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Full Length Research Paper

Genetic diversity of *Ustilago scitaminea* Syd. in Southern China revealed by combined ISSR and RAPD analysis

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The polymorphism and similarity relationships among 35 mating-type isolates of *Ustilago scitaminea* collected from Southern China were determined with random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analyses. These fungal isolates were collected from 16 sugarcane cultivars including F134 that is resistant to the physiological race 1 but susceptible to the race 2 of *U. scitaminea,* and N: Co376 that is immune to both races 1 and 2. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis revealed that the *U. scitaminea* isolates could be divided into 2 groups with a coefficient of 0.74. The first group comprises two isolates collected from the sugarcane cultivar F134, while the remaining 33 isolates were clustered into the second group. The second group was further divided into two subgroups with most of the isolates from Guangdong Province which clustered in the same subgroup, and all the isolates from Guangxi and Yunnan Provinces were clustered in another subgroup. Given that the member of the second group could infect the cultivar N:Co376, which is immune to the races 1 and 2, our results suggest that majority of *U. scitaminea* in sugarcane-producing regions of Southern China may belong to or genetically similar to race 3.

Key words: *Ustilago scitaminea*, sugarcane, inter-simple sequence repeat (ISSR), random amplified polymorphic deoxyribonucleic acid (DNA) (RAPD), genetic diversity.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important economic crop for sugar and ethanol production. Mainland China is currently the third largest producer of sugarcane in the world, followed by Brazil and India. Southern China, including Guangxi Zhuang autonomous region, Yunnan and Guangdong Province, is the major sugarcane-producing region in mainland China (Chen and Yuan, 2010). Smut disease, caused by the fungus *U. scitaminea*, is an important disease of sugarcane worldwide, leads to considerable yield loss and reduction in cane quality (Ferreira and Comstock, 1989). In China, sugarcane smut was first reported in 1932 (Antoine, 1961) and the disease has caused serious problems in sugarcane plantation and sugar production (Wang, 2007). A range of sugarcane cultivars cultivated in China were originated from Taiwan, where three *U. scitaminea* physiological races were identified based on their varied infectivity against different sugarcane cultivars (Leu et al., 1976; Hsieh and Lee, 1978; Lee et al., 1999). However, the existence of these *U. scitaminea* physiological races in mainland China has not been systemically investigated. Random amplified polymorphic DNA (RAPD) (Willams et al., 1990) and inter-simple sequence repeat (ISSR) (Zietkiemicz et al., 1994) fingerprints are two main kinds of molecular markers for kinship and population studies. The methods have advantages of high-efficiency, sharp sensibility and easy-detection. These techniques have now been widely used for line identification, genetic map construction, genetic diversity

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analysis and other studies in various organisms, including animals, plants, mammalians and microorganisms (Zhang et al., 2008; Subramanyam et al., 2009; Zamani et al., 2011). On the other hand, combinations of RAPD and ISSR markers have been used for genetic diversity studies (Qian et al., 2001; Pradeep et al., 2005; Josiah et al., 2008), which utilizes the advantages of the two molecular marker techniques, reduces potential errors connected with each method and hence improve the reliability of the results (Korbin M et al., 2002; Josiah et al., 2008). By using RAPD, amplified fragment length polymorphisms (AFLP) or simple sequence repeat (SSR) molecular marker techniques, several studies on genetic diversity of *U. scitaminea* have been conducted in China (Xu et al., 2004), Australia (Braithwaite et al., 2004), Southern Africa (Singh et al., 2005) and France (Raboin et al., 2007), respectively. The previous study in China was based on RAPD analysis and used only 18 isolates of *U. scitaminea* from different locations. To increase the representativeness and provide a detailed analysis linking the inherent variability to differentiation of physiological races, this study collected 35 mating-type isolates of *U. scitaminea* from 16 different sugarcane cultivars of varied *U. scitaminea* susceptibility in the main sugarcane-producing regions of Southern China, including Guangdong Province, Guangxi Zhuang autonomous region and Yunnan Province and used a combination of ISSR and RAPD for sample analysis at the molecular level. The results present useful information on the genetic diversity of sugarcane smut pathogen, and the protocol developed in this study may serve as a valuable molecular tool for identification of new physiological race of *U. scitaminea* and for breeding of smut-resistant sugarcane varieties.

MATERIALS AND METHODS

U. scitaminea sample collection

A total of 35 monosporidial mating-type isolates derived from 28 single-whips (sori) of sugarcane smut collected from Southern China were used in this study and listed in Table 1. Monosporidial mating-type isolates of *U. scitaminea* were prepared following the method of Moosawi-Jorf and Mahin (2007).

