Journal of
Plant Pathology & Microbiology

Zhang et al., J Plant Pathol Microb 2013, 4:10
DOI: 10.4172/2157-7471.1000210

Research Article Open Access

Cultural Characteristics and Pathogenic Variations among *Cochliobolus carbonum* Isolates in Yunnan Province of China

Jun-Xiang Zhang[1], Yi-Xin Wu[2], Hon-Hing Ho[3], Can-Hua Lu[2], Yue-Qiu He[1,2]*

[1]*Faculty of Plant Protection, Yunnan Agricultural University, Kunming, China*
[2]*Faculty of Agriculture and Biotechnology, Yunnan Agricultural University, Kunming, China*
[3]*Department of Biology, State University of New York, New Paltz, NY 12561, USA*

Abstract

Northern leaf spot, caused by *Cochliobolus carbonum*, is an important corn disease that is favored by the temperate mountain climate, and is the most important factor limiting the production of corn in Yunnan Province, China. Cultural characteristics and pathogenic diversity of 25 isolates of *C. carbonum* from Yunnan were studied. The isolates showed some variation in colony morphology and sporulation on potato lactose agar (PLA) medium. Pathogenicity test of the isolates on 8 differential corn inbred lines demonstrated that races 2 and 3 of *C. carbonum* caused the greatest losses in maize production in Yunnan. However, lines MU-4 and W-8053 were resistant to all race 3 isolates, whereas line s37 showed resistance response to all race 2 isolates. The pathogencity and virulence of all isolates were studied on 40 cultivars. The virulence varied with the fungal isolates. In general, race 3 isolates were more virulent than race 2 isolates, but 2 isolates of race 2 proved to be highly virulent and could cause great damage to corn. This information will be useful in the selection of cultivars/lines with different resistance genes for use in designing resistant corn breeding programs.

Keywords: *Bipolaris zeicola*; Conidia; Corn; Cultivar; Leaf spot; Virulence

Introduction

Cochliobolus carbonum Nelson (anamorph, *Bipolaris zeicola*), an ascomycetous fungus, can cause northern leaf spot (NLS), a ubiquitous foliar disease of corn (maize) and grasses in many regions of the world [1-3]. NLS is one of the important factors limiting the production of corn in Yunnan Province of China due to the lack of resistant cultivars and temperate mountain climate favorable to the disease development, even though it is not considered to be a serious problem in corn production in other countries of the world [4].

C. carbonum can infect the leaf, leaf sheath, husks, and ears of corn, and the differentiation of pathogenic races is based primarily on the lesion characters on host leaves, such as the shape and size of the lesion spot. Race 0 is avirulent on corn and causes only flecks or minute lesions on inoculated leaves [5]. Race 1 produces the HC-toxin and induces oval to circular lesions (11.4×3.8 mm) with a dark center and dark-watery margins on corn with genotypes homozygous for the *hm1* gene [6-8]. Race 2 induces necrotic, dark to brown, oval to irregular lesions (1.9×0.7 mm) on susceptible maize and chlorotic or necrotic, circular to oval lesions on resistant maize [7], and Race 3 causes linear lesions (3.5×0.5 mm) on susceptible maize and small oval or short linear chlorotic lesions on resistant cultivars [7,9,10]. Race 4 is characterized by circular to oval lesions (5-10 mm) on maize inbred with a B73 background and low virulence on inbred line W64A [11-13].

Several studies have documented variability in the pathogenicity of the fungus, and different pathotypes have been reported from many countries using a range of differential corn lines [11]. Little is known about the pathogenic complexity of *C. carbonum* in China, and understanding the pathotypes of the pathogen is essential for the development of appropriate disease management strategies. Determination of both host specificity and genetic diversity in the pathogen population is also prerequisite to breed for durable resistance in corn cultivars.

The objectives of present study were to (1) compare the cultural characteristics of *C. carbonum* isolates from Yunnan; (2) determine the extent of pathogenic variability among *C. carbonum* isolates; (3) ascertain races of *C. carbonum* based on pathogenicity tests using eight corn inbred lines from Yunnan.

Materials and Methods

Pathogen isolation

Isolates of *C. carbonum* were obtained from infected plant leaves of different varieties collected from corn fields in 10 states of Yunnan Province, China, in 2010 and 2011. The pathogen was isolated by following standard tissue isolation procedure [14]. Single conidium was located microscopically and picked up by a sterile needle. Each spore was eventually transferred to potato lactose agar (PLA) slants for storage.

Studies of cultural characteristics

The cultural characteristics of different all isolates were studied on 6-cm diameter PLA plates. The morphology of the colony, mycelia and asexual structures were studied after the plates were incubated for 10 days at 25°C. Morphological identifications of isolates were based on the description of Nelson [15], and Zhang and Sun [16]. The size and shape of 100 conidia were measured under a light microscope.

***Corresponding author:** Yue-qiu HE, Faculty of Plant Protection, Yunnan Agricultural University, Kunming 650201, Yunnan, China, Tel: 86-0871-65228221; Fax: 86-0871-65228532; E-mail: ynfh2007@163.com; heyueqiu1955@hotmail.com

Received: November 07, 2013; **Accepted** November 28, 2013; **Published** November 30, 2013

Citation: Zhang JX, Yi-Xin Wu, Hon-Hing Ho, Can-Hua Lu, Yue-Qiu He (2013) Cultural Characteristics and Pathogenic Variations among *Cochliobolus carbonum* Isolates in Yunnan Province of China. J Plant Pathol Microb 4: 210. doi:10.4172/2157-7471.1000210

Citation: Zhang JX, Yi-Xin Wu, Hon-Hing Ho, Can-Hua Lu, Yue-Qiu He (2013) Cultural Characteristics and Pathogenic Variations among *Cochliobolus carbonum* Isolates in Yunnan Province of China. J Plant Pathol Microb 4: 210. doi:10.4172/2157-7471.1000210

Race identification

The race identification of isolates was based on the lesion types on 8 inbred corn lines grown and inoculated in the greenhouse. Inoculation method followed that described by Welz and Leonard [8,17] with minor modification. The inoculum was prepared by washing the conidia from a 10-days old 6-cm diameter PLA cultures plate with 10

Isolate	Geographic origin	Host	Hypha color	Colony reverve color	Conidia production[a]
s3	Shuangjiang, Lincang city	ND-108	greyish-green	reddish violet	8.4×10^6
s20	Zhenkang, Lincang city	BEJZ	greyish-green	reddish violet	1.4×10^6
s27	Yongde, Lincang city	LPZ	greyish-green	greyish-green	6.4×10^5
s33	Dali, Dali city	YY-22	greyish-green	greyish-green	3.4×10^5
s44	Jianchuan, Dali city	BY-7	greyish-green	greyish-green	1.6×10^6
s46	Dali, Dali city	XH-201	greyish-green	greyish-green	6.3×10^5
s53	Yangbi, Dali city	ZD-808	greyish-green	greyish-green	4.6×10^6
s56	Yangbi, Dali city	BY-16	greyish-green	greyish-green	3.7×10^6
s59	Lufeng, Chuxiong city	HD-4	grayish-white	greyish-green	4.4×10^5
s61	Longling, Baoshan city	TY-7	grayish-white	greyish-green	3.3×10^6
s68	Yiliang,Chaotong ctiy	BDPZ	grayish-white	greyish-green	7.8×10^6
s74	Gucheng, Lijiang city	XX-201	greyish-green	greyish-green	9.1×10^5
s92	Hongta, Yuxi City	HD-4	greyish-green	greyish-green	1.1×10^6
s111	Longyang, Baoshan City	BY-2	greyish-green	greyish-green	3.3×10^5
s118	Yuxi, Yuxi City	unknown	grayish-white	reddish violet	5.7×10^6
s120	Songming, Kunming city	unknown	grayish-white	reddish violet	2.3×10^6
s144	Eryuan, Dali city	DY-8	grayish-white	reddish violet	3.0×10^6
s146	Wenshan, Wenshan ctiy	LD-8	grayish-white	reddish violet	1.4×10^6
s149	Wenshan, Wenshan ctiy	YD-90	greyish-green	greyish-green	1.3×10^5
s151	Zhanyi, Qujing city	unknown	greyish-green	greyish-green	7.5×10^6
s155	Zhanyi, Qujing city	unknown	greyish-green	Light red	1.6×10^6
s156	Zhanyi, Qujing city	unknown	greyish-green	greyish-green	3.2×10^5
s157	Zhanyi, Qujing city	unknown	greyish-green	greyish-green	3.2×10^5
s159	Zhanyi, Qujing city	unknown	greyish-green	greyish-green	5.5×10^5
s167	Zhanyi, Qujing city	2-A	greyish-green	Light red	3.3×10^5

