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RESEARCH ARTICLE

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Distribution of mating types and genetic diversity induced by sexual recombination in *Setosphaeria turcica* in northern China

Abstract Mature ascocarps and ascospores in the heterothallic ascomycete fungus, *Setosphaeria turcica*, were successfully produced in Sach's medium with barley culm as the mating stimulator after four weeks' coincubation of two opposite mating type isolates at 25°C in darkness. A single isolate could not produce ascospores or ascocarps. The ascocarps were produced on the exposed surface and embedded parts of barley culm or in the upper layer of the medium. The asci linked themselves to ascocarp with their short handles and assembled at the bottom of the ascocarp. Many asci had four to six colorless mature ascospores with one to six septa. But asci with eight ascospores were also found. Using isolate 9914 and isolate 9961 as standard testers for mating types (MAT1 and MAT2), respectively, 94 isolates of *S. turcica* collected from northern China in 1999, 2003, and 2004 were grouped into three mating types: MAT1 (53 isolates), MAT2 (31 isolates) and MAT12 (10 isolates). The MAT12 isolates, which were first found in China, were compatible with not only MAT1 isolates but also MAT2 isolates. No MAT12 isolates were found in 1999, but 2 MAT12 isolates and 8 MAT12 isolates were found in 2001 and 2003, respectively. The geographic distribution of different mating types was unequal among locations. Generally the frequency of MAT1 was significantly higher than that of MAT2 and MAT12. The unequal distribution of mating types suggested a low frequency of genetic recombination. The pathogenicity of different mating type isolates was tested on the susceptible corn inbred B37 and the results revealed that the disease latency period, disease incidence, lesion area and conidia production were not significantly different among the three mating type groups. However, the pathogenicity of the progeny isolates of isolate 99-12 (MAT2, race 1) and isolate 99-15 (MAT1, race 0) was significantly different from the parent isolates, isolate 99-12 and isolate 99-15, suggesting that sexual recombination could cause significantly virulence variation in *S. turcica*. Random amplification of polymorphic DNA (RAPD) analysis also revealed high genotype diversity among the progeny isolates, indicating that the sexual recombination could also produce significant genetic variation in the fungal pathogen.

Keywords *Zea mays*, *Setosphaeria turcica*, Northern Corn Leaf Blight (NCLB), pathogenicity, mating type

1 Introduction

The phytopathogenic fungus *Setosphaeria turcica* (Luttrell) Leonard & Suggs (agamous *Exserohilum turcicum*), the causal agent of Northern Corn Leaf Blight (NCLB), is a heterothallic ascomycete having a single-locus and two-allele mating system (Nelson, 1959). Its perfect stage has never been found on corn leaves in nature although it was successfully induced in the laboratory by Luttrell (1957). The studies of Rodriguez & Ullstrup (1962) and Nelson (1965) revealed that this pathogen could increase its pathogenicity and host range through sexual recombination. Fallah and Patak (1994) and Gianas et al. (1996) had confirmed that isolates belonging to different races could mate and lead to the production of new races with different levels of pathogenicity. Therefore, sexual recombination is possibly one of the major sources for genetic variation in *S. turcica*. After many physiological races had been discovered (Windes and Pedersen, 1991; Gianas et al., 1996; Liu et al., 1996; Li et al., 1999) and would probably continue to be discovered in many countries, more and more attention was paid to the studies on the distribution and genetic structure of mating type in *S. turcica* by scientists

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and corn breeders (Borchardt et al., 1998; Ferguson and Carson, 2004; Oliari et al., 2005).

Northern Corn Leaf Blight is one of the most important corn diseases in northern China, especially after the emergence of *S. turcica* race 1 which was virulent to corn lines with *Ht1* resistant gene in 1983 (Wu et al., 1983). Since then, many new races, such as race 23, race 1N, race 123, and race 23N, had been discovered in Shandong, Hebei, Liaoning, Yunnan and other provinces in China (Welz and Geiger, 1993; Li, 1995; Liu and Zhang, 1996; Liu et al., 1996; Yang and Wang, 2002). Although the disease is not so severe, it happens widely every year. NCLB is always considered in the corn-breeding program. High resistance to NCLB is indispensable to the governmental certification of new corn varieties in China. However, the genetic variation of *S. turcica* populations is still a challenge to develop corn resistance. Therefore, monitoring the change of genetic structure in *S. turcica* populations is necessary for disease control. This study aims at (1) inducing the perfect stage of *S. turcica* in laboratory; (2) elucidating the geographic distribution of mating types by identifying the mating type of isolates collected in northern China; and (3) clarifying the possible effects of sexual recombination on pathogenicity variation and genetic diversity of *S. turcica* by pathogenicity tests and RAPD analyses of their progenies.