DNA extraction

Sporidial DNA samples were prepared by using cetyl trimethylammonium bromide (CTAB) method as described by Shen et al. (2006). The purified DNA was quantified by measuring absorbance at 260 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. The DNA samples were then diluted to a concentration of 30 ng/µl for PCR analysis.

ISSR analysis

The PCR reaction mixture contained 1 µl of genomic DNA (about 30 ng), 0.3 µl of rTaq DNA polymerase (5 U/µl), 2.5 µl of 10 × PCR reaction buffer (with Mg^{2+}), 2 µl of 2.5 mM dNTP mixture, 1 µl of 5 µM random primer and 18.2 µl of ddH_2O in a total volume of 25 µl. PCR amplification was performed on a PTC-100™ amplifier (MJ Inc.). The thermal cycling reaction started by denaturation at 94°C for 5 min, followed by 38 cycles of 94°C for 30 s, 50°C for 45 s, 72°C for 2 min, and then a final extension at 72°C for 8 min. The amplification products were electrophoresed on a 1.8% agarose gels buffer with 0.5 × TBE at 100 V for 2 h along with the DL 2000 DNA markers. The separated DNA fragments were stained with ethidium bromide and photographed with a Gene Genius Biomaging System. The experiment was repeated for at least 2 times.

RAPD analysis

The PCR reaction mixture contained 1 µl of genomic DNA (about 30 ng), 0.4 µl of rTaq DNA polymerase (5 U/µl), 2.5 µl of 10 × PCR reaction buffer (with Mg^{2+}), 2 µl of 2.5 mM dNTP mixture, 1 µl of 5 µM random primers, and ddH_2O 18.1 µl in a total volume of 25 µl. PCR amplification was conducted on a PTC-100™ amplifier. The thermal cycling reaction was started by denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 60 s, 38°C for 45 s, 72°C for 1 min, and then a final extension at 72°C for 8 min. The amplification products were analyzed by electrophoresis as described above. The experiment was repeated for at least twice.

Data analysis

Based on the electrophoresis results of amplification products by ISSR-PCR and RAPD-PCR, the samples with DNA band were marked as '1', whereas those without DNA band were marked "0" (only the repeatable bands in DNA electrophoresis analysis were recorded). Jaccard similarity coefficient and UPGMA cluster analyses were conducted by using NTSYS-pc2.10 analytical software (Rohlf, 2000).

RESULTS

ISSR analysis of *U. scataminea* isolates

Based on the preliminary results of amplification, 18 primers (Table 2) were selected for further study out of the 100 arbitrarily designed primers. The selected primers generated clear bands and good polymorphisms in ISSR analysis of *U. scataminea* (35 mating type isolates) (Figure 1). A total of 128 ISSR bands were generated and among them 93 were polymorphic bands. The polymorphic rate was at 72.7% and the sizes of bands were varied from 200 to 3000 bp. The number of ISSR bands generated by each primer was in the range of 4 to 15 with an average number of 7.1. Among the primers used in this study, the primer 884 generated the most number of bands with 12 polymorphic bands, while the primers 827 and 891 produced only 3 polymorphic bands, respectively.

RAPD analysis of *U. scataminea* isolates

Similar to ISSR analysis, we firstly screened 50 random

Table 1. List of *U. scitanminea* isolates used in this study.

Isolate	Geographical origin	Host (sugarcane variety)	Mating type
1	Guangzhou, Guangdong	F134	+
2	Guangzhou, Guangdong	F134	-
3	Guangzhou, Guangdong	YT 97-639	+
4	Guangzhou, Guangdong	YT 97-639	-
5	Guangzhou, Guangdong	ROC22	+
6	Guangzhou, Guangdong	ROC22	-
7	Zhanjiang, Guangdong	ROC10	+
8	Zhanjiang, Guangdong	ROC10	-
9	Guangzhou, Guangdong	CP94-1100	+
10	Guangzhou, Guangdong	CP94-1100	-
11	Zhanjiang, Guangdong	ROC22	+
12	Zhanjiang, Guangdong	ROC22	-
13	Zhanjiang, Guangdong	ROC16	+
14	Zhanjiang, Guangdong	ROC16	-
15	Shaoguan, Guangdong	ROC22	-
16	Guangzhou, Guangdong	N:Co376	-
17	Honghe, Yunnan	YT 00-236	-
18	Honghe, Yunnan	YT 00-236	-
19	Honghe, Yunnan	ROC26	-
20	Honghe, Yunnan	ROC10	-
21	Honghe, Yunnan	ROC20	-
22	Honghe, Yunnan	ROC16	-
23	Honghe, Yunnan	ROC7	-
24	Baise, Guangxi	ROC22	-
25	Chongzuo, Guangxi	ROC22	-
26	Chongzuo, Guangxi	ROC22	-
27	Shaoguan, Guangdong	HoCP95-988	-
28	Shaoguan, Guangdong	FN 28	-
29	Zhanjiang, Guangdong	Yin 0518	-
30	Zhanjiang, Guangdong	ROC22	-
31	Zhanjiang, Guangdong	Taitang 1626	-
32	Zhanjiang, Guangdong	ROC22	-
33	Zhanjiang, Guangdong	ROC22	-
34	Zhanjiang, Guangdong	YT 89-113	-
35	Zhanjiang, Guangdong	ROC22	-