a=number of conidia per 6-mm petridish.

Table 1: Cultural characteristics of isolates of *Cochliobolus carbonum* on potato lactose agar medium incubated for 10 days at 25°C.

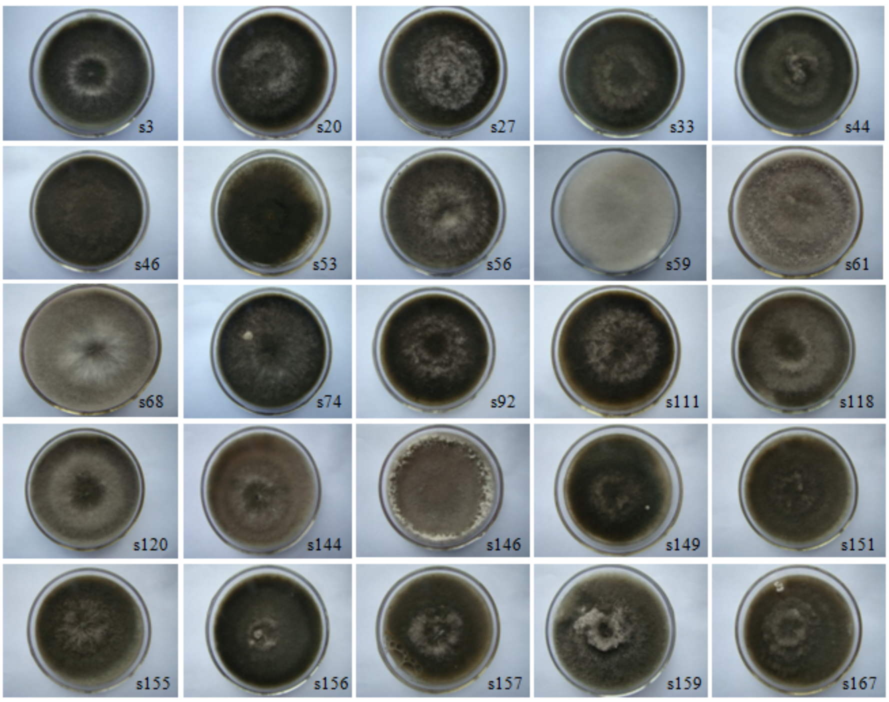

Figure 1: Different cultural characteristics of *Cochliobolus carbonum* isolates on potato lactose agar (PLA) plates. The isolates number is shown at the bottom right corner.

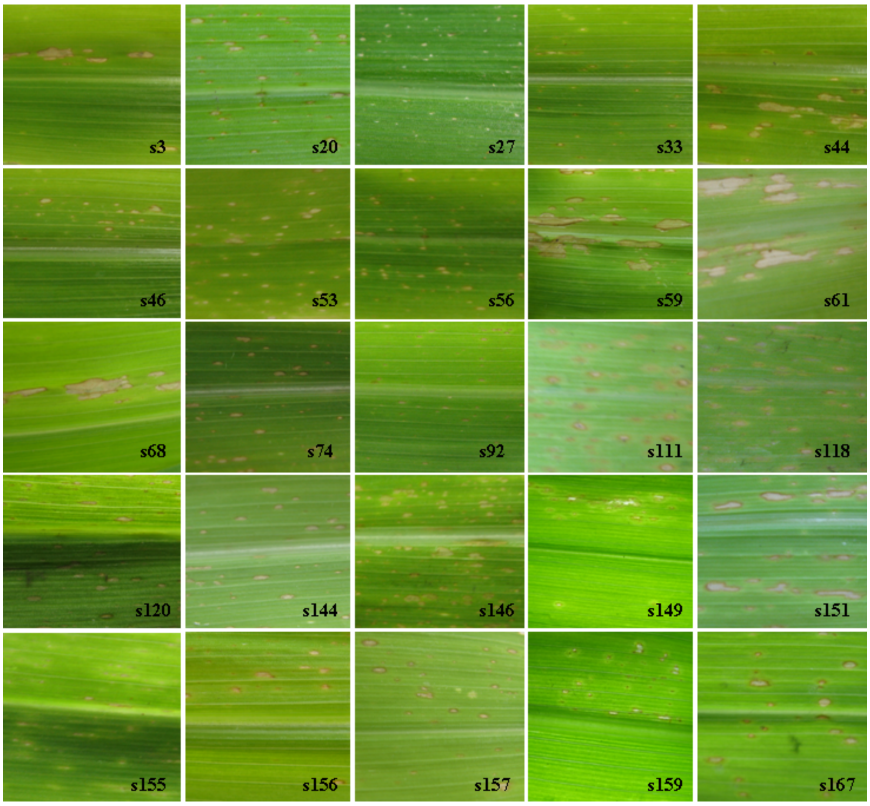

Figure 2: Symptoms of *Cochliobolus carbonum* isolates on corn inbred line MU-6. The isolates number is shown at the bottom right corner.

Isolates	Corn inbred lines								Race
	MU-6	W-0809	MU-4	SU-3-X	S37	W-8053	SY-2134	SY-10-1	
s3	LS*	LS	SOL	LS	LS	ALCS	LS	SOL	3
s20	OS	OS	NO	OS	SOL	OS	OS	OS	2
s27	SOL	LS	SOL	SOL	SOL	SOL	SOL	SOL	3
s33	SOL	SOL	SOL	LS	SOL	SOL	SOL	SOL	3
s44	LS	LS	ALCS	LS	ALCS	ALCS	LS	NO	3
s46	OS	OS	OS	OS	OS	ALCS	OS	OS	3
s53	OS	OS	ALCS	LS	OS	ALCS	OS	OS	3
s56	OS	OS	OS	OS	SOL	OS	OS	OS	2
s59	LS	LS	ALCS	OS	OS	OS	OS	OS	3
s61	LS	LS	ALCS	OS	OS	OS	OS	OS	3
s68	LS	LS	ALCS	ALCS	ALCS	ALCS	ALCS	ALCS	3
s74	OS	OS	ALCS	OS	SOL	OS	OS	OS	2
s92	ALCS	OS	ALCS	OS	SOL	OS	OS	OS	2
s111	OS	OS	ALCS	LS	LS	ALCS	OS	OS	3
s118	OS	OS	ALCS	LS	LS	ALCS	OS	OS	3
s120	OS	OS	ALCS	LS	LS	ALCS	OS	OS	3
s144	ALCS	ALCS	ALCS	ALCS	SOL	ALCS	ALCS	ALCS	2
s146	OS	OS	OS	OS	SOL	OS	OS	OS	2
s149	ALCS	ALCS	NO	ALCS	LS	ALCS	ALCS	ALCS	3
s151	LS	OS	OS	LS	LS	ALCS	OS	OS	3
s155	OS	OS	OS	OS	SOL	OS	OS	OS	2
s156	OS	OS	OS	OS	SOL	OS	OS	OS	2
s157	OS	OS	OS	OS	SOL	OS	OS	OS	2
s159	ALCS	ALCS	OS	LS	LS	ALCS	ALCS	ALCS	3
s167	OS	OS	SOL	OS	SOL	OS	NO	OS	2

*LS=linear lesions (3.5 x 0.5 mm) (Susceptible to race 3); SOL= Small oval spots(≤0.5 x 0.5 mm) (Resistant to race 2); OS=Oval spots (1.9 x 0.7 mm) (Susceptible to race 2 and resistant to race 3); ALCS=Short linear lesions (1.0 x 0.2 mm) (Resistant to race 3); NO=No lesions (Resistant to both races 2 and 3).