2 Materials and methods

2.1 Study area

The research region was located in northern China where corn was a staple feed for livestock (Table 1). The corn was grown as monoculture or in mixed stand including wheat, soybean or peanuts. All corn cultivars were hybrids with *Ht1*, *Ht2*, and/or *Ht3* resistant genes or without any resistant genes to *S. turcica*. Most farmers in the region had discontinuous holdings of less than 1 hectare, so hybrids with different resistant genes were often grown mixed in one plot.

2.2 Isolates collection

A total of 94 mono-conidial isolates were purified from diseased leaves from 71 sites in northern China, out of which 42 isolates were collected from 37 sites in 1999, 37 isolates were collected from 31 sites in 2001, and 15 isolates were collected from 14 sites in 2003 (Table 1). The regions were Heilongjiang, Jilin, Liaoning, Hebei (including Beijing and Tianjin cities), Shandong, Shanxi and Henan provinces, where NCLB was serious.

2.3 Perfect stage induction

Twelve isolates (Table 2) were mated with each other to determine which pairs could produce ascocarps following the method previously described (Luttrell, 1958). Each test was repeated at least three times. A mating pair of isolates was inoculated onto the opposite sides of the Petri dish ($d = 9.0$ cm) with Sach's medium (1 000 mL Sach's medium contains 200 g Potato extract, 0.25 g K_2HPO_4, 4.0 g $CaCO_3$, 1 g $Ca(NO_3)_2$, 0.25 g $MgSO_4$, some $FeCl_3$, and 15 g agar powder). Autoclaved barley culm was used as a mating stimulator and half-embedded into the middle of a plate, at a distance of 1.5–2.0 cm to the isolates at each side. The plates were then incubated at 25°C in darkness for four weeks. Ascocarps were examined and maturity was determined by whether or not they could produce asci with ascospores. The isolates unable to mate with each other were classified into one mating type group. One or two isolates with high mating ability were selected from each mating type group as the tester for other isolates. The mating ability was determined by the average number of mature ascocarps per Petri dish.

2.4 Mating type determination

The mating types of other 82 isolates in Table 1 were determined by pairing a small piece of a culture with the same size of the mating type MAT1 or mating type MAT2 tester culture in a Petri dish according to the method described above. The isolates that formed mature ascocarps when paired with the mating type MAT1 tester were designated as mating type MAT2. Similarly, the isolates that formed mature ascocarps when paired with mating type MAT2 tester were designated as mating type MAT1. Isolates compatible with both mating type MAT1 tester and mting type MAT2 tester were designated as mating type MAT12.

2.5 Progeny culture isolation

An ascocarp was carefully picked up from the barley or the agar medium and placed onto a sterilized glass plate. A drop of sterile distilled water was added and the ascocarp was gently crushed to release the ascospores into the water. The ascospore suspension was then spread onto the surface of 2% agar medium and a single ascospore, with a dissecting microscope, was picked off the medium using a sterile needle and plated on potato dextrose agar (PDA) medium with 100 mg/L ampicillin to avoid bacterial contamination. After incubation at 25°C for five to ten days, mycelia of *S. turcica* germinating from the ascospore were transferred to PDA medium for further growth.

2.6 Pathogenicity test

The pathogenicity of parent and progeny isolates was tested as outlined by Warren (1975). The isolates were cultured on PDA medium at 25°C about seven to ten days before inoculation. The conidia were dislodged from the surface of the colonies and then washed with 10 mL of sterile distilled water into a beaker. The conidium suspension was filtered through double-layered gauze and the conidia were adjusted to 10^5–10^6 spores per mL for inoculation on susceptible corn

