkali* [g], *Zuo-Yi Liu* [h,i,*]

[a] *The Provincial Key Laboratory for Agricultural Pest Management of Mountainous Region, Guizhou University, Guiyang, Guizhou 550025, PR China*
[b] *Agricultural College of Guizhou University, Guiyang, Guizhou 550025, PR China*
[c] *Chongqing Entry-Exit Inspection and Quarantine Bureau, Chongqing 400020, PR China*
[d] *Department of Biology and Geography, Liupanshui Normal University, Shuicheng, Guizhou 553006, PR China*
[e] *Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, PR China*
[f] *Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[g] *King Saud University, College of Science, Botany and Microbiology Department, Riyadh, Saudi Arabia*
[h] *Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, PR China*
[i] *Guizhou Key Laboratory of Agricultural Biotechnology, Guiyang, Guizhou 550006, PR China*

ARTICLE INFO

Article history:
Received 17 October 2011
Received in revised form
9 April 2012
Accepted 3 May 2012
Available online 7 September 2012

Keywords:
Anthracnose
Multilocus phylogeny
New species
Pathogenicity
Vitaceae

ABSTRACT

Twenty-six strains representing three species of *Colletotrichum* were isolated from leaf and fruit lesions of vitaceous plants in Guizhou and Yunnan provinces, China. The strains were characterized by morphology and phylogenetic analyses of actin, β-tubulin, calmodulin, glutamine synthetase, glyceraldehyde-3-phosphate dehydrogenase and rDNA internal transcribed spacer gene sequences. The combined dataset showed that 20 of 26 strains represented a novel species, the rest being *Colletotrichum fructicola* (four strains) and *Colletotrichum gloeosporioides* (two strains). The new species is described herein as *Colletotrichum viniferum*. Its conidia, compared with similar *Colletotrichum* species are cylindrical and 12–16 μm long. Based on pathogenicity tests, *C. viniferum* caused leaf spots and anthracnose of table grape but was not host-specific.

1. Introduction

Grape (*Vitis* spp.) is one of the most widely planted fruit trees worldwide (Sung et al. 2008) and the table grape (*Vitis vinifera*) is one of the most important grape vines in China. In recent years, there has been a rapid increase in areas planted with *V. vinifera* throughout China. In 2003, the total area with grape vines was 421,000 hm^2 with a yield of 517,600 tons. Due to the warm and rainy climate in southern China, yield losses of up to 50% have been reported due to disease and insects (Lu 2005). Ripe rot of grape caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum acutatum* J.H. Simmonds ex J.H. Simmonds were considered to be serious diseases occurring in most vineyards, and caused big loss and deterioration of grape vines (Sung et al. 2008).

* *Corresponding author*. Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, PR China.
E-mail address: liuzuoyi@yahoo.com.cn (Z.-Y. Liu).

1340-3540/$ – see front matter © 2012 The Mycological Society of Japan. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.myc.2012.07.006

Southworth (1891) first reported grape ripe rot disease caused by *C. gloeosporioides* in the USA. *C . acutatum* was also reported as a pathogen of the disease in Australia, Japan, Korea and USA (Sung et al. 2008). The taxonomy and nomenclature of *Colletotrichum* remained confusing (Cai et al. 2009; Hyde et al. 2009) and a study of anthracnose of tropical fruits showed that *C. gloeosporioides* was not the causative agent as previously recorded (Phoulivong et al. 2010a). It was difficult to identify the species of *Colletotrichum* due to instability of its morphological characters, which depended upon experimental methods and conditions (Cai et al. 2009; Hyde et al. 2010). Many *Colletotrichum* strains have being successfully identified and epitypified by the application of morphology and multilocus phylogeny (Than et al. 2008; Moriwaki and Tsukiboshi 2009; Prihastuti et al. 2009; Shivas and Tan 2009; Yang et al. 2009; Phoulivong et al. 2010a, 2010b; Weir and Johnston 2010; Su et al. 2011; Wikee et al. 2011). That suggested molecular phylogeny could lead to better understanding and identification of *Colletotrichum* species (Cai et al. 2009; Hyde et al. 2010), which in turn was of great importance to correctly identify phytopathogen species for the sake of plant disease control (Hyde et al. 2010; Cai et al. 2011; Ko Ko et al. 2011; Udayanga et al. 2011).

The objective of the present was to identify *Colletotrichum* species causing disease on plants of Vitaceae in Guizhou and Yunnan provinces, China. Strains were collected from lesions in vineyards for lab experiment. Molecular and morphological data were used to identify the *Colletotrichum* species and revealed two known species and one new species.

