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Full Length Research Paper

ISSR for comparison of cross-inoculation potential of *Colletotrichum capsici* causing chilli anthracnose

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Thirty-four isolates of *Colletotrichum* spp. including 2 species, *C. gloeosporioides* and *C. capsici*, from anthracnose on Bell pepper, Long cayenne pepper and Bird's eye chilli were isolated and their pathogenicity was proven via fruit inoculation. Pathogenicity tests divided pathogenic potential into low, medium and high virulence groups. It is clearly revealed that *C. capsici* from the three tested hosts expressed the highest virulent isolates. Cross-inoculation of three high virulent isolates of *C. capsici* in accordance with three chilli varieties showed that all isolates could produce anthracnose symptom in the same lesions. All tested isolates developed lesions after co-inoculation of all hosts. Inter simple sequence repeat (ISSR) analysis indicated that there are two distinct groups of *C. gloeosporioides* and *C. capsici*. Furthermore, genetic diversity was correlated with geographic distribution, while there was no clear relationship between genetic diversity and pathogenic variability. But it is clearly demonstrated that whereas *C. gloeosporioides* appears in the same geographic area as *C. capsici*, it causes lower disease incidence.

Key words: Anthracnose, chilli, *Colletotrichum capsici*, cross-inoculation.

INTRODUCTION

Anthracnose of chilli pepper (*Capsicum annuum*) is an economically important disease which affects chilli pepper planting in Thailand. It is caused by several species of the fungal genus *Colletotrichum*, of which *C. capsici* and *C. gloeosporioides* crop up widely. *C. capsici* and *C. gloeosporioides* have been reported to cause anthracnose disease in India, Indonesia, Korea and Thailand (Kim et al., 1999; Oh et al., 1999; Ahn et al., 2003; Oanh et al., 2004; Voorrips et al., 2004; Pakdeevaraporn et al., 2005; Gopinath et al., 2006). Moreover, *C. acutatum* has also been reported in Australia and Indonesia (Nirenberg et al., 2002; Whitelaw-Weackert et al., 2007) and *C. coccodes* in Korea and New Zealand (Johnston and Jones, 1997; Ahn et al., 2003). These fungi are capable of causing disease on all parts of the chilli pepper plant throughout at any stage of plant growth. However, fruit rot disease is the most economically devastating disease.

Single species of *Colletotrichum* may infect multiple hosts e.g. *C. gloeosporioides* infects wide variety of fruits, including almond, avocado, apple and strawberry (Freeman et al., 1998). Moreover, the cross-inoculation potential of *Colletotrichum* spp. has been reported e.g. *C. gloeosporioides* isolates from avocadoes and mangoes were reported to produce lesions on strawberries, peppers, guavas and papayas which often cultivated in adjacent orchards (Sanders and Korsten, 2003). *C. acutatum* isolates from pepper which often cultivate with persimmon trees produced symptoms of anthracnose on the fruits of pepper and twigs of persimmon (Kim et al., 2009). Because morphology and pathology are not enough to distinguish between *Colletotrichum* species, molecular markers have been applied to *Colletotrichum* sp. diversity studies include internal transcribed spacer (ITS) regions (Freeman et al., 2001b; Moriwaki et al.,

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2002; Martinez-Culebras et al., 2003; Sanders and Korsten, 2003; Lee et al., 2007), as well as restriction fragment length polymorphism (RFLP) (Balardin et al., 1999; Martín and García-Figueres, 1999; Saha et al., 2002) and random amplified polymorphic DNA (RAPD) markers (Sicard et al., 1997; Denoyes-Rothan et al., 2003; Weeds et al., 2003; Agosteo et al., 2005; Camargo-Junior et al., 2007). Of these, RAPD analysis has been widely applied to study *Colletotrichum* sp. diversity. Molecular markers such as inter simple sequence repeat (ISSR) markers have also been successfully used as tools for understanding the phylogenetic relationships of fungi (Chadha and Gopalakrishna, 2007; Schwarzenbach et al., 2007). ISSR is a simple technique, requires no sequence information and is carried out using a single primer based on a simple repeat. Only small amounts of DNA template are required and the results are clearly scorable and reproducible (Nagaoka and Ogihara, 1997). In this study, the potential for cross-inoculation of isolates of *C. capsici* obtained from three varieties of chilli (Bell pepper, Long cayenne pepper and Bird's eye chilli) was examined by inoculating each alternative host. In addition, the different isolates were analysed by ISSR-PCR to determine genetic variability.