Table 2: Race identification of 25 isolates of *Cochliobolus carbonum* on eight corn inbred lines.

Cultivars	Isolates												
	s3	**s20**	**s27**	**s33**	**s44**	**s46**	**s53**	**s56**	**s59**	**s61**	**s68**	**s74**	**s92**
AN-4	6.1 ± 0.9 S*	4.3 ± 1.9[a,b] S	5.8 ± 1.7S	6.3 ± 0.3 S	7.5 ± 0.5 S	4.5 ± 0.8 S	4.3 ± 1.1 S	2.7 ± 1.3 S	5.8 ± 1.1 S	1.2 ± 0.6 R	1.0 ± 0.0 R	1.0 ± 0.0 R	4.6 ± 1.2 S
YY-68	5.8 ± 1.7S	1.0 ± 0.0 R	4.5 ± 1.2 S	7.7 ± 0.2 S	6.6 ± 0.4 S	3.4 ± 0.4 S	4.8 ± 0.6 S	3.4 ± 1.5 S	7.7 ± 0.7 S	5.6 ± 0.7 S	4.3 ± 1.1 S	2.3 ± 1.9 S	3.6 ± 0.9 S
YH-1	4.6 ± 1.2 S	3.5 ± 1.0 S	6.6 ± 0.4 S	8.1 ± 0.2 S	6.8 ± 0.2 S	4.4 ± 0.9 S	5.7 ± 0.8 S	3.3 ± 1.2 S	6.5 ± 0.8 S	1.3 ± 0.5 R	1.3 ± 0.7 R	1.0 ± 0.0 R	1.2 ± 0.3 R
QK-973	5.5 ± 1.1 S	2.3 ± 0.9 S	4.7 ± 1.3 S	2.9 ± 0.3 R	7.3 ± 0.6 S	4.7 ± 1.1 S	4.9 ± 0.9 S	3.4 ± 0.7 S	5.4 ± 1.2 S	1.2 ± 0.4 R	4.8 ± 1.2 S	1.0 ± 0.0 R	4.3 ± 1.3 S
LH-25	6.6 ± 1.3 S	3.5 ± 0.8 S	4.4 ± 0.2 S	6.4 ± 0.7 S	7.5 ± 0.5 S	4.2 ± 0.4 S	6.1 ± 0.7 S	2.6 ± 0.4 S	6.1 ± 1.3 S	1.0 ± 0.0 R	1.0 ± 0.0 R	1.0 ± 0.0 R	1.0 ± 0.0 R
GY-10	4.9 ± 1.4S	1.2 ± 0.2 R	5.8 ± 1.3 S	6.5 ± 0.5 S	6.5 ± 0.3 S	5.1 ± 0.5 S	5.4 ± 0.6 S	2.5 ± 0.9 S	6.2 ± 0.7 S	1.0 ± 0.0 R	5.8 ± 0.9 S	2.5 ± 0.7 S	4.6 ± 0.4 S
BY-7	5.1 ± 1.4 S	2.3 ± 0.4 S	4.9 ± 1.1 S	5.4 ± 0.7 S	6.2 ± 0.4 S	5.3 ± 0.8 S	3.2 ± 0.6 S	2.7 ± 0.8 S	7.4 ± 0.6 S	1.0 ± 0.0 R	2.3 ± 0.3 R	1.0 ± 0.0 R	3.4 ± 0.5 S
LY-26	6.2 ± 1.9 S	1.2 ± 0.2 R	6.4 ± 0.3 S	8.3 ± 0.3 S	6.5 ± 0.8 S	5.7 ± 0.3 S	3.3 ± 0.7 S	3.1 ± 0.7 S	4.8 ± 0.5 S	5.1 ± 0.6 S	2.8 ± 0.4 R	1.1 ± 0.5 R	3.9 ± 0.7 S
SB-9	6.2 ± 1.3 S	1.0 ± 0.0 R	5.2 ± 0.5 S	7.3 ± 0.7 S	8.2 ± 0.2 S	4.6 ± 0.6 S	5.7 ± 0.4 S	2.9 ± 0.8 S	5.3 ± 0.6 S	6.3 ± 0.3 S	3.6 ± 0.4 R	1.0 ± 0.0 S	1.0 ± 0.0 R
TY-67	7.2 ± 1.5 S	2.5 ± 2.2 S	5.9 ± 0.6 S	5.7 ± 1.1 S	6.7 ± 0.3 S	4.3 ± 0.8 S	6.7 ± 0.3 S	1.0 ± 0.0 R	6.2 ± 0.9 S	4.9 ± 0.5 S	4.4 ± 1.2 S	1.0 ± 0.0 R	2.8 ± 0.8 S
S-70	5.3 ± 1.6S	1.0 ± 0.0 R	6.1 ± 0.8 S	6.1 ± 0.3 S	6.6 ± 0.6 S	4.7 ± 0.5 S	4.3 ± 0.9 S	3.4 ± 1.3 S	7.2 ± 0.5 S	1.1 ± 0.3 R	1.2 ± 0.3 R	2.2 ± 0.6 S	2.6 ± 0.6 S
Q259	6.8 ± 1.2 S	1.0 ± 0.0 R	2.8 ± 0.5 R	3.4 ± 0.3 R	5.9 ± 0.3 S	5.2 ± 0.7 S	3.7 ± 0.7 S	2.2 ± 0.5 S	2.6 ± 0.5 R	1.1 ± 0.5 R	1.3 ± 0.4 R	1.0 ± 0.0 R	3.7 ± 0.8 S
WY-1	4.8 ± 1.5 S	3.2 ± 0.3 S	7.7 ± 0.8 S	5.7 ± 1.2 S	7.2 ± 0.4 S	4.9 ± 0.3 S	4.6 ± 0.6 S	2.5 ± 0.5 S	4.3 ± 1.1 S	1.0 ± 0.0 R	1.0 ± 0.0 R	1.0 ± 0.0 R	3.4 ± 0.5 S
XD-5	6.5 ± 1.2 S	1.1 ± 0.2 R	6.3 ± 0.6 S	4.8 ± 0.3 S	6.4 ± 0.3 S	4.3 ± 0.6 S	4.3 ± 0.8 S	2.3 ± 0.3 S	5.7 ± 0.9 S	1.2 ± 0.3 R	5.4 ± 0.8 S	1.0 ± 0.0 R	4.2 ± 0.7 S
XH-206	5.8 ± 0.9 S	2.5 ± 0.9 S	5.6 ± 1.2 S	5.4 ± 0.4S	7.3 ± 0.4 S	5.8 ± 0.4 S	3.7 ± 0.6 S	4.2 ± 0.6 S	6.2 ± 0.4 S	1.0 ± 0.0 R	3.9 ± 1.1 S	2.4 ± 0.8 S	3.3 ± 0.8 S
CY-1	7.2 ± 0.5S	2.6 ± 0.4 S	5.1 ± 1.1 S	6.6 ± 0.8 S	6.2 ± 0.4 S	5.3 ± 0.7 S	4.7 ± 0.8 S	3.8 ± 0.7 S	6.6 ± 0.5 S	7.1 ± 0.6 S	4.3 ± 1.2 S	2.5 ± 0.7 S	3.5 ± 1.2 S
LY-9	6.4 ± 0.6 S	2.7 ± 0.1 S	5.6 ± 0.7 S	7.3 ± 0.3 S	6.7 ± 0.6 S	5.6 ± 0.4 S	4.6 ± 0.5 S	3.8 ± 0.8 S	7.4 ± 0.6 S	5.6 ± 0.7 S	5.8 ± 1.0 S	2.8 ± 0.6 S	3.7 ± 0.7 S
ZJ-3	5.7 ± 0.6 S	1.1 ± 0.3R	5.3 ± 0.4 S	3.5 ± 1.2 R	6.9 ± 0.5 S	3.6 ± 0.3 S	5.5 ± 0.8 S	2.4 ± 0.5 S	7.8 ± 0.3 S	6.4 ± 0.4 S	4.2 ± 1.2 S	2.4 ± 0.3 S	1.8 ± 0.4 S
SF-169	7.3 ± 0.8 S	2.8 ± 0.9 S	4.8 ± 0.8 S	6.8 ± 0.3 S	7.3 ± 0.6 S	5.2 ± 0.5 S	5.6 ± 0.6 S	3.2 ± 0.7 S	5.7 ± 0.4 S	5.7 ± 0.3 S	1.0 ± 0.0 R	2.7 ± 0.5 S	1.3 ± 0.4 R