Table 1 Distribution of mating types of *Setosphaeria turcica* in northern China

Province	District	Isolate code a)	Mating type
Hebei	Baoding	99-34	MAT1
		01-04	MAT1
		03-23	MAT1
	Chengde	99-12	MAT1
		01-06	MAT1
		01-29	MAT2
		03-08	MAT12 b)
		03-11	MAT12
	Chicheng	99-11	MAT2
		99-16	MAT1
	Cixian	01-07	MAT2
	Dacheng	01-24	MAT1
	Dingzhou	99-18	MAT1
	Fengning	03-16	MAT12
	Fuping	01-18	MAT2
	Fucheng	99-48	MAT1
	Kuancheng	01-22	MAT2
	Laiyuan	01-00	MAT1
		01-01	MAT1
		01-10	MAT1
		01-11	MAT1
		01-12	MAT2
		01-34	MAT1
	Longhua	99-08	MAT1
	Lulong	01-32	MAT1
	Luannan	03-09	MAT12
	Luanping	99-13	MAT1
	Nanhe	99-21	MAT1
	Pingshan	03-17	MAT1
	Raoyang	99-26	MAT1
	Shangyi	99-42	MAT2
	Shijiazhuang	03-18	MAT2
	Tangxian	01-20	MAT1
	Weixian	99-61	MAT2
	Weichang	01-13	MAT2
	Xianghe	01-26	MAT2
	Xinji	01-05	MAT2
	Xinglong	99-20	MAT1
		01-28	MAT1
		03-06	MAT12
	Xuanhua	99-14	MAT1
	Yixian	03-13	MAT12
	Zaoqiang	99-43	MAT2
	Zhangjiakou	01-19	MAT1
	Zhengding	03-14	MAT1
	Zunhua	03-22	MAT1
	Zhuolu	99-53	MAT2
Heilongjiang	Beian	99-51	MAT2
	Dongning	03-03	MAT12
	Gannan	99-29	MAT2
		01-16	MAT1
	Jixian	01-30	MAT1
	Longjiang	99-25	MAT2
	Mudanjiang	01-21	MAT1
		03-12	MAT12
	Ningan	99-03	MAT2
		01-18	MAT1
	Shangzhi	99-47	MAT2
	Yichun	99-30	MAT2
Henan	Anyang	01-03	MAT1
	Lingbao	01-15	MAT1
	Luoning	99-59	MAT1
	Zhoukou	01-35	MAT1
Shandong	Jining	99-40	MAT1
	Laiyang	99-05	MAT1
	Ningjin	99-32	MAT1
		99-56	MAT1
	Rushan	99-09	MAT2
		99-27	MAT1
Liaoning	Xintai	99-39	MAT2
	Zhucheng	99-36	MAT1
	Benxi	01-25	MAT1
	Dashiqiao	01-36	MAT1
	Fushun	99-50	MAT1
	Jianchang	99-23	MAT2
	Jianping	99-62	MAT2
	Kazuo	99-24	MAT2
	Kuandian	99-22	MAT1
		01-02	MAT12
		99-46	MAT1
	Lingyuan	01-30	MAT2
		03-01	MAT2
	Pulandian	01-09	MAT1
	Suizhong	01-14	MAT1
	Xinbin	01-23	MAT2
	Xingcheng	01-33	MAT1
	Youyan	99-49	MAT1
Jilin	Antu	01-17	MAT1
	Baicheng	01-27	MAT12
	Jiaohe	99-57	MAT2
	Qianan	99-52	MAT1
		99-28	MAT2
Shanxi	Changzi	03-19	MAT2
	Yangquan	99-17	MAT1

Note: a) indicates the former two digit of isolate code standing for the collecting year, i.e. "99", "01" and "03" indicate the year of 1999, 2001 and 2003, respectively; and b) means that mating type MAT12 is compatible with both MAT1 and MAT2.

inbred B37 (kindly supplied by Professor Dai from National Plant Resources of China) seedlings with four to six leaves. To enhance the ability of spore adhesion to corn leaves, a few drops of Tween-20 and sucrose (3.0% w/v) were added to the solution just prior to inoculation. The corn seedlings were inoculated by spraying the spore suspension onto their leaves with a hand-held sprayer. Inoculated plants were then covered with polythene sheets to maintain the moisture up to 48 h to facilitate infection before the corn seedlings were placed in a greenhouse. Three replicates were used for each treatment. To avoid cross contamination, inoculation area was treated with 5% carbolic acid before inoculating each isolate and the sprayer was sterilized before next inoculation.

The pathogenicity tests included the examination of disease latency period (the days from inoculation to lesion appearance), disease incidence (%), lesion area (the lesion width × length × leaf area index 0.7), and the conidium production. The disease incidence (%) was determined by the