MATERIALS AND METHODS

Isolation and identification of pathogens from infected chilli

Colletotrichum isolates were collected from fields in Bangkok and Ratchaburi provinces, Thailand. Isolation of causal agent was done using the tissue transplanting technique described by Ratanacherdchai et al. (2007). The diseased plant parts were cut at the advanced margin of lesions into small pieces (5 × 5 mm) and then surface disinfected with 10% Clorox for 1 min, followed by rinsing with sterile distilled water two times and transferred onto isolating water agar (WA) medium. The mycelia growing out of the plant tissue were sub-cultured to potato dextrose agar (PDA) medium and incubated at room temperature (approximately 28 - 30°C) for 7 - 10 days. Single spore isolation was performed to obtain pure cultures. The isolate was first identified to species by morphological observation under a compound microscope.

Pathogenicity tests

Pathogenicity tests were done separately for each isolate from each host plant using plug inoculation methods following a modified protocol by Sanders and Korsten (2003). Prior to inoculation, all fruits were swabbed with 70% (v/v) ethanol to reduce surface contamination and left for air-dry in laboratory by 3 - 4 min. Fruits were then wounded by gentle pricking with a sterilized needle.

Inocula were prepared by culturing each isolate on PDA at room temperature (approximately 28 - 30°C) for 10 days. Plugs (5 mm diameter) were cut from actively-sporulating areas near to colony periphery by using a sterilized cork borer and placed over the wounded part of chilli fruits. Wounded chilli fruits inoculated by placing only a PDA plug over the wound were used as a control. The inoculated fruits were kept in moist chamber at room temperature (28 - 30°C) for 10 days. The experiments were done using a Completely Randomized Design with four replications. Data was collected as lesion diameter of tested fruits (mm) and the coefficient of variation was computed. Treatment means were statistically compared using Duncan's New Multiple Range Test. *Colletotrichum* isolates were grouped into three categories based on lesion size as follows: 0 - 5.0 mm = low virulent isolates, 5.1 - 20.0 mm = moderately virulent isolates, and > 20 mm = high virulent isolates.

Cross-inoculation tests

Three isolates of *C. capsici* pathogenic to Bell pepper, Long cayenne pepper and Bird's eye chilli were selected for the cross-inoculation test. Conidial suspensions were generated from the pure culture of *C. capsici* and numbers of conidia were adjusted to 1×10^6 conidia ml^{-1} with sterile distilled water using haemacytometer according to method described by Hong and Hwang (1998). All pepper fruits were thoroughly washed under running water, swabbed with 70% (v/v) ethanol and air-dried in the laboratory. Inoculum droplets (10 µl) containing ca. 10^6 conidia ml^{-1} were separately placed onto 2 mm deep prick wounds on the fruit surfaces. Each of the three isolates was placed onto a separate wound along the longitudinal axis of the fruit with four replications. Wounded fruits receiving only a sterile distilled water droplet served as a control. Inoculated fruits were placed in moist chamber at room temperature. The experiments were arranged using a Completely Randomized Design with four replications. Data was collected as lesion size (mm) of each fruit tested and the coefficient of variation was calculated. Treatment means were statistically compared by Duncan's Multiple Range Test.

Inter simple sequence repeat (ISSR) analysis

Genomic DNA extraction

Total genomic DNA was isolated from fungal mycelia. Isolates were agitated at 180 r/min, 28°C for 4 days in tubes containing 20 ml of potato dextrose broth (PDB). Mycelia were harvested by filtration through filter paper, dried between two layers of filter paper and stored at –80°C for future use. Dried mycelia were ground to a fine powder with mortar and pestle using liquid nitrogen and transferred to 1.5 ml Eppendorf tubes. DNA extraction was performed using CTAB (hexadecyltrimethylammonium bromide) method described by Wang et al. (2005). The obtained DNA pellet was dissolved in 50 µl of sterile double distilled water and quality of extracted DNA checked by using 1% agarose gel electrophoresis.

PCR amplification of DNA

Inter simple sequence repeat polymerase chain reaction (ISSR-PCR) was performed using a 50 µl reaction mixture consisting of 5 µl of 10 × PCR buffer, 4 µl of 25 mM $MgCl_2$, 1.5 µl of dNTP mix (10 mM each of dATP, dCTP, dGTP, dTTP), 0.3 µl Taq DNA polymerase, 2 µl of primer, 1 µl of genomic DNA and 36.2 µl of sterile double-distilled water. The amplification was carried out in a MyGene™ Series Peltier Thermal Cycle using three temperature profiles, programmed for initial DNA denaturation at 94°C for 2 min, followed by 35 cycles of DNA denaturation for 1 min at 94°C, 1 min at annealing temperature specific for each primer (Table 3) and extension for 1.5 min at 72°C, with a 6 min final extension at 72°C. Amplification products were separated by electrophoresis on 1.5% agarose gels in 1× TAE buffer at 90 V for 1.5 h and stained with ethidium bromide, then visualized with UV light and photographed. The 1 kb DNA ladder (Fermentas) (0.5 µg/µl) was run for weight size comparison.