Symbol: +positive mating type, -negative mating type. A total of 28 single-whips of sugarcane smut were collected from June 2009 to June 2011.

decamer primers using the genomic DNA of isolate No. 1 (Table 1) as the template for RAPD analysis. Among them, 18 primers were found to produce reproducible bands and thus selected for further analysis of *U. scataminea* isolates (Table 3). An example of RAPD profiles is shown in Figure 2. RAPD analysis of 35 isolates revealed a total of 110 bands with 79 polymorphic bands (71.8 %) (Table 3), on average, each primer generated about 6.1 DNA bands of which 4.4 were polymorphic. The number of RAPD bands amplified by each primer ranged from 3 to 8, and the size of bands varied from 150 to 3500 bp. Among these primers, OPC08 and AG13 generated most DNA bands with 7 polymorphic bands, respectively; while S471 produced only 1 polymorphic band (Table 3).

Genetic diversity analysis

We first conducted UPGMA dendrogram separately with ISSR and RAPD data. According to ISSR data, the 35 isolates of *U. scitaminea* were divided into 3 main groups (G1, G2 and G3) based on the Jaccard's similarity coefficient of 0.75 (Figure 3A), whereas using RAPD data, these isolates were grouped into two (G1, G2) with a Jaccard's similarity coefficient of 0.69 (Figure 3B).

Table 2. *U. scataminea* polymorphism determined by ISSR analysis.

Primer code	Sequence (5′ to 3′)	Number of amplified band	Number of polymorphic band	Percentage of polymorphic (%)	Size of amplified bands (bp)
104	ATGATGATGATGATGATG	8	6	75.0	400-3000
113	AGAGAGAGAGAGAGAGTC	7	6	85.7	400-2500
880	GGAGAGGAGAGGAGA	8	5	62.5	300-1800
826	ACACACACACACACACC	7	4	57.1	550-2200
827	ACACACACACACACACG	5	3	60.0	300-2200
812	GAGAGAGAGAGAGAGAA	9	5	55.6	300-2000
855	ACACACACACACACACYT	7	6	85.7	500-2500
859	TGTGTGTGTGTGTGTGRC	7	6	85.7	550-1900
815	CTCTCTCTCTCTCTCTG	4	4	100.0	500-2300
811	GAGAGAGAGAGAGAGAC	7	4	57.1	400-2000
835	AGAGAGAGAGAGAGAGYC	7	6	85.7	500-3000
884	HBHAGAGAGAGAGAGAG	15	12	80.0	200-3000
890	VHVGTGTGTGTGTGTGT	7	4	57.1	300-2500
891	HVHTGTGTGTGTGTGTG	7	3	42.9	450-2300
873	GACAGACAGACAGACA	6	4	66.7	500-2500
117	AGAAGAAGAAGAAGAAGAAGA	6	6	100.0	300-1800
857	ACACACACACACACACYG	6	4	66.7	550-3000
876	GATAGATAGACAGACA	5	5	100.0	300-2200
Total		128	93	-	-
Average		7.1	5.2	72.7	-