2. Materials and methods

2.1. *Isolation of* Colletotrichum *species*

Colletotrichum samples were collected from anthracnose lesions on fruits or leaves of Vitaceae at locations in Guizhou and Yunnan provinces between June and September 2008 (Table 1). Single-spore isolations from infected leaves or fruits with sporulation were carried with the methods described by Choi et al. (1999) and Than et al. (2008). Spore masses were placed in sterilized water and streaked on to the surface of water agar (WA) plates which were then incubated overnight at 22–24°C. Single germinated spore was picked up with sterilized needles under the microscope and transferred onto potato dextrose agar plates (PDA). Pure cultures were stored at 4°C on PDA slants. Isolates were deposited in Agricultural College of Guizhou University, China. The ex-holotype and ex-paratype living culture were deposited at CBS, the Netherlands.

2.2. *Morphological and cultural characters*

Morphological and cultural characterization follow the methods of Cai et al. (2009). Mycelial discs (5 mm diam.) were taken from the growing edge of 5-day-old cultures and transferred onto PDA plates (Petri dishes diameter: 9 cm) and incubated in the dark at 25°C. Four replicate cultures of each isolate were investigated.

Colony diameter was measured daily for 8 days, growth rate (mm per day) was calculated and its colour, conidial masses and

Table 1 – Synopsis of characters of *Colletotrichum* isolates from Vitaceae.

Species	Specimen no.	Host	Symptom	Location
C. viniferum	[a]GZAAS5.08601	*V. vinifera* cv. shuijing	Fruit anthracnose	Kunming, Yunnan, China
	GZAAS5.08602	*V. vinifera* cv. meiguixiang	Fruit anthracnose	Kunming, Yunnan, China
	GZAAS5.08603	*V. vinifera* cv. hongti	Fruit anthracnose	Binchuan, Yunnan, China
	GZAAS5.08604	*V. vinifera* cv. jinya	Fruit anthracnose	Binchuan, Yunnan, China
	GZAAS5.08605	*V. vinifera* cv. shuijing	Fruit anthracnose	Pingba, Guizhou, China
	GZAAS5.08606	*V. vinifera* cv. baixiangjiao	Fruit anthracnose	Pingba, Guizhou, China
	GZAAS5.08607	*V. vinifera* cv. kyoho	Fruit anthracnose	Pingba, Guizhou, China
	GZAAS5.08608	*V. vinifera* cv. hongti	Fruit anthracnose	Guiyang, Guizhou, China
	GZAAS5.08609	*V. vinifera* cv. hongfushi	Fruit anthracnose	Guiyang, Guizhou, China
	GZAAS5.08611	*V. vinifera* cv. shuijing	Fruit anthracnose	Guiyang, Guizhou, China
	GZAAS5.08612	*V. vinifera* cv. baixiangjiao	Fruit anthracnose	Jinsha, Guizhou, China
	GZAAS5.08613	*V. vinifera* cv. shuijing	Fruit anthracnose	Jinsha, Guizhou, China
	GZAAS5.08614	*V. vinifera* cv. shuijing	Fruit anthracnose	Xifeng, Guizhou, China
	GZAAS5.08615	*V. vinifera* cv. heimeigui	Fruit anthracnose	Xifeng, Guizhou, China
	GZAAS5.08616	*V. vinifera* cv. shuijing	Fruit anthracnose	Zunyi, Guizhou, China
	GZAAS5.08617	*V. vinifera* cv. kyoho	Fruit anthracnose	Zunyi, Guizhou, China
	GZAAS5.08621	*C. japonica*	Leaf lesion	Libo, Guizhou, China
	GZAAS5.08626	*Ampelopsis* sp.	Leaf lesion	Libo, Guizhou, China
	GZAAS5.08622	*V. vinifera* cv. shuijing	Fruit anthracnose	Luodian, Guizhou, China
	GZAAS5.08623	*V. vinifera* cv. shuijing	Fruit anthracnose	Leishan, Guizhou, China
C. fructicola	GZAAS5.08610	*V. vinifera* cv. kyoho	Fruit anthracnose	Guiyang, Guizhou, China
	GZAAS5.08618	*V. vinifera* cv. shuijing	Fruit anthracnose	Dunyun, Guizhou, China
	GZAAS5.08627	*Ampelopsis* sp.	Leaf lesion	Dunyun, Guizhou, China
	GZAAS5.08628	*Ampelopsis* sp.	Leaf lesion	Dushan, Guizhou, China
C. gloeosporioides	GZAAS5.08624	*Ampelopsis* sp.	Leaf lesion	Zunyi, Guizhou, China
	GZAAS5.08620	*V. vinifera* cv. shuijing	Fruit anthracnose	Dushan, Guizhou, China

a GZAAS: Guizhou Academy of Agricultural Sciences, Guizhou Province, China.