Cluster analysis

ISSR bands generated by using six set of primers in 34 isolates of *Colletotrichum* spp. were scored for their presence (1) or absence (0). Data was analysed using the Numerical Taxonomy System, NTSYS-pc version 2.10e program (Rohlf, 2000). A similarity matrix was generated from the binary data using similarity coefficient for qualitative (nominal) data (SIMQUAL). Cluster analysis (SAHN) was performed for the data matrix using the unweighed pair group method with the arithmetic averaging (UPGMA) algorithm and 25 iterations and dendrogram was constructed. The validity of the clustering was determined by comparing the similarity and cophenetic (ultrametric) value matrices using the matrix comparison unit of NTSYS-pc.

RESULTS

Isolation and identification of pathogens from infected chilli

Thirty-four isolates of *Colletotrichum* spp. were obtained from peppers showing anthracnose symptoms in Bangkok and Ratchaburi provinces. Of these, 7 were isolated from Bell pepper (*Capsicum annuum*), 17 from Long cayenne pepper (*C. annuum* var *acuminatum*) and 10 from Bird's eye chilli (*C. frutescens*) (Table 1).

Pathogenicity tests

Pathogenicity test of the isolates from Bell pepper fruits showed that the lesion size produced by *C. capsici* isolate C108 was 29.25 mm, giving it the highest virulence for disease incidence. This lesion size was significantly larger than those produced by the other six isolates (P = 0.01). The sizes of lesions caused by *C. gloeosporioides* isolates C104, C102, C105, and C101 were 11.25, 9.25, 6.00 and 5.50 mm, respectively. Two Bell pepper isolates (C103 and C107) did not produce any symptoms when inoculated onto Bell pepper fruits (Table 1). Pathogenicity tests of the isolates from Long cayenne pepper fruits showed that all isolates produced lesions when inoculated onto Long cayenne. The lesion size produced by *C. capsici* isolate C208 was 23.90 mm, which gave it the highest virulence for disease incidence, and this lesion size was significantly larger than those caused by the other 16 isolates (P = 0.01).

Similar to the Long cayenne pepper isolates, all Bird's eye chilli isolates caused lesions when inoculated onto Bird's eye chilli fruits. The lesion size produced by *C. capsici* isolate C308 was 11.00 mm, giving it the highest virulence for disease incidence and differing significantly from the other nine isolates (P = 0.01).

Pathogenic variability of *Colletotrichum* isolates was grouped into three categories based on their reactions as shown in Table 1. Two isolates from Bell pepper were grouped in low virulent group, four in moderately virulent group and one in high virulent group. For *Colletotrichum* spp. isolated from Long cayenne pepper, 13 isolates were placed in the moderately virulent group and 4 in the high virulent group. Whereas, four isolates from Bird's eye chilli showed low virulence while six isolates were moderately virulent.

Cross-inoculation test

Cross-inoculation test of *C. capsici* isolates from Bell pepper (C108), Long cayenne pepper (C208), and Bird's eye chilli (C308) onto Bell pepper fruits showed that all tested isolates produced lesions after co-inoculation onto Bell pepper fruits, which differed significantly from the control (P = 0.05). The mean lesion size developed by isolates C108, C208 and C308 were 19, 17 and 13.75 mm, respectively. However, the mean lesion size developed by all tested isolates did not differ significantly (P = 0.05).

Similar to the Bell pepper, all tested isolates produced lesions after co-inoculation onto Long cayenne pepper fruits. The mean lesion size produced by isolates C108, C208 and C308 were 11.75, 11.75 and 10.75 mm, respectively, which was not significantly differ (P = 0.05). All tested isolates produced lesions after co-inoculated onto Bird's eye chilli fruits. The mean lesion size produced by isolate C308 was 5.75 mm. Whereas, the mean lesion sizes developed by isolates C108 and C208 were 4.75 and 525 mm, respectively which differed significantly from the control (P = 0.05). Overall, mean lesion sizes produced by all tested isolates differed significantly on the different hosts but did not differ significantly among the three isolates (Table 2 and Figure 1).