CL-98	5.3 ± 0.7S	3.3 ± 0.6 S	5.8 ± 0.4 S	2.7 ± 0.3 R	6.1 ± 0.4 S	3.3 ± 0.5 S	4.4 ± 0.7 S	3.3 ± 0.9 S	5.2 ± 0.5 S	6.3 ± 0.7 S	1,0 ± 0.0 R	3.1 ± 0.8 S	2.5 ± 1.1 S
CX-6	5.7 ± 0.4 S	2.6 ± 0.9 S	5.3 ± 1.3 S	5.8 ± 0.8 S	5.3 ± 0.5 S	4.3 ± 1.1 S	4.7 ± 0.3 S	2.7 ± 0.6 S	5.6 ± 0.8 S	5.5 ± 0.6 S	5.2 ± 0.7 S	1.9 ± 1.6 S	3.3 ± 0.7 S
ZH-8	6.4 ± 0.1 S	2.2 ± 0.6 S	5.5 ± 0.6 S	5.2 ± 0.5 S	6.7 ± 0.7 S	4.7 ± 0.9 S	4.9 ± 0.4 S	3.3 ± 0.4 S	5.3 ± 0.5 S	4.8 ± 0.3 S	3.6 ± 0.6 S	2.6 ± 1.3 S	3.7 ± 1.2 S
JY-2	5.9 ± 0.6 S	1.0 ± 0.0 R	4.7 ± 0.7 S	5.5 ± 0.4 S	6.4 ± 0.6 S	4.9 ± 0.4 S	3.5 ± 1.3 S	2.4 ± 0.5 S	5.7 ± 0.6 S	6.6 ± 0.5 S	3.7 ± 0.8 S	2.7 ± 0.6 S	4.3 ± 1.5 S
DY-5	5.7 ± 0.4 S	1.0 ± 0.0 R	6.9 ± 0.4 S	6.3 ± 0.5 S	7.6 ± 0.3 S	5.0 ± 0.0 S	5.3 ± 1.1 S	2.5 ± 0.5 S	5.7 ± 0.3 S	7.2 ± 0.7 S	3.5 ± 0.7 S	1.0 ± 0.0 R	3.4 ± 0.6 S
SQ-1	6.9 ± 0.2 S	3.3 ± 1.1 S	5.3 ± 0.6 S	6.2 ± 0.7 S	7.1 ± 0.2 S	2.2 ± 0.3 R	6.2 ± 0.9 S	3.8 ± 0.6 S	6.3 ± 0.7 S	5.7 ± 0.9 S	3.5 ± 0.5 S	3.2 ± 0.8 S	2.6 ± 1.1 S
XY-696	8.4 ± 0.4 S	2.6 ± 0.7 S	5.4 ± 0.7 S	7.6 ± 0.4 S	5.6 ± 0.6 S	4.6 ± 0.7 S	5.3 ± 1.3 S	4.5 ± 0.9 S	6.7 ± 0.8 S	4.6 ± 0.5 S	4.5 ± 0.8 S	2.3 ± 1.3 S	2.9 ± 0.9 S
ZD-277	6.7 ± 1.0 S	1.9 ± 0.8 S	4.7 ± 1.1 S	3.3 ± 0.3 R	8.2 ± 0.3 S	4.3 ± 0.8 S	5.5 ± 0.9 S	2.7 ± 0.8 S	4.6 ± 0.4 S	4.7 ± 0.4 S	1.1 ± 0.4 R	2.6 ± 1.9 S	3.4 ± 1.3 S
GHD-9	7.4 ± 0.2 S	3.2 ± 1.1 S	2.7 ± 0.6 R	6.2 ± 0.7 S	7.4 ± 0.3 S	4.7 ± 0.7 S	4.7 ± 0.9 S	3.4 ± 0.5 S	5.3 ± 0.7 S	4.8 ± 0.3 S	4.9 ± 0.8 S	1.0 ± 0.0 R	3.7 ± 0.8 S
WG-11	5.7 ± 1.1 S	2.5 ± 0.8 S	5.3 ± 0.8 S	2.6 ± 0.4 R	7.9 ± 0.7 S	4.9 ± 0.5 S	4.8 ± 0.8 S	3.6 ± 0.6 S	5.5 ± 0.6 S	4.9 ± 0.8 S	5.7 ± 0.6 S	1.0 ± 0.0 R	3.2 ± 0.5 S
MZ-63	8.3 ± 0.3S	2.6 ± 1.0 S	5.7 ± 1.3 S	5.8 ± 0.3 S	6.3 ± 0.8 S	3.7 ± 0.6 S	4.3 ± 1.3 S	3.3 ± 0.8 S	6.3 ± 0.4 S	5.0 ± 0.7 S	1.0 ± 0.0 R	2.7 ± 1.3 S	3.5 ± 1.3 S
SY-1	6.4 ± 1.0 S	2.5 ± 0.5 S	4.4 ± 1.4 S	6.4 ± 0.7 S	7.1 ± 0.5 S	4.2 ± 0.7 S	4.2 ± 0.4 S	2.4 ± 0.4 S	6.7 ± 0.5 S	6.8 ± 0.5 S	1.0 ± 0.0 R	2.2 ± 1.5 S	3.3 ± 1.4 S
GY-8	6.1 ± 0.9 S	3.2 ± 0.6 R	4.7 ± 0.6 S	6.8 ± 0.6 S	6.3 ± 0.7 S	4.6 ± 0.3 S	5.7 ± 0.5 S	2.6 ± 0.7 S	6.9 ± 0.7 S	7.4 ± 0.4 S	4.6 ± 1.5 S	2.4 ± 1.7 S	2.7 ± 1.5 S
YD-1	4.8 ± 1.1 S	2.3 ± 0.8 R	4.1 ± 0.7 S	6.3 ± 0.4 S	6.7 ± 0.4 S	4.6 ± 0.6 S	4.6 ± 0.7 S	1.0 ± 0.6 R	6.1 ± 0.5 S	6.6 ± 0.7 S	1.4 ± 0.4 R	2.3 ± 1.4 S	3.8 ± 1.3 S
LD-99	5.4 ± 0.5 S	1.0 ± 0.0 R	5.5 ± 0.4 S	2.5 ± 0.2 R	6.4 ± 0.5 S	5.3 ± 0.3 S	5.2 ± 0.8 S	1.0 ± 0.8 R	5.3 ± 0.8 S	5.3 ± 0.7 S	3.6 ± 0.6 S	2.5 ± 1.5 S	2.9 ± 1.7 S
LX-4	5.5 ± 0.6S	3.2 ± 0.7 S	6.3 ± 0.4 S	5.9 ± 0.9 S	7.2 ± 0.6 S	3.2 ± 0.8 S	3.5 ± 0.5 S	2.5 ± 0.6 S	5.7 ± 0.4 S	5.2 ± 0.8 S	1.2 ± 0.5 R	1.0 ± 0.0 R	2.4 ± 1.5 S
TC-2	6.7 ± 0.3 S	1.0 ± 0.0 R	6.1 ± 0.7 S	6.2 ± 1.1 S	6.3 ± 0.7 S	5.8 ± 0.4 S	3.8 ± 0.7 S	3.3 ± 0.8 S	7.1 ± 0.7 S	5.7 ± 0.7 S	1.0 ± 0.0 R	1.0 ± 0.0 R	2.6 ± 1.5 S
HY-92	7.6 ± 0.4 S	1.0 ± 0.0 R	2.4 ± 0.2 R	3.5 ± 0.5 R	6.2 ± 0.3 S	4.3 ± 1.1 S	4.4 ± 0.5 S	3.6 ± 1.3 S	6.2 ± 0.4 S	7.1 ± 0.7 S	4.7 ± 0.7 S	1.0 ± 0.0 R	2.5 ± 1.3 S
DK-007	6.4 ± 0.3 S	1.0 ± 0.0 R	2.8 ± 0.5 R	5.6 ± 0.7 S	7.4 ± 0.5 S	3.9 ± 0.4 S	4.2 ± 0.6 S	3.1 ± 1.4 S	6.3 ± 0.5 S	6.3 ± 0.6 S	1.4 ± 0.9 R	1.0 ± 0.0 R	2.7 ± 1.2 S
HD-4	6.9 ± 0.5 S	2.3 ± 0.6 S	5.6 ± 1.1 S	6.3 ± 0.8 S	7.7 ± 0.8 S	3.2 ± 0.6 S	5.7 ± 0.8 S	2.9 ± 1.5 S	5.6 ± 0.3 S	5.5 ± 0.4 S	5.2 ± 0.8 S	2.1 ± 1.7 S	2.3 ± 1.6 R
HD-5	6.6 ± 0.5 S	2.6 ± 0.8 S	4.5 ± 0.7 S	6.7 ± 0.4 S	8.0 ± 0.6 S	4.3 ± 1.2 S	4.3 ± 0.5 S	2.6 ± 0.8 S	5.2 ± 1.1 S	5.2 ± 0.7 S	4.5 ± 0.5 S	2.2 ± 1.5 S	2.4 ± 1.8 S