Table 2 Production of ascocarp of *Setosphaeria turcica*

Isolate	99-11	99-14	99-23	99-24	99-27	99-30	99-36	99-39	99-48	99-50	99-53	99-61
99-11	−	+	−	−	+	−	+	−	+	+	−	−
99-14	+	−	+	+	−	+	−	+	−	−	+	+
99-23	−	+	−	−	+	−	+	−	+	+	−	−
99-24	−	+	−	−	+	−	+	−	+	+	−	−
99-27	+	−	+	+	−	+	−	+	−	−	+	+
99-30	−	+	−	−	+	−	+	−	+	+	−	−
99-36	+	−	+	+	−	+	−	+	−	−	+	+
99-39	−	+	−	−	+	−	+	−	+	+	−	−
99-48	+	−	+	+	−	+	−	+	−	−	+	+
99-50	+	−	+	+	−	+	−	+	−	−	+	+
99-53	−	+	−	−	+	−	+	−	+	+	−	−
99-61	−	+	−	−	+	−	+	−	+	+	−	−

Note: "−" indicates no mature ascocarp production and "+" indicates mature ascocarp production.

formula: (infected leaves / total leaves inoculated) × 100. The conidium production was determined by the following method: the diseased leaves were punched into discs by a puncher ($\Phi = 0.4$ cm), and five discs at the junction of diseased and healthy parts of the leaf were selected and placed onto a glass plate in a Petri dish with a small amount of distilled water on its floor to maintain moist conditions. The Petri dish was then sealed with parafilm and incubated at 25°C for 48 h. The conidia on the leaf discs were washed off by 1 mL distilled water and 5 μL conidium suspension was used to examine microscopically the number of conidia. The amount of conidium production was calculated by the formula

$$N = (N_{5\mu\text{L}} \times (1\,000/5))/(\pi \times 0.2^2 \times 5) = 318.5 \times N_{5\mu\text{L}}$$

where N indicates the number of conidia in 1 cm^2 diseased leaf area; $N_{5\mu\text{L}}$ means the number of conidia in 5 μL of conidium suspension.

The above tests were at least repeated three times, and in each repeat there were at least five records for one treatment. Then the average number of each treatment was subjected to the analysis of variance (ANOVA) test between different isolates.

2.7 RAPD analysis

Random amplification of polymorphic DNA analysis was applied to determine genetic variation among progeny isolates of *S. turcica.* Genomic DNA was extracted from hyphae culture in liquid potato dextrose (PD) medium (1 000 mL PD medium includes 200 g potato extract and 20 g dextrose) by the cetyltriethyl ammonium bromide (CTAB) method (An et al., 2001). DNA relative purity and concentration were determined by electrophoresis with a known concentration marker and fluorometry at the ratio of OD_{260}/OD_{280} prior to RAPD amplification (Sambrook et al., 1989).

Polymerase chain reaction (PCR) amplification was carried out in 25 μL reaction mixtures, containing 10 ng of template DNA, 0.2 mmol 10-base random primer (Sangon, Shanghai, China), 100 mmol of each dNTP, 2 mmol $MgCl_2$, 0.1% Triton X-100, 0.2 unit of Taq DNA polymerase (TaKaRa, Japan). Twenty-six 10-base primers (Sangon, Shanghai, China) were used in this study. The amplification reaction was performed in a Biometra 4 800 (Whatman Corp., Germany) with a program consisting of a predenature at 94°C for 3 min, 40 cycles (at 94°C for 1 min, at 41°C for 2 min, at 72°C for 2 min) and a final extension of 6 min at 72°C. Reaction products were electrophoresed on 1.6% agarose gels at 3 V/cm and stained with ethidium bromide before visualization under ultraviolet light.

The banding patterns produced by RAPD markers were scored manually using a binary system ("1" stands for presence and "0" for absence at each band position). The proportion of common bands was used to analyze the genetic polymorphism within races and calculate similarity index to study genetic variation between races, using the following formula: $F_{ij} = 2B_{ij}/A_{ij}$, where A_{ij} was the number of total bands observed for the ith and jth races, and B_{ij} was the common bands observed between the given pair of races (Nei, 1978).

Phylogenetic relationships between races were examined by cluster analysis and phylogenetic dendrograms construction. The dendrograms were generated by the UPGMA method (Sneath and Sokal, 1973) based on the average similarity index between races using the SAHN program of the NTSYS-pc package (Rohlf, 1992). The confidence limits of clusters in the UPGMA-based phenograms were determined by performing bootstrap of the binary data using the program WinBoot (Yap and Nelson, 1996). Each phenogram was reconstructed 2 000 times by repeated sampling with replacement, and the frequency, with which particular grouping was formed, was considered to reflect the robustness of the group.