Cluster analysis

Six ISSR primers showed multi band patterns for each of the isolates (Table 3). The primers amplified a total of 103 bands from 34 isolates tested. The average number of bands per primer was 17.17 which ranged in size from 250 to 3500 bp. Among 34 isolates, 70 of the total 103 ISSR bands were polymorphic. The percentage of polymorphic fragments per primer was 67.96%. UPGMA analysis based on total ISSR character difference was carried out to group the 34 isolates of *Colletotrichum* spp. According to the dendrogram, *Colletotrichum* spp. can be divided into three main groups (Figure 2). Group 1 contained *C. gloeosporioides* isolates obtained from Ratchaburi province. Whereas, groups 2 and 3 consisted of *C. capsici* isolates. Group 2 consisted of *C. capsici* isolates obtained from Ratchaburi province while group 3 consisted of *C. capsici* isolates obtained from Bangkok.

The clustering of the *Colletotrichum* isolates based on ISSR data showed a relationship with geographical distribution of isolates. Moreover, group 1, (*C. gloeosporioides* isolates) can be divided into different subgroups based on host. It was shown that original host was important for phylogenetic grouping. The within-

Table 1. Characteristics of *Colletotrichum* spp. isolates studied.

Host	Provinces	Isolate ID		Lesion diameter (mm)	Virulence[1]	ISSR group
Bell pepper	Ratchaburi	*C. gloeosporioides*	C101	5.50bc[2]	M	I
		C. gloeosporioides	C102	9.25b	M	I
		C. gloeosporioides	C103	0.00c	L	I
		C. gloeosporioides	C104	11.25b	M	I
		C. gloeosporioides	C105	6.00bc	M	I
		C. gloeosporioides	C107	0.00c	L	I
		C. capsici	C108	23.25a	H	II
C.V. (%)				41.41		
Long cayenne pepper	Ratchaburi	*C. capsici*	C201	6.00ef	M	II
		C. capsici	C202	20.80abc	H	II
		C. gloeosporioides	C203	6.20ef	M	I
		C. capsici	C204	7.50def	M	II
		C. capsici	C205	11.20cde	M	II
		C. capsici	C206	13.00bcde	M	II
		C. capsici	C207	16.80abcd	M	II
		C. capsici	C208	23.90a	H	II
		C. capsici	C209	22.10ab	H	II
		C. capsici	C210	15.50abcde	M	II
	Bangkok	*C. capsici*	C211	12.10cde	M	III
		C. capsici	C212	12.50bcde	M	III
		C. capsici	C213	8.50def	M	III
		C. capsici	C214	8.40def	M	III
Long cayenne pepper	Bangkok	*C. capsici*	C215	10.90cde	M	III
		C. capsici	C216	20.80abc	H	III
		C. capsici	C217	12.20cde	M	III
C.V. (%)				36.30		
Bird's eye chilli	Ratchaburi	*C. gloeosporioides*	C301	3.40de	L	I
		C. gloeosporioides	C302	2.20de	L	I
		C. gloeosporioides	C304	2.80de	L	I
		C. gloeosporioides	C306	1.20e	L	I
	Bangkok	*C. capsici*	C307	9.50ab	M	III
		C. capsici	C308	11.00a	M	III
		C. capsici	C310	5.20cd	M	III
		C. capsici	C311	7.50bc	M	III
		C. capsici	C312	5.80cd	M	III
		C. capsici	C314	5.80cd	M	III
C.V. (%)				33.81		

[1]Lesion size was divided into three categories as follows: L, low virulent = 0 - 5.0 mm; M, moderately virulent = 5.1 - 20.0 mm; H, high virulent = > 20 mm.
[2]Average of four replications. Means followed by the common letters in each column within each host were not significantly different at P = 0.01.

group similarity of *C. gloeosporioides* isolates and the within-group similarity of *C. capsici* isolates were 0.57 - 1.00 and 0.63 - 1.00, respectively. There was less similarity (0.54 - 0.72) between the *C. gloeosporioides* isolates and *C. capsici* isolates.

DISCUSSION

The *Colletotrichum* spp. causing chilli anthracnoses in this study were identified as two species: *C. gloeosporioides* (Penz.) Penz. and Sacc. and *C. capsici* (Syd.) E.J. Butler and Bisby (Oanh et al., 2004; Ratanacherdchai et al., 2007). In addition, there was a clear distinction between the *C. gloeosporioides* and *C. capsici* isolates based on the ISSR data. These groups correlated with morphological characters as described by Moriwaki et al. (2002):*C. gloeosporioides* isolates produce conidia with obtuse ends while *C. capsici* isolates produce falcate conidia. However, chilli anthracnose in Thailand has been reported not only by causal agents *C. gloeosporioides* and *C. capsici* but also by *C. acutatum* (Than et al., 2008a). The pathogenicity test by plug inoculation on chilli fruits showed variation in virulence in

Table 2. Comparison of lesion size produced on different test hosts by three *Colletotrichum capsici* isolates from Bell pepper, Long cayenne pepper, and Bird's eye chilli.