R=Resistant, S=Susceptible.
aDisease severity was recorded on a 1-9 scale on the fourth and fifth inoculated leaves. 1=0%; 2=1-10%; 3=11-20%; 4=21-30%; 5=31-40%; 6=41-50%; 7=51-65%; 8=65-80%; 9=>80% leaf area covered with lesions.
bMean and standard deviations were calculated from 10 plants.

Table 3: Pathogenicity and virulence (disease severity on a 1-9 scales) of 25 isolates of Cochliobolus carbonum on 40 corn cultivars.

ml sterile distilled water (SDW) and filtering the suspension through two layer of cheesecloth. The concentration of the spore suspension was determined with the aid of a hemocytometer and the suspension was diluted with SDW to obtain a concentration of $5×10^5$ conidia/ml. A 5-6 leaf-stage corn plant was sprayed with the spore suspension containing 2 μl/l of polyoxyethylene sorbitan monolaureate 20 (Tween 20). Control plants were treated similarly with plain SDW. The inoculated plants were incubated overnight at 25°C in a dark moist chamber with 100% relative humidity and then returned to the greenhouse. Lesion types were recorded 6 days after inoculation, and the identification of races followed the method developed by Stanković et al. [18].

Pathogenicity test

Pathogenic variability of the isolates was evaluated in a greenhouse by testing all 25 isolates on 40 differential corn cultivars. The plants were spray-inoculated as described above. Plants were scored for disease reaction and disease severity 10 days after inoculation. Disease reaction was recorded as R=resistant (no lesions, small oval or short linear lesions); and S=susceptible (linear or oval lesions). Disease severity (Isolate virulence) was recorded on a 1-9 scale on the fourth and fifth inoculated leaves: 1=0%; 2=1-10%; 3=11-20%; 4=21-30%; 5=31-40%; 6=41-50%; 7=51-65%; 8=65-80%; 9=>80% leaf area covered with lesions. The experiment was conducted with 10 plants per test isolate, and it was repeated once.

Results

Cultural characteristics

The isolates of *Cochliobolus* sp. which were obtained from the lesions on corn leaves in the field were identified as *C. carbonum* based primarily on the size and shape of conidia [15,16]. The isolates of *C. carbonum* showed differences in cultural characteristics (Table 1 and Figure 1). After 7 days of incubation various isolates produced grayish-white to greyish-green hyphae on PLA whereas the reverse of the colonies was reddish violet to greyish-green. Conidia were mostly 3-9 septate and variable in shape, straight or moderately curved, dark or olivaceous brown, and the cells on the ends sometimes appeared paler than those in the middle, and measured 47.44-103.77×9.39-16.66 µm (av. 66.43×12.70 µm). All isolates of *C. carbonum* produced abundant conidia on PLA medium (number of conidia >10^5/6 mm agar plate).

Race identification

Races 2 and 3 of *C. carbonum* were identified among the 25 isolates tested. Isolates of race 3 accounted for 60% of the isolates tested and predominated in all the regions surveyed (Table 2 and Figure 2).

Pathogenicity variability

The variable pathogenicity of *C. carbonum* isolates to corn leaves was summarized in Table 3. The reaction of 40 corn cultivars indicated that most cultivars showed susceptibility to most of the 25 isolates of *C. carbonum*. Isolates S53, s146 and s151 were pathogenic to all corn cultivars, whereas LY-9, CX-6, XY-696 and HD-5 of corn cultivars showed susceptible response to all isolates. The reaction of 8 corn inbred lines to the 25 isolates indicated that MU-4 and W-8053 showed resistance response to all race 3 isolates, and s37 was resistant to all race 2 isolates. The remaining cultivars showed considerable variation in disease reactions. The virulence of *C. carbonum* isolate towards all the test cultivars exhibited considerable variability. The highly virulent isolates were s3, s27, s33, s44, s59, s118, s146, s151 and s167, and they belonged to race 3 except for s146 and s167; the less virulent isolates