3 Results

3.1 Production of ascocarps in *S. turcica*

Twelve isolates were used in mating to induce ascocarp production in *S. turcica* in Sach's medium with barley culm as the stimulator. After four weeks of incubation in darkness, each mating pair was examined under microscope for presence of ascocarps. No isolates produced ascocarps when self-mated, indicating that this pathogen was self-sterile. All mating pairs could produce ascocarps, and accordingly the isolates tested were classified into two groups: mating type (MAT1) including isolates 99-14, 99-27, 99-36, 99-48, and 99-50 and mating type (MAT2) including isolates 99-61, 99-11, 99-23, 99-24, 99-30, 99-39, and 99-53. According to the number of ascocarps producted, isolate 99-14 and isolate 99-61, which produced the most ascocarps, were chosen as the testers for MAT1 and MAT2, respectively.

3.2 Characteristics of ascocarps

The ascocarps and asci were cylindrical with typical double cystic walls. A few asci with eight mature ascospores were found in the experiment, but most of mature asci contained less than eight ascospores. The mature ascospores were colorless with one to six septa. The immature asci had no ascospores, but a group of sticky debris.

3.3 Distribution of mating types

Isolates 99-14 and 99-61 were used as the typical testers of MAT1 and MAT2, respectively, to determine the mating type of 94 isolates collected from northern provinces of China. Generally, three mating types, MAT1 (53 isolates), MAT2 (31 isolates), and MAT12 (10 isolates) with the ratio of 5.3 : 3.1 : 1.0 were identified (Table 1). MAT1 could be found in all the provinces as shown in Fig. 1, MAT2 was not found only in Henan Province, and MAT12 could only be found in Hebei, Liaoning, Heilongjiang, and Jilin Provinces. Although there were not enough isolates for analyses of the distribution of mating types in Henan and Shanxi Provinces, it still could be found that the mating types were unbalanced among provinces, and different mating type isolates could coexist in a same district (Fig. 1).

3.4 Pathogenicity variation in progeny of sexual recombination in *S. turcica*

Five isolates of each mating type “A” and mating type “a” were chosen to test their pathogenicity in the susceptible corn inbred line B37. Although the pathogenicity of each isolate was different regardless of mating types, the disease latency period, disease incidence, lesion area, and sporulation were generally similar between the two mating types (Table 3) and there was no significant difference in pathogenicity between the two mating types ($F_{0.05} = 0.003\,539\,209 < F_{\text{crit}} = 4.098\,168\,915$). There were different levels of pathogenicity among the isolates in either mating type groups.

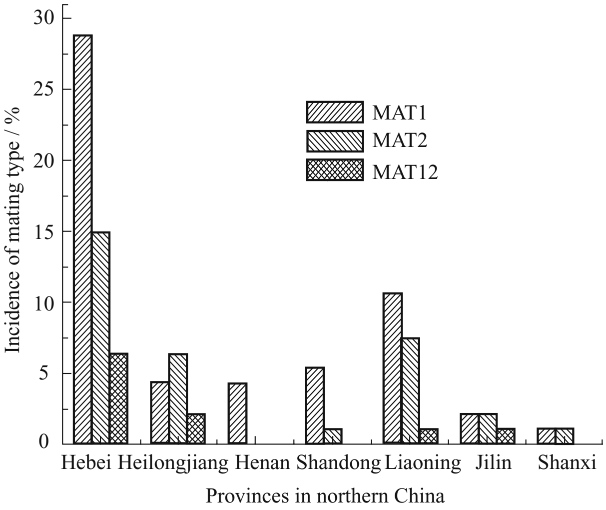


Fig. 1 Distribution of mating types in northern China

Nineteen single-ascospore isolates produced by the mating of isolate 01-12 (MAT2, race 1) and isolate 01-15 (MAT1, race 0) were used for the pathogenicity test on the susceptible inbred B37. Among the progeny cultures, isolates 42, 50, 55, 56, 58, 65, 73, and 83 were non-pathogenic, while isolates 44, 48, 60 and 69 had significantly increased their pathogenicity when compared with their parents (Table 4).

3.5 Genetic variation in progeny of sexual recombination

Twenty-one primers (Sangon Technologies Inc. Shanghai, China) were used to randomly amplify the sequences from 36 progeny isolates of sex recombination between isolate 01-12 (MAT2) and isolate 01-15 (MAT1). Average 6.85 bands per primer were obtained and 126 (87.5%) out of the total 144

Table 3 Pathogenicity tests of mating types of *Setosphaeria turcica* on corn inbred B37