Test hosts	Lesion size (mm)			C.V. (%)
	C108	C208	C308	
Bell pepper	17.00a[1]	13.75a	19.00a	28.68
Long cayenne pepper	11.75a	11.75a	10.75a	20.30
Bird's eye chilli	4.75b	5.25ab	5.75a	15.11

[1]Average of four replications. Means followed by the common letters in each row were not significantly different at P = 0.05.

Table 3. Sequences of ISSR primers that revealed polymorphism in *Colletotrichum* spp.

Primer identifier	Sequence*	Annealing temperature (°C)	Fraction polymorphic fragments**
AF80820	AGAGAGAGAGAGAGAGT	52	13/18
AF80821	AGAGAGAGAGAGAGAGC	57	10/15
AF80822	GAGAGAGAGAGAGAGAT	55	12/17
AF80823	TGTGTGTGTGTGTGTGA	55	9/17
AF80824	GAGAGAGAGAGAGAGAYG	57	13/19
AF80825	GTGTGTGTGTGTGTGTYC	57	12/17

*Single letter abbreviation for mixed-base position: Y = C, T
**Number of polymorphic fragments/number of fragments amplified.

Figure 1. Cross-inoculation test of three highly virulent isolates of *Colletotrichum capsici* from Bell pepper, Long cayenne pepper and Bird's eye chilli on fruits of Bell pepper (*Capsicum annuum*), Long cayenne pepper (*C. annuum* var *acuminatum*) and Bird's eye chilli fruits (*C. frutescens*).

virulence in *Colletotrichum* spp. isolates. The aggressiveness of these isolates generally took place in the moderately virulent group (23 isolates). Six isolates showed low virulence while five isolates showed high virulence.