Cultivars	Isolates										
	s111	s118	s120	s144	s146	s149	s151	s155	s156	s157	s159	s167
AN-4	5.7 ± 0.8 S*	7.3 ± 0.6[a,b] S	1.0 ± 0.0 R	2.3 ± 0.3 S	7.6 ± 0.4 S	3.8 ± 0.7 S	7.4 ± 0.3 S	3.8 ± 0.6 S	2.8 ± 0.7 S	3.4 ± 0.5 S	4.6 ± 0.4 S	7.4 ± 0.5 S
YY-68	5.6 ± 0.6 S	6.7 ± 0.4 S	4.5 ± 0.8 S	2.6 ± 0.5 S	5.6 ± 0.7 S	4.5 ± 0.5 S	5.8 ± 0.4 S	4.5 ± 0.7 S	3.9 ± 0.6 S	4.3 ± 0.7 S	5.3 ± 0.6 S	6.4 ± 0.7 S
YH-1	6.3 ± 1.6 S	7.5 ± 0.5 S	3.8 ± 0.6 S	3.5 ± 0.6 S	6.3 ± 0.9 S	4.2 ± 0.7 S	6.5 ± 0.5 S	3.5 ± 0.5 S	3.3 ± 0.3 S	2.8 ± 1.3 S	5.9 ± 0.7 S	6.7 ± 0.7 S
QK-973	4.7 ± 1.1 S	6.4 ± 0.3 S	2.3 ± 0.4 R	3.2 ± 0.5 S	5.4 ± 1.7 S	4.7 ± 0.9 S	4.6 ± 0.7 S	1.6 ± 0.4 R	1.0 ± 0.0 R	2.5 ± 0.8 S	6.2 ± 0.3 S	7.4 ± 0.8 S
LH-25	7.2 ± 0.5 S	5.7 ± 0.5 S	2.6 ± 0.5 R	3.8 ± 0.8 S	5.7 ± 0.4 S	3.3 ± 0.4 S	7.6 ± 0.6 S	1.0 ± 0.0 R	4.1 ± 0.7 S	2.8 ± 0.5 S	3.8 ± 0.6 S	5.7 ± 0.4 S
GY-10	6.3 ± 0.6 S	5.9 ± 0.7 S	4.5 ± 0.4 S	2.3 ± 0.2 S	6.5 ± 0.7 S	4.6 ± 0.5 S	5.4 ± 0.5 S	3.7 ± 0.7 S	4.6 ± 0.9 S	3.5 ± 0.4 S	7.4 ± 0.8 S	5.3 ± 0.6 S
BY-7	5.4 ± 0.9 S	2.9 ± 1.3 R	4.8 ± 0.6 S	2.6 ± 0.3 S	6.8 ± 0.3 S	5.5 ± 0.7 S	4.7 ± 0.3 S	4.5 ± 0.9 S	3.9 ± 0.5 S	3.6 ± 0.6 S	7.1 ± 0.6 S	6.2 ± 0.3 S
LY-26	6.4 ± 1.2 S	6.4 ± 0.4 S	4.8 ± 0.3 S	2.4 ± 0.4 S	5.3 ± 0.6 S	1.2 ± 0.3 R	5.3 ± 0.6 S	1.0 ± 0.0 R	3.4 ± 0.3 S	3.9 ± 0.7 S	6.5 ± 0.5 S	6.4 ± 0.6 S
SB-9	5.2 ± 0.3 S	5.7 ± 0.3 S	4.1 ± 0.5 S	3.6 ± 0.6 S	5.7 ± 0.4 S	1.0 ± 0.0 R	6.7 ± 0.7 S	1.0 ± 0.0 R	3.3 ± 0.6 S	2.4 ± 0.6 S	5.4 ± 0.3 S	6.7 ± 0.2 S
TY-67	5.6 ± 0.5 S	5.4 ± 0.6 S	3.9 ± 0.6 S	3.6 ± 0.5 S	6.3 ± 0.7 S	1.0 ± 0.0 R	5.9 ± 0.5 S	3.5 ± 0.4 S	3.7 ± 0.5 S	2.7 ± 0.9 S	5.9 ± 0.2 S	6.8 ± 0.9 S
S-70	6.1 ± 0.7 S	7.3 ± 0.6 S	3.7 ± 0.3 R	3.5 ± 0.7 S	5.7 ± 0.6 S	3.7 ± 0.5 S	6.4 ± 0.6 S	4.8 ± 0.6 S	2.7 ± 0.6 S	2.8 ± 0.4 S	4.7 ± 0.6 S	7.2 ± 0.5 S
Q259	7.3 ± 0.4 S	6.3 ± 0.7 S	3.9 ± 0.3 S	2.1 ± 0.6 S	6.8 ± 0.5 S	3.2 ± 0.6 S	5.7 ± 0.4 S	2.5 ± 0.7 S	2.6 ± 0.7 S	2.7 ± 0.6 S	4.3 ± 0.5 S	5.9 ± 0.7 S
WY-1	2.6 ± 0.3 R	5.5 ± 0.5 S	3.5 ± 0.4 S	3.4 ± 0.7 S	5.5 ± 0.6 S	4.6 ± 0.4 S	5.4 ± 0.7 S	3.8 ± 0.5 S	3.8 ± 0.5 S	3.6 ± 0.3 S	5.7 ± 0.8 S	6.4 ± 0.5 S
XD-5	5.4 ± 0.9 S	6.8 ± 0.6 S	3.6 ± 0.5 S	3.4 ± 0.2 S	5.5 ± 0.5 S	1.0 ± 0.0 R	6.2 ± 0.6 S	2.8 ± 0.7 S	2.6 ± 0.6 S	3.4 ± 0.6 S	6.3 ± 0.6 S	7.3 ± 0.7 S
XH-206	6.2 ± 0.5 S	6.5 ± 0.7 S	4.2 ± 0.6 S	2.9 ± 0.3 S	5.2 ± 1.3 S	6.2 ± 0.3 S	6.8 ± 0.5 S	3.3 ± 0.5 S	3.3 ± 0.7 S	3.3 ± 0.5 S	6.8 ± 0.3 S	7.8 ± 0.9 S
CY-1	1.8 ± 0.6 R	5.2 ± 0.5 S	4.6 ± 0.7 S	2.2 ± 0.4 S	6.7 ± 0.4 S	5.6 ± 0.5 S	6.5 ± 0.6 S	1.0 ± 0.0 R	2.9 ± 0.5 S	3.7 ± 0.4 S	6.3 ± 0.7 S	6.4 ± 0.2 S
LY-9	4.5 ± 0.7 S	6.4 ± 0.6 S	4.7 ± 0.9 S	2.5 ± 0.7 S	6.4 ± 0.5 S	5.2 ± 0.6 S	7.5 ± 0.7 S	3.6 ± 0.8 S	2.8 ± 0.2 S	4.2 ± 0.7 S	5.7 ± 0.6 S	5.5 ± 0.5 S
ZJ-3	5.7 ± 1.6 S	6.7 ± 0.7 S	4.4 ± 0.7 S	2.7 ± 0.6 S	7.3 ± 0.7 S	4.8 ± 0.5 S	4.7 ± 0.5 S	3.7 ± 0.7 S	3.3 ± 0.7 S	3.6 ± 0.9 S	5.4 ± 0.4 S	5.7 ± 0.6 S
SF-169	4.2 ± 0.9 S	5.6 ± 0.9 S	3.6 ± 0.8 S	2.8 ± 0.7 S	5.6 ± 0.7 S	4.6 ± 0.6 S	4.8 ± 0.8 S	2.5 ± 0.6 S	2.8 ± 0.6 S	3.4 ± 0.5R	5.9 ± 0.7 S	5.3 ± 0.7 S
CL-98	4.9 ± 0.7 S	5.2 ± 0.5 S	2.1 ± 0.3 R	2.4 ± 0.6 S	6.2 ± 0.6 S	6.3 ± 0.7 S	4.1 ± 0.6 S	3.6 ± 0.7 S	4.3 ± 0.4 S	2.8 ± 0.6 S	7.2 ± 0.6 S	5.7 ± 0.5 S