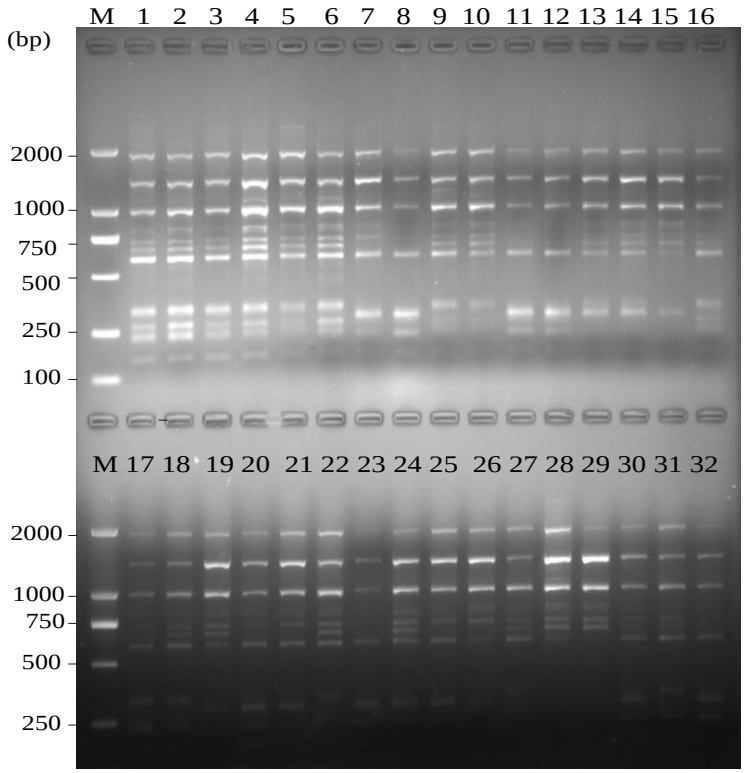

Figure 1. ISSR analysis of *U. scitanminea* isolates with primer 884. Lane M: DL2000 DNA marker, lanes 1-32 were the ISSR-PCR bands amplified with the 32 isolates listed in Table 1, respectively.

Table 3. RAPD-PCR analysis of *U. scataminea* polymorphism.

Primer code	Sequence (5′ to 3′)	Number of amplified band	Number of polymorphic band	Percentage of polymorphic (%)	Size of amplified bands(bp)
UBC203	CACGGCGAGT	7	4	57.14	500-3500
OPC08	TGGACCGGTG	7	7	100.00	700-3500
AA10	TGGTCGGGTG	7	5	71.43	300-2000
OPH19	CTGACCAGCC	4	2	50.00	1300-2200
Z07	CCAGGAGGAC	6	4	66.67	500-2300
OPM13	GGTGGTCAAG	7	5	71.43	300-1800
S471	AACGAGTCGG	3	1	33.33	1200-1700
UBC220	GTCGATGTCG	6	4	66.67	750-1500
UBC230	CGTCGCCCAT	6	4	66.67	550-3500
T5	GGGTTTGGCA	7	6	85.71	300-2200
OPR12	ACAGGTGCGT	8	6	75.00	150-2000
OPM14	AGGGTCGTTC	7	5	71.43	600-3000
AG13	GGCTTGGCGA	8	7	87.50	300-2000
S105	AGTCGTCCCC	4	2	50.00	1500-2500
AB01	CCGTCGGTAG	6	5	83.33	600-2200
K7	AGCGAGCAAG	7	4	57.14	300-3000
S307	GAGCGAGGCT	7	6	85.71	650-2300
S104	GGAAGTCGCC	3	2	66.67	700-2500
Total		110	79	-	-
Average		6.1	4.4	71.8	-

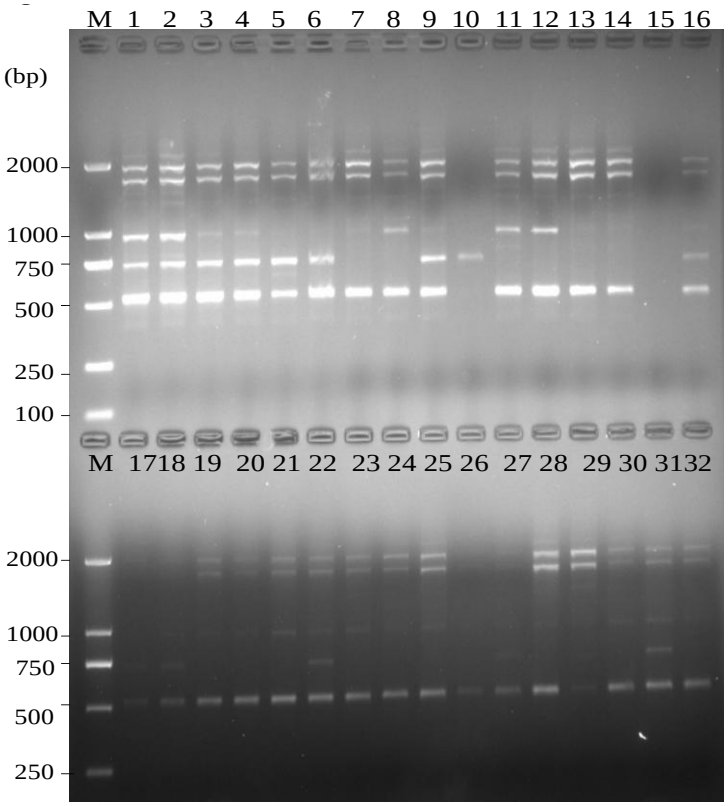

Figure 2. RAPD analysis of *U. scitanminea* isolates with primer Z07. Lane M: DL2000 DNA marker, lanes 1-32 showed the RAPD-PCR bands amplified from 32 *U. scitanminea* isolates, respectively.