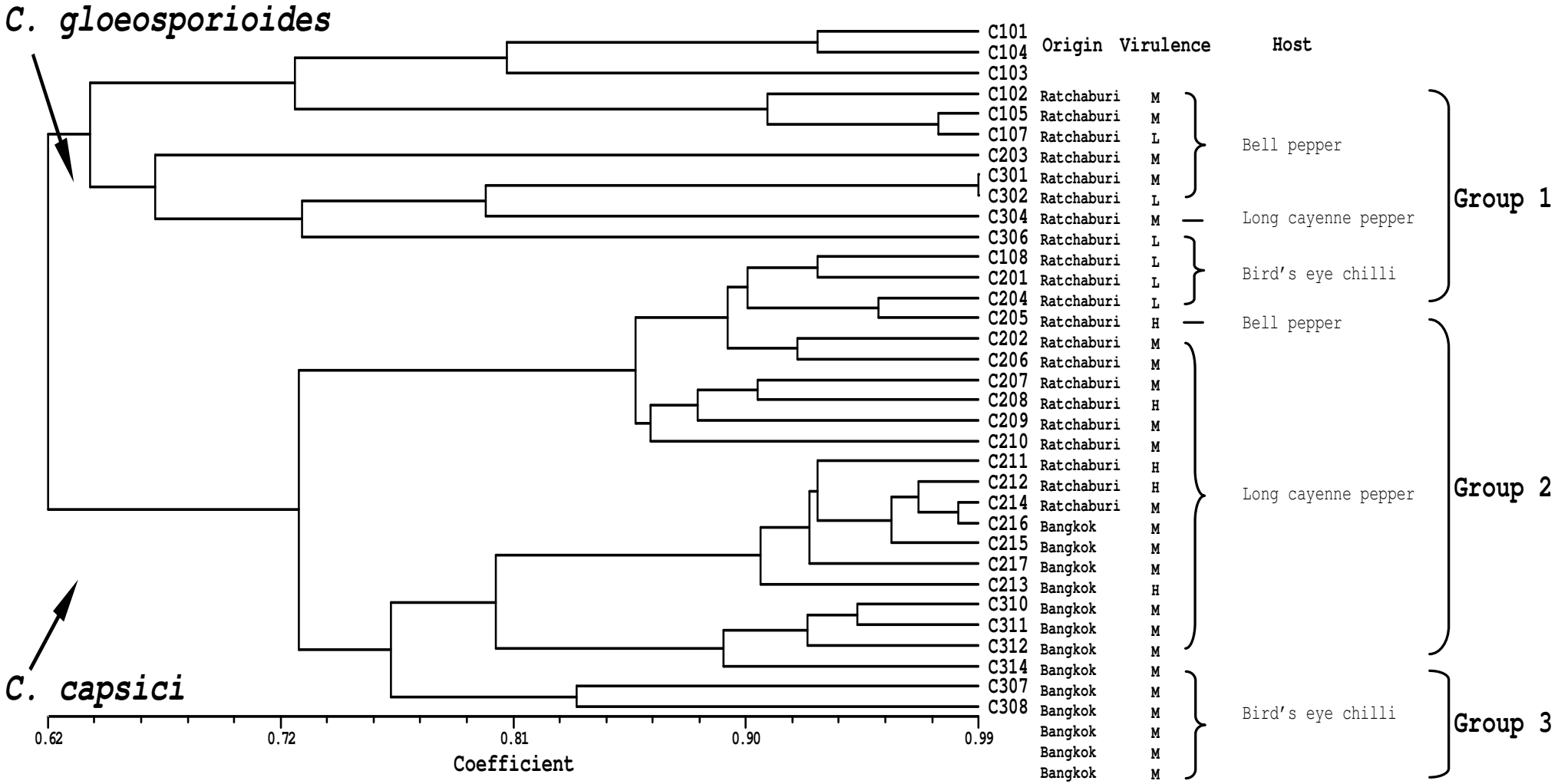

Figure 2. Generated dendrogram from ISSR data for all six selected primers used showing the clustering of strains of *Colletotrichum* spp.

Moreover, *C. capsici* isolates appeared to be more virulent than *C. gloeosporioides* isolates. This difference could be supported by Than et al. (2008b) reported that different *Colletotrichum* species cause diseases of different organs of the chilli fruits and different maturity stages of chilli fruit as well. *C. gloeosporioides* infects chilli fruit at the developmental stage, while *C. capsici* infects mature red chilli fruit. In addition, Amusa et al. (2004) reported that *C. capsici*was found associated with the fruit anthracnose of hot pepper fruit production areas in the humid rainforest zone of

South-western Nigeria and reported to affect both green and ripe pepper fruits. The clustering of *Colletotrichum* isolates based on ISSR data failed to reveal a relationship between clustering in dendrogram and pathogenic variability. For instance, the low and moderately virulent isolates of *C. gloeosporioides* were clustered into the same ISSR group. Moreover, ISSR groups 2 and 3 contained both moderately and highly virulent isolates of *C. capsici*. This result is in agreement with the results obtained by Denoyes-Rothan et al. (2003), Sharma et al. (2005) and Bayraktar et al. (2008).

Virulence of cross-inoculation of *C. capsici* isolates onto Bell pepper, Long cayenne pepper and Bird's eye chilli was determined by comparing mean lesion size on each host. All tested isolates produced lesions after co-inoculation onto all hosts. The virulence, based on lesion size, among all tested isolates did not differ significantly (P = 0.05) among hosts. However, these three isolates did not cluster into the same ISSR group. Thus, no clear relationship between ISSR grouping and pathogenic variability was demonstrated. However, all isolates developed larger lesions on Bell pepper than on other chilli fruits after co-inoculation onto each chilli fruit. This may be due to the different fruit types rather than a difference in host preference by the fungal isolates. Several previous reports have also discussed cross-infection potential of *Colletotrichum* isolates. For instance, *C. gloeosporioides* isolates from almond, apple, avocado, mango, pecan, and yam produced lesions on almond, apple, avocado, guavas, mango, nectarine, papaya, pepper, strawberry fruits (Freeman and Shabi, 1996; Sanders and Korsten, 2003; Abang et al., 2006). Similar to *C. gloeosporioides*, it has also been reported that *C. acutatum* from strawberry is able to cause lesions on various fruits such as anemone, apple and peach (Freeman et al., 2001a); moreover, Kim et al. (2009) found that *C. acutatum* isolates from pepper which cultivated with persimmon were able to infect both hosts.

Furthermore, the genetic diversity of *Colletotrichum* spp. based on ISSR markers showed correlation between genetic and geographical distribution which is supported by the previous report. Than et al. (2008a) reported that *C. acutatum* isolates collected in Australia from strawberry and papaya formed a subcluster distinct from the isolates from Thailand. In contrast, the RAPD analysis of Sharma et al. (2005) suggested that the classification of *C. capsici* isolates causing fruit rot of chilli is not correlated with virulence or geographic origin.