CX-6	4.9 ± 0.8 S	6.7 ± 0.6 S	3.3 ± 0.7 S	2.5 ± 0.7 S	6.7 ± 0.4 S	5.7 ± 0.4 S	7.2 ± 0.4 S	3.9 ± 0.5 S	3.6 ± 0.5 S	2.6 ± 0.5 S	7.3 ± 0.3 S	6.7 ± 0.7 S
ZH-8	5.2 ± 0.7 S	2.2 ± 0.7 R	3.6 ± 0.5 S	3.4 ± 0.9 S	5.5 ± 0.4 S	4.5 ± 0.6 S	4.8 ± 0.6 S	1.0 ± 0.0 R	3.8 ± 0.7 S	3.8 ± 0.4 S	4.7 ± 0.4 S	6.4 ± 0.9 S
JY-2	4.6 ± 0.6 S	6.3 ± 0.7 S	3.7 ± 0.6 S	2.3 ± 0.7 S	6.3 ± 0.6 S	5.2 ± 0.6 S	5.4 ± 0.7 S	2.6 ± 0.7 S	4.6 ± 0.5 S	4.1 ± 0.8 S	4.3 ± 0.6 S	5.8 ± 0.6 S
DY-5	4.9 ± 0.7 S	6.4 ± 0.4 S	3.9 ± 0.5 S	2.6 ± 0.7 S	5.7 ± 0.7 S	4.8 ± 0.4 S	6.3 ± 0.9 S	2.9 ± 0.5 S	3.8 ± 0.6 S	3.7 ± 0.3 S	5.8 ± 0.5 S	7.8 ± 0.5 S
SQ-1	2.8 ± 0.5 R	5.6 ± 0.5 S	4.2 ± 0.6 S	3.7 ± 0.6 S	5.3 ± 0.7 S	1.3 ± 0.6 R	4.8 ± 0.3 S	2.5 ± 0.7 S	4.2 ± 0.3 S	3.3 ± 0.2 S	6.4 ± 0.3 S	5.4 ± 0.4 S
XY-696	6.3 ± 0.5 S	5.7 ± 0.6 S	4.6 ± 0.4 S	3.2 ± 0.5 S	6.6 ± 0.9 S	6.3 ± 0.5 S	5.9 ± 0.5 S	3.5 ± 0.9 S	4.4 ± 0.3 S	3.6 ± 0.8 S	6.1 ± 0.7 S	5.7 ± 0.7 S
ZD-277	3.8 ± 0.4 S	5.3 ± 0.4 S	3.2 ± 0.3 S	2.8 ± 0.4 S	7.2 ± 0.7 S	5.4 ± 0.8 S	4.6 ± 0.7 S	1.0 ± 0.0 R	3.2 ± 0.9 S	4.2 ± 0.5 S	5.7 ± 0.4 S	6.3 ± 0.3 S
GHD-9	5.4 ± 0.3 S	6.1 ± 0.7 S	3.4 ± 0.4 S	3.3 ± 0.6 S	5.4 ± 0.5 S	3.8 ± 0.6 S	4.5 ± 0.4 S	2.5 ± 0.4 S	2.7 ± 0.4 S	4.5 ± 0.5 S	5.3 ± 0.5 S	6.7 ± 0.4 S
WG-11	5.5 ± 0.7 S	5.8 ± 0.3 S	3.1 ± 0.5 S	2.5 ± 0.7 S	5.3 ± 0.6 S	4.3 ± 0.3 S	5.7 ± 0.6 S	1.0 ± 0.0 R	3.6 ± 0.4 S	3.2 ± 0.6 S	6.7 ± 0.5 S	6.2 ± 0.5 S
MZ-63	4.1 ± 0.5 S	2.9 ± 0.5 R	1.0 ± 0.0 R	2.7 ± 0.6 S	7.8 ± 0.7 S	5.7 ± 0.5 S	5.8 ± 0.7 S	1.0 ± 0.0 R	4.6 ± 0.6 S	3.7 ± 0.7 S	6.2 ± 0.6 S	7.3 ± 0.2 S
SY-1	4.6 ± 0.3 S	5.7 ± 0.7 S	2.3 ± 0.0 S	3.3 ± 0.5 S	6.4 ± 0.3 S	6.5 ± 0.7 S	7.8 ± 0.5 S	1.0 ± 0.0 R	3.7 ± 0.7 S	2.9 ± 0.5 S	7.3 ± 0.4 S	6.4 ± 0.5 S
GY-8	6.3 ± 0.5 S	2.4 ± 0.6 R	3.2 ± 0.5 S	3.6 ± 0.6 S	5.7 ± 0.5 S	3.5 ± 0.8 S	5.4 ± 0.6 S	1.0 ± 0.0 R	4.6 ± 0.8 S	4.3 ± 0.7 S	4.7 ± 0.7 S	5.7 ± 0.7 S
YD-1	5.8 ± 0.3 S	5.4 ± 0.7 S	4.3 ± 0.6 S	2.8 ± 0.8 S	4.7 ± 0.5 S	3.2 ± 0.7 S	6.8 ± 0.4 S	1.0 ± 0.0 R	3.8 ± 0.9 S	2.3 ± 0.5 S	5.6 ± 0.5 S	5.8 ± 0.4 S
LD-99	6.3 ± 0.3 S	6.5 ± 0.5 S	2.5 ± 0.4 S	3.4 ± 0.3 S	6.3 ± 0.6 S	3.9 ± 0.5 S	5.3 ± 0.6 S	1.0 ± 0.0 R	3.3 ± 0.4 S	3.8 ± 0.7 S	7.8 ± 0.6 S	6.4 ± 0.8 S
LX-4	4.7 ± 0.9 S	6.8 ± 0.6 S	3.4 ± 0.6 S	3.5 ± 0.4 S	6.5 ± 0.4 S	4.3 ± 0.6 S	6.4 ± 0.7 S	3.5 ± 0.6 S	1.0 ± 0.0 R	3.6 ± 0.3 S	6.3 ± 0.4 S	6.8 ± 0.5 S
TC-2	7.2 ± 0.4 S	5.2 ± 0.3 S	1.8 ± 0.4 R	3.5 ± 0.6 S	7.2 ± 0.7 S	4.6 ± 0.6 S	6.3 ± 0.5 S	1.0 ± 0.0 R	3.3 ± 0.6 S	3.4 ± 0.6 S	6.4 ± 0.7 S	6.1 ± 0.6 S
HY-92	5.4 ± 0.6 S	5.3 ± 0.7 S	3.2 ± 0.7 S	3.1 ± 0.7 S	5.9 ± 0.9 S	6.7 ± 0.4 S	5.8 ± 0.3 S	3.4 ± 0.7 S	4.3 ± 0.4 S	4.1 ± 0.4 S	6.8 ± 0.5 S	6.5 ± 0.5 S
DK-007	4.2 ± 0.7 S	5.8 ± 0.5 S	2.7 ± 0.5 S	3.8 ± 0.5 S	6.3 ± 0.3 S	5.5 ± 0.6 S	6.0 ± 0.2 S	2.8 ± 0.5 S	3.7 ± 0.7 S	3.9 ± 0.4 S	5.4 ± 0.3 S	6.7 ± 0.7 S
HD-4	4.3 ± 0.5 S	5.5 ± 0.6 S	2.3 ± 0.4 S	3.0 ± 0.4 S	6.8 ± 0.4 S	3.8 ± 0.7 S	5.7 ± 0.6 S	3.4 ± 0.3 S	3.6 ± 0.5 S	3.5 ± 0.7 S	6.2 ± 0.6 S	5.6 ± 0.6 S
HD-5	4.7 ± 0.7 S	5.1 ± 0.9 S	2.2 ± 0.6 S	2.9 ± 0.6 S	5.9 ± 0.4 S	4.7 ± 0.5 S	4.9 ± 0.7 S	3.7 ± 0.5 S	3.7 ± 0.2 S	3.8 ± 1.1 S	5.4 ± 0.4 S	5.5 ± 0.5 S