Isolate code	Mating type	Disease latency period[a)] /d	Lesion area[b)] /cm^2	Disease incidence[c)] /%	Sporulation[d)] /Conidia · cm^{-2}
99-48	MAT1	8	1.40	70.5	6 970
99-50	MAT1	9	1.12	50.0	4 260
99-12	MAT1	10	0.49	34.2	580
99-56	MAT1	10	0.77	60.0	2 780
99-41	MAT1	7	0.77	77.5	3 220
99-42	MAT2	9	1.61	66.4	6 000
99-29	MAT2	10	1.26	70.5	2 700
99-24	MAT2	14	0.42	42.5	350
99-57	MAT2	10	1.33	65.0	7 000
99-25	MAT2	11	1.61	42.5	2 500

Note: [a)] means the average days from inoculation to lesion appeared; [b)] means the lesion width (cm) × length (cm) × 0.7 (leaf area index); [c)] means infected leaves / total leaves inoculated × 100; and [d)] means the number of conidia in 1 cm^2 diseased leaf area.

Table 4 Pathogenicity tests of progeny cultures of *Setosphaeria turcica* on corn inbred B37

Isolate code	Mating type	Disease latency period /d	Lesion area /cm^2	Disease incidence /%	Sporulation /Conidia · cm^{-2}	
01-12[a]	MAT2	10	1.02	5.0	4 423	b[d]
01-15[b]	MAT1	12	1.80	7.5	5 308	b
22[c]	MAT2	11	1.35	10.0	3 715	b
42	MAT2	–	0.00	0.0	0	c
44	MAT1	11	2.79	10.0	13 800	a
48	MAT1	15	1.12	5.0	58 334	a
50	MAT2	–	0.00	0.0	0	c
51	MAT1	14	1.85	7.5	5 258	b
54	MAT2	10	0.98	10.0	5 485	b
55	MAT1	–	0.00	0.0	0	c
56	MAT2	–	0.00	0.0	0	c
58	MAT2	–	0.00	0.0	0	c
60	MAT2	14	3.37	7.5	8 492	b
64	MAT1	13	0.29	2.5	1 415	b
65	MAT2	–	0.00	0.0	0	c
68	MAT2	13	1.00	7.5	5 485	b
69	MAT12	9	4.09	10.0	10 262	a
73	MAT2	–	0.00	0.0	0	c
82	MAT1	10	0.59	2.5	1 592	b
83	MAT2	–	0.00	0.0	0	c
84	MAT2	11	0.27	2.5	177	c

Note: [a] 01-12, and [b] 01-15, are parent isolates; [c] others are their progeny isolates; [d] ANOVA test of different isolates.

bands were shown polymorphic and scored as molecular markers (Table 5). Primers S33 and S2024 amplified the most bands (11 bands). Primers S48 and S43612 amplified the least bands (only three bands). The number of progeny isolates, whose band patterns were identical to each parent isolate or different from parent isolates, was also shown in Table 5. In the total 36 × 21 (756) isolate and primer combinations, the banding patterns of 148 (19.58%) combinations were identical to parent isolate 01-12, 104 (13.76%) identical to parent isolate 01-15, and 504 (66.66%) different from both parent isolates.

The genetic similarity between parent isolates was 0.675 2, and the genetic similarity among progeny isolates was from 0.625 0 to 0.791 4. Genetic similarity between

Table 5 Characteristics of RAPD primers and their amplification results

Primer code[a]	Sequence 5′-3′	Average RAPD bands	Polymophic RAPD bands	Number of band patterns of progeny isolates		
				Identical to parent isolate 01-12	Identical to parent isolate 01-15	Different from parent isolates
S2021	ACACTGGCTG	6	5	1	2	33
S2023	TCACGTGGCT	4	3	6	9	21
S2024	ACCAGGTCAC	11	10	3	5	28
S2026	CTGAAGCTGG	5	5	1	2	33
S2027	GAAGGCTGGG	9	9	0	0	36
S2029	AGGCCGGTCA	10	10	0	0	36
S2035	AAGTGCCCTG	7	6	0	2	34
S2037	ACACCGTGCC	9	9	15	1	20
S27	GAAACGGGTG	5	3	8	8	20
S30	GTGATCGCAG	6	4	11	9	16
S33	CAGCACCCAC	11	8	17	12	7
S43612	AGCGTGTCTG	3	3	13	12	11
S43613	CTGAGACGGA	8	8	2	0	34
S43614	GTGCCTAACC	8	8	10	0	26
S43615	GAACCTGCGG	6	5	4	5	27
S43623	ACTGGGACTC	7	6	2	1	33
S43624	AGCGTCCTCC	7	7	3	2	31
S43625	ACGACCGACA	9	8	5	3	28
S46	ACCTGAACGG	4	2	12	12	12
S48	GTGTGCCCCA	3	1	22	14	0
S5	TGCGCCCTTC	6	6	13	5	18
Σ		144	126	148	104	504

Note: [a] Primers were synthesized by Sangon Technologies Inc. Shanghai, China.

parent isolate 01-12 and its progeny isolates was from 0.647 5 to 0.791 4. Among the progeny isolates, isolate 25 was the least similar to parent isolate 01-12, and isolate 16 was the most similar to isolate 01-12. Genetic similarity between parent isolate 01-15 and its progeny isolates was from 0.625 0 to 0.781 6. Among the progeny isolates, isolate 77 was the least similar to parent isolate 01-15, and isolate 87 was the most similar to isolate 01-15.