Conclusion

Colletotrichum spp. causing anthracnose of Bell pepper, Long cayenne pepper and Bird's eye chilli were identified as *C. gloeosporioides* and *C. capsici*. Cross-inoculation tests indicated that all isolates tested produced lesions after co-inoculation onto each host. However, this cross-inoculation test was carried out under the optimum conditions for artificial inoculation in the laboratory such as high inoculum concentration, detached and wounded fruits and optimized humidity, thus it remains to be determined whether isolates from different hosts cause serious disease in the field. The phylogenetic grouping based on ISSR showed a relationship between clustering in dendrogram and geographical distribution of isolates. However, the pathological and ISSR grouping of isolates was suggested on correlation among the tested isolates. Therefore, ISSR markers are a useful method of studying genetic diversity in *Colletotrichum* spp. PCR-based technique like ISSR used in this study are rapid, reproducible and produce a large number of polymorphic bands. Such techniques aid in the understanding of pathogen population dynamics, which can facilitate the development of effective control strategies.

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REFERENCES

Abang MM, Asiedu R, Hoffmann P, Wolf GA, Mignouna HD, Winter S (2006). Pathogenic and genetic variability among *Colletotrichum gloeosporioides* isolates from different yam hosts in the agroecological zones in Nigeria. J. Phytopathol. 154(1): 51-61.

Agosteo GE, Macri C, Cacciola SO (2005). Characterization of *Colletotrichum acutatum* isolates causing anthracnose of strawberry in Calabria. J. Plant Pathol. 87(4): 287.

Ahn IP, Uhm KH, Kim S, Lee YH (2003). Signaling pathways involved in preinfection development of *Colletotrichum gloeosporioides, C. coccodes,* and *C. domatiums* pathogenic on red pepper. Physiol. Mol. Plant Pathol. 63(5): 281-289.

Amusa NA, Kehinde IA, Adegbite AA (2004). Pepper (*Capsicum frutescens*) fruit anthracnose in the humid forest region of south-western Nigeria. Nutr. Food Sci. 34(3): 130-134.

Balardin RS, Smith JJ, Kelly JD (1999). Ribosomal DNA polymorphism in *Colletotrichum lindemuthianum.* Mycol. Res. 103(7): 841-848.

Bayraktar H, Dolar FS, Maden S (2008). Use of RAPD and ISSR markers in detection of genetic variation and population structure among *Fusarium oxysporum* f. sp. *Ciceris* isolates on chickpea in Turkey. J. Phytopathol. 156(3): 146-154.

Camargo-Junior OA, Souza EA, Mendes-Costa MC, Santos JB, Soares MA (2007). Identification of *Glomerella cingulata* f.sp *phaseoli* recombinants by RAPD markers. Genet. Mol. Res. 6(3): 607-615.

Chadha S, Gopalakrishna T (2007). Comparative assessment of REMAP and ISSR marker assays for genetic polymorphism studies in *Magnaporthe grisea.* Curr. Sci. 93(5): 688-692.

Denoyes-Rothan B, Guérin G, Délye C, Smith B, Minz D, Maymon M, Freeman S (2003). Genetic diversity and pathogenic variability among isolates of *Colletotrichum* species from strawberry. Phytopathol. 93(2): 219-228.

Freeman S, Horowitz S, Sharon A (2001a). Pathogenic and nonpathogenic lifestyles in *Colletotrichum acutatum* from strawberry and other plants. Phytopathol. 91(10): 986-992.

Freeman S, Katan T, Shabi E (1998). Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant

Dis. 82(6): 596-605.
Freeman S, Minz D, Maymon M, Zveibil A (2001b). Genetic diversity within *Colletotrichum acutatum sensu* Simmonds. Phytopathol. 91(6): 586-592.
Freeman S, Shabi E (1996). Cross-infection of subtropical and temperate fruits by *Colletotrichum* species from various hosts. Physiol. Mol. Plant Pathol. 49(6): 395-404.
Gopinath K, Radhakrishnan NV, Jayaral J (2006). Effect of propiconazole and difenoconazole on the control of anthracnose of chili fruit caused by *Colletotrichum capsici*. Crop Protection 25(9): 1024-1031.
Hong JK, Hwang BK (1998). Influence of inoculum density, wetness duration, plant age, inoculation method, and cultivar resistance on infection of pepper plants by *Colletotrichum coccodes*. Plant Dis. 82(10): 1079-1083.
Johnston PR, Jones D (1997). Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequence. Mycologia 89(3): 420-430.
Kim HR, Lim TH, Kim J, Kim YH, Kim HT (2009). Potential of cross-infection of *Colletotrichum* species causing anthracnose in persimmon and pepper. Plant Pathol. J. 25(1): 13-20.
Kim KD, Oh BJ, Yang J (1999). Differential interactions of a *Colletotrichum gloeosporioides* isolates with green and red pepper fruits. Phytoparasitica 27(2): 1-10.
Lee DH, Kim DH, Jeon YA, Uhm JY, Hong S.B (2007). Molecular and cultural characterization of *Colletotrichum* spp. causing bitter rot of apples in Korea. Plant Pathol. J. 23(2): 37-44.
Martín MP, García-Figueres F (1999). *Colletotrichum acutatum* and *C. gloeosporioides* cause anthracnose on olives. Eur. J. Plant Pathol. 105(8): 733-741.
Martinez-Culebras PV, Querol A, Suarez-Fernandez MB, Garcia-Lopez MD, Barrio E (2003). Phylogenetic relationships among *Colletotrichum* pathogens of strawberry and design of PCR primers for their identification. J. Phytopathol. 151(3): 135-143.
Moriwaki J, Tsukiboshi T, Sato T (2002). Grouping of *Colletotrichum* species in Japan based on rDNA sequences. J. Gen. Plant Pathol. 68(4): 307-320.
Nagaoka T, Ogihara Y (1997). Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94(5): 597-602.
Nirenberg HI, Feiler U, Hagedorn G (2002). Description of *Colletotrichum lupini* comb. nov. In modern trems. Mycologia. 94(2): 307-320.
Oanh LTK, Korpraditskul V, Rattanakreetakul C (2004). A pathogenicity of anthracnose fungus, *Colletotrichum capsici* on various Thai chilli varieties. Kasetsart J. Nat. Sci. 38(6): 103-108.
Oh BJ, Ko MK, Kim YS, Kim KS, Kostenyuk I, Kee HK (1999). A cytochrome P450 gene is differentially expressed in compatible and incompatible interactions between pepper (*Capsicum annuum*) and the anthracnose fungus, *Colletotrichum gloeosporioides*. Mol. Plant Microbe Interact. 12(12): 1044-1052.
Pakdeevaraporn P, Wasee S, Taylor PWJ, Mongkolporn O (2005). Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. Plant Breed. 124(2): 206-208.
Ratanacherdchai K, Wang HK, Lin FC, Soytong K (2007). RAPD analysis technique of *Colletotrichum* species causing chilli anthracnose disease in Thailand. J. Agric. Technol. 3(2): 211-219.
Rohlf FJ (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software: Setauket, NY.
Saha T, Kumar A, Ravindran M, Jacob CK, Roy B, Nazeer MA (2002). Identification of *Colletotrichum acutatum* from rubber using random amplified polymorphic DNAs and ribosomal DNA polymorphisms. Mycol. Res. 106(2): 215-221.
Sanders GM, Korsten L (2003). Comparison of cross inoculation potential of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. Microbiol. Res. 158(2): 143-150.
Schwarzenbach K, Widmer F, Enkerli F (2007). Cultivation-independent analysis of fungal genotypes in soil by using simple sequence repeat markers. Appl. Environ. Microbiol. 73(20): 6519-6525.
Sharma PN, Kaur M, Sharma OP, Sharma P, Pathania A (2005). Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western India. J. Phytopathol. 153(4): 232-237.
Sicard D, Michalakis Y, Dron M, Neema C (1997). Genetic diversity and pathogenicity variation of *Colletotrichum lindemuthianum* in the three centers of diversity of its host, *Phaseolus vulgaris.* Phytopathol. 87(8): 807-813.
Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PWJ (2008a). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chilli (*Capsicum* spp.) in Thailand. Plant Pathol. 57(3): 562-572.
Than PP, Prihastuti H, Phoulivong S, Taylor PWJ, Hyde KD (2008b). Chilli anthracnose disease caused by *Colletotrichum* species. J. Zhejiang Univ. Sci. B. 9(10): 764-778.
Voorrips RE, Finkers R, Sanjaya L, Groenwold R (2004). QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. Theor. Appl. Genet. 109(6): 1275-1282.
Wang JY, Liu XH, Lu JP, Lin FC (2005). Sequence analysis and expression pattern of *MGTA1* gene in rice blast pathogen *Magnaporthe grisea*. J. Zhejiang Univ. Sci. B. 6(8): 817-824.
Weeds PL, Chakraborty S, Fernandes CD, Charchar MJ d'A, Ramesh CR, Kexian Y, Kelemu S (2003). Genetic diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centers of origin and utilization. Phytopathol. 93(2): 176-185.
Whitelaw-Weckert MA, Curtin SJ, Huang R, Steel CC, Blanchard CL, Roffey PE (2007). Phylogenetic relationships and pathogenicity of *Colletotrichum acutatum* isolates from grape in subtropical Australia. Plant Pathol. 56(3): 448-463.