*R=Resistant, S=Susceptible

aDisease severity was recorded on a 1-9 scale on the fourth and fifth inoculated leaves. 1=0%; 2=1-10%; 3=11-20%; 4=21-30%; 5=31-40%; 6=41-50%; 7=51-65%; 8=65-80%; 9=>80% leaf area covered with lesions.

bMean and standard deviations were calculated from 10 plants.

Table 3: (continued) Pathogenicity and virulence (disease severity on a 1-9 scales) of 25 isolates of *Cochliobolus carbonum* on 40 corn cultivars.

were s20, s56, s74, s120, s144 and s156, and they were identified as race 2 except for s120.

Discussion

The cultural characteristics of 25 isolates of *C. carbonum* from Yunnan were in agreement with those in a previous study [19] and thus their identification was confirmed. Differences between isolates of a pathogen in characters like growth, and sporulation can be critical for determining disease incidence, because these characters help define the fitness of the pathogen. Variation in conidial production has been reported by Welz et al. [17] who showed that race 2 produced significantly more conidia on PLA than race 3. However, no such association was observed in present study. Moreover, there was no clear relationship between the cultural characters and pathogenicity. For example, isolates s44 and s68 were similar morphologically but different in virulence, even though they belonged to the same race. Isolates showed different symptoms on the same inbred line, indicating that there is considerable variation in symptom production by the isolates (Table 2). Conversely, an isolate did not show the same virulence on every cultivar or line. Similarly, Nelson [2] reported that isolates induced different symptoms on the same host and an isolate induced more than one symptom on different hosts.

The occurrence and distribution of physiological races in the field provided important information for the management of northern leaf spot of corn in Yunnan. The information would help to determine, control strategies such as selection and rotation of crop cultivars. In this study, 15 out of 25 isolates belonged to race 3, and 10 belonged to race 2 (Table 2). No isolate of race 1 was recovered from any of the regions surveyed, presumably due to the wide-spread utilization of corn hybrids resistant to race 1 in China. Also, races 0 and 4 were not found, probably due to the small number of maize genotypes susceptible to these races. The findings demonstrated the existence of race 2 and 3 of *C. carbonum* as the major cause of great losses in maize production in Yunnan Province.

Pathogenic variability of *C. carbonum* has been demonstrated by Nelson et al. [9], Leonard [6,10], Tsukiboshi et al. [20], Dodd and Hooker [11], Dodd [13], as well as Welz and Leonard [8]. Some of these authors characterized the pathogenic types as races of different cultivars, but differences in virulence of the isolates are little known. Race 3 isolates were considered more virulent than race 2 isolates in the previous studies [21,22]. Other contributing factors might involve the larger and contiguous lesion spots of race 3 and the lack of selection pressure on this race due to the monoculture of corn with a narrow genetic base with most cultivars being susceptible. In this study, most race 3 isolates were more virulent than isolates of race 2. However, 2 highly virulence strains (s146 and s167) of race 2 were also isolated suggesting that race 2 might also have the potential to cause great damage to corn in Yunnan.

Acknowledgements

The research was financially supported by the Department of Agriculture, Yunnan Province under the program (TX2009001) of Maize Production System.

References

1. Shoemaker RA (1959) Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from *Helminthosporium*. Can J Bot 37: 879-887.
2. Nelson RR (1970) Genes for pathogenecity in *Cochliobolus carbonum*. Phytopathology 60: 1335-1337.
3. Welz HG, Leonard KJ (1994) Genetic analysis of two race 0 × race 2 crosses in *Cochliobolus carbonum*. Phytopathology 84: 83-90.
4. Smith IM, Dunez J, Lelliott RA, Phillips DH, Archer SA (1988) European handbook of plant diseases. Blackwell Scientific Publications, Oxford, UK.
5. Welz HG, Leonard KJ (1995) Gametic phase disequilibria in populations of race 2 and race 3 of *Cochliobolus carbonum*. Eur J Plant Pathol 101: 301-310.
6. Leonard KJ (1987) The host population as a selective factor. In: Wolfe MS and Caten CE (eds), Populations of Plant Pathogens: Their Dynamics and Their Genetics. Blackwell Scientific Publications, Oxford. 163-179.
7. Welz HG, Leonard KJ (1988) Genetic variation in field population of races 0, 2 and 3 of *Bipolaris zeicola* in 1987. (Abstr). Phytopathology 78: 1574.
8. Welz HG, Leonard KJ (1993) Phenotypic variation and parasitic fitness of races of *Cochliobolus carbonum* on corn in North Carolina. Phytopathology 83: 593-601.
9. Nelson RR, Blanco M, Dalmacio S, Moore BS (1973) A new race of *Helminthosporium carbonum* on corn. Plant Dis Rep 57: 822-823.
10. Leonard KJ (1978) Polymorphisms for lesion type, fungicide tolerance, and mating capacity in *Cochliobolus carbonum* isolates pathogenic to corn. Can J Bot 56: 1809-1815.
11. Dodd JL, Hooker AL (1990) Previously undescribed pathotype of *Bipolaris zeicola* on corn. Plant Dis 74: 530.
12. Lević J, Penčić V (1992) Response of maize inbred B73 to a new pathotype of *Bipolaris zeicola* (Stout) Shoemaker. Maydica 37: 355-361.
13. Dodd JL (1993) Recent developments in the maize pathogen *Bipolaris zeicola* Shoemaker. Maydica 38: 201-204.
14. Tuite J (1969) Plant Pathological Methods, Fungi and Bacteria. Burges Publishing Company, USA
15. Nelson RR (1959) *Cochliobolus carbonum*, the perfect stage of *Helminthosporium carbonum*. Phytopathology 49: 807-810.
16. Zhang TY, Sun GY (2000) Flora fungorum sinicorum. (Vol 30), Helminthosporioid Hyphomycetes. Beijing, Science Press.
17. Welz HG, Leath S, Leonard KJ (1993) Sporulation by races 0, 2, and 3 of *Cochliobolus carbonum* on synthetic medium and sterilized corn leaves. Plant Dis 77: 1153-1157.
18. Stanković S, Lević J, Ivanović D (2007) Genetic variability of maize pathogens in Serbia. Genetika 39: 227-240.
19. Lević J, Penčić V (1993) Morphology of a new pathotype of *Bipolaris zeicola* (Stout) Shoemaker. J Phytopathol 139: 339-346.
20. Tsukiboshi T, Kimigafukuro T, Sato T (1987) Identification of races of *Bipolaris zeicola*, the causal fungus of *Helminthosporium* leaf spot on corn in Japan. Ann Phytopathol Soc Japan 53: 647-649.
21. Castor LL, Ayers JL, Nelson RR, Schreiber AJ (1976) The reaction of several corn inbreeds to race 3 of *Helminthosporium carbonum*. Plant Dis Rep 60: 827-830.
22. Halseth DE, Pardee WD, Viands DR (1991) Inheritance of resistance to *Helminthosporium carbonum* race 3 in maize. Crop Sci 31: 612-617.