In order to determine the overall genetic relationship between isolates, a dendrogram (Fig. 2) was constructed by the UPGMA method (Sneath and Sokal, 1973) based on the genetic similarities. Three clusters, A including parent isolates (01-12 and 01-15) and their 12 progeny isolates, B including seven progeny isolates, and C including 17 progeny isolates, were resolved at the 0.69 similarity level, indicating that 24 (66.7%) progeny isolates had a distinctly genetic difference from their parent isolates. The results also suggested that the sex recombination in *S. turcica* could lead a significantly genetic differentiation in the progeny isolates.

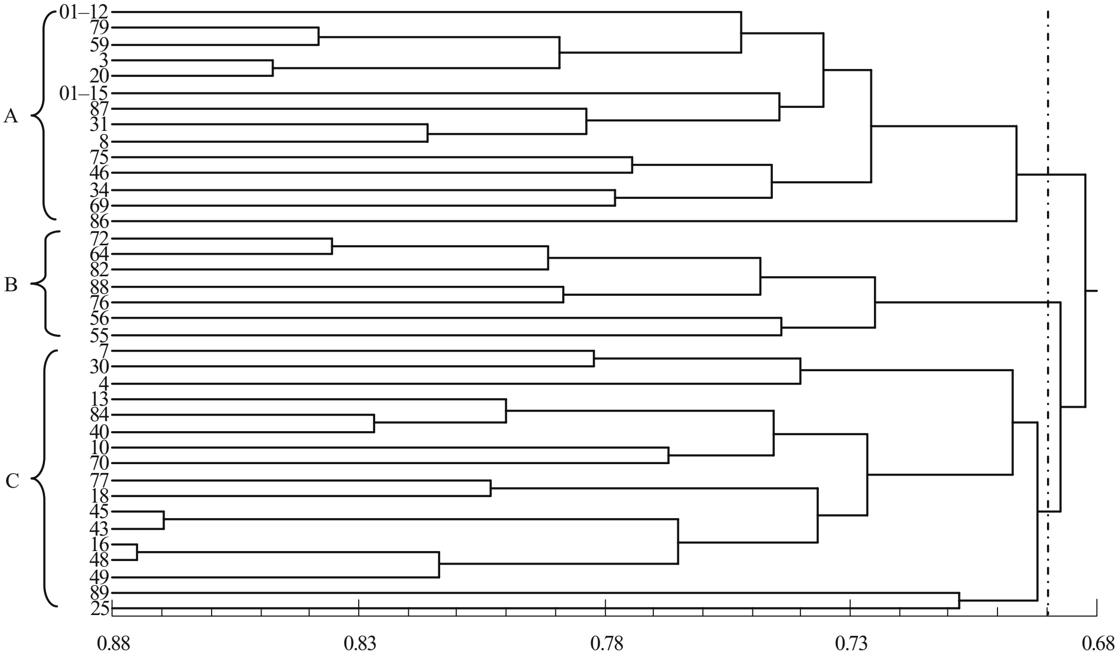
4 Discussion

4.1 *In vitro* induction of perfect stage of *S. turcica*

Although *S. turcica* is known to be heterothallic, its mature perithecia could not be induced when the opposite mating type isolates were co-incubated in a Petri dish without a certain kind of plant tissues as stimulators (Luttrell, 1958). In the absence of stimulators, the isolates could mate but would not be able to produce perithecia with asci in them. One of the possible reasons for the phenomenon was in lack of special nutrients or chemical signals from the plant tissues. The perithecia were mainly produced on the exposed part or embedded part of barley culm just as supposed by Luttrell (1958) and Nelson (1957), but they were also able to be induced in the middle of plate away from the culm in this study. However, no perithecia were found on the surface of medium. Failure to produce eight full ascospores in a ascus was common for *S. turcica*, as revealed in this experiment and previous studies (Luttrell, 1957, 1958; Knox-Davies and Dickson, 1960). It was cytologically explained that some nuclei had degenerated before they developed into ascospores (Knox-Davies and Dickson, 1960). Experimental results supported the fact that successful sexual recombination of *S. turcica* required some strict preconditions (An et al., 2001); for example, contact of two opposite mating types, presence of special nutrients or chemical signals, proper temperature, humidity and darkness during the long period of ascospore development. As a result, ascospores were seldom fully developed, which might explain the fact that the perfect stage of this fungus has never been discovered in field.