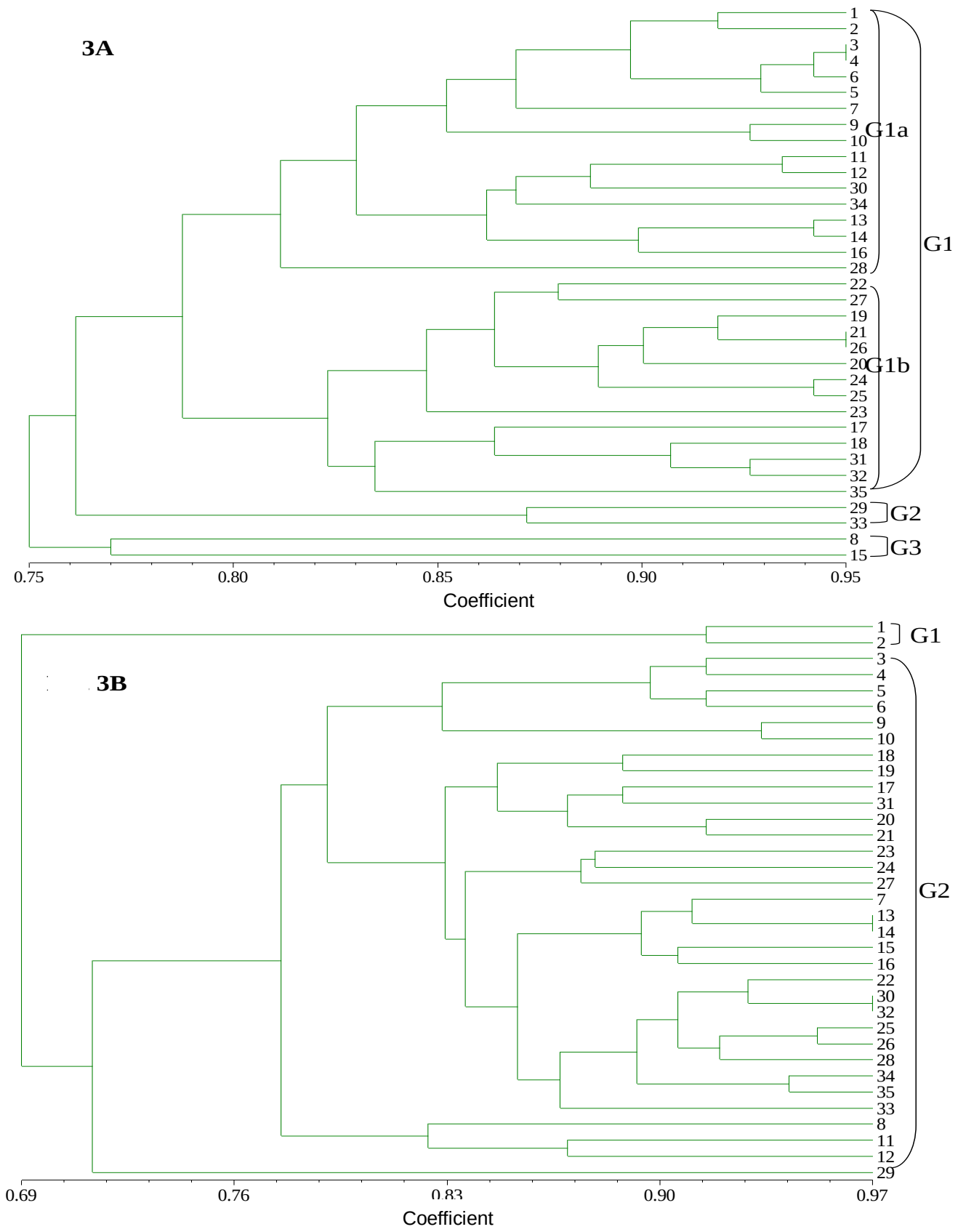

Figure 3. UPGMA dendrogram of cluster analysis of *U. scitanminea* isolates. (**A**) ISSR dada; (**B**) RAPD data; (**C**) RAPD and ISSR combined data.