4.2 Distribution of *S. turcica* mating types in northern China

Isolates collected from northern China could be classified into three major mating types: MAT1, MAT2 and MAT12. Isolates in MAT12 group could mate MAT1 or MAT2 isolates, but they could not mate each other or themselves. Infertile isolates, or "neutral isolates" which could not mate



Note: Clustering was carried out based on the genetic similarity between the isolates; three clusters, A (including parent isolates (01-12 and 01-15) and their 12 progeny isolates), B (including 7 progeny isolates), and C (including 17 isolates), were resolved at the 0.69 similarity level.

Fig. 2 UPGMA dendrogram of *Setosphaeria turcica* 36 progeny isolates and their parent isolates 01-12 and 01-15 based on RAPD analysis with both MAT1 and MAT2 isolates (Pedersen and Brandenburg, 1986; Abadi et al., 1993), were not discovered in our study. The MAT12 was first discovered in China, and its discovery made it more difficult to understand the genetic control of mating types in *S. turcica*, because the MAT12 might increase the mating frequency of *S. turcica* in field. Although only two isolates of MAT12 were found in 2001 and eight such isolates were found in 2003 because of the relatively low sample size in this study, it could be conceived that the pathogen had been developing its genetic population structure to adapt the environment and host population structure changes.

As it is known, having multiple mating types can greatly improve the chances of meeting a compatible mating partner. In some mushrooms, there are several thousands of mating types developmentally generated by the cell type switch and genetically controlled by mating type genes (Kües and Casselton, 1993). However, there are no reports on the mechanism of the bi-sexual and self-sterile individual production in fungal plant pathogen. Further studies on the MAT12 isolates from the genetic and developmental perspective will definitely help the clarification of the mating mechanism in *S. turcica*.

Mating types of MAT1, MAT2 and MAT12, with the ratio of about 5 : 3 : 1, were uneven distributed in northern China, indicating that there was a low frequency of sexual recombination of *S. turcica* in this region. However, two opposite mating types were also found in some locations of China, for example, in Chengde City, Chicheng County, Laiyuan County and Xinglong County of Hebei Province, Gannan City, Mudanjiang City and Ningan County of Heilongjiang Province, Rushan City of Shandong Province, Kuandian City of Liaoning Province, and Qianan County of Jilin Province.

The coexistence of two opposite mating types did not mean that the sexual recombination happened, because the incidence of the two mating types took a great part in the efficiency of sexual recombination. It was proposed that the closer the incidences of the two opposite mating types, the higher the frequency of the sexual recombination (Nelson 1957; Luttrell, 1958).

Notwithstanding the attempt to sample as broad geographic areas as possible to obtain an estimate of mating type distribution of *S. turcica* population throughout northern China, because of the discontinuous distribution of corn-grown areas and the difficulties of pathogen isolation and fungal conservation for some samples, only a few isolates were collected from some provinces. Therefore, the above results could not completely answer for the mating type distribution in some provinces, such as Shanxi, Shandong, Henan and Jilin Provinces. However, it could still be found that Hebei and Liaoning Provinces were sensitive areas where the sexual recombination of *S. turcica* was prone to take place.

4.3 Virulence variation and genetic differentiation between progeny and parent isolates

Results of pathogenicity tests indicated that the pathogenicity had little correlation with mating types in *S. turcica*, and their progeny isolates could have significantly increased or decreased pathogenicity in comparison to their parents. Our results confirmed the former studies that sexual recombination was a possible way of virulent variation (Hamid and Aragaki, 1975; Welz and Geiger, 1993). RAPD analysis of progeny isolates of *S. turcica* indicated that the virulence variation in progeny isolates was derived from the genetic differentiation between them. The results of pathogenicity tests and RAPD analyses also revealed that the sex recombination of *S. turcica* could lead to significant virulence and genetic variation in their progenies, and it was possible that a progeny isolate with high virulence and great genetic change could come forth and subsequently lead to a big resistance loss in corn. The above results strongly suggested that the sexual recombination should be frequently observed in *S. turcica*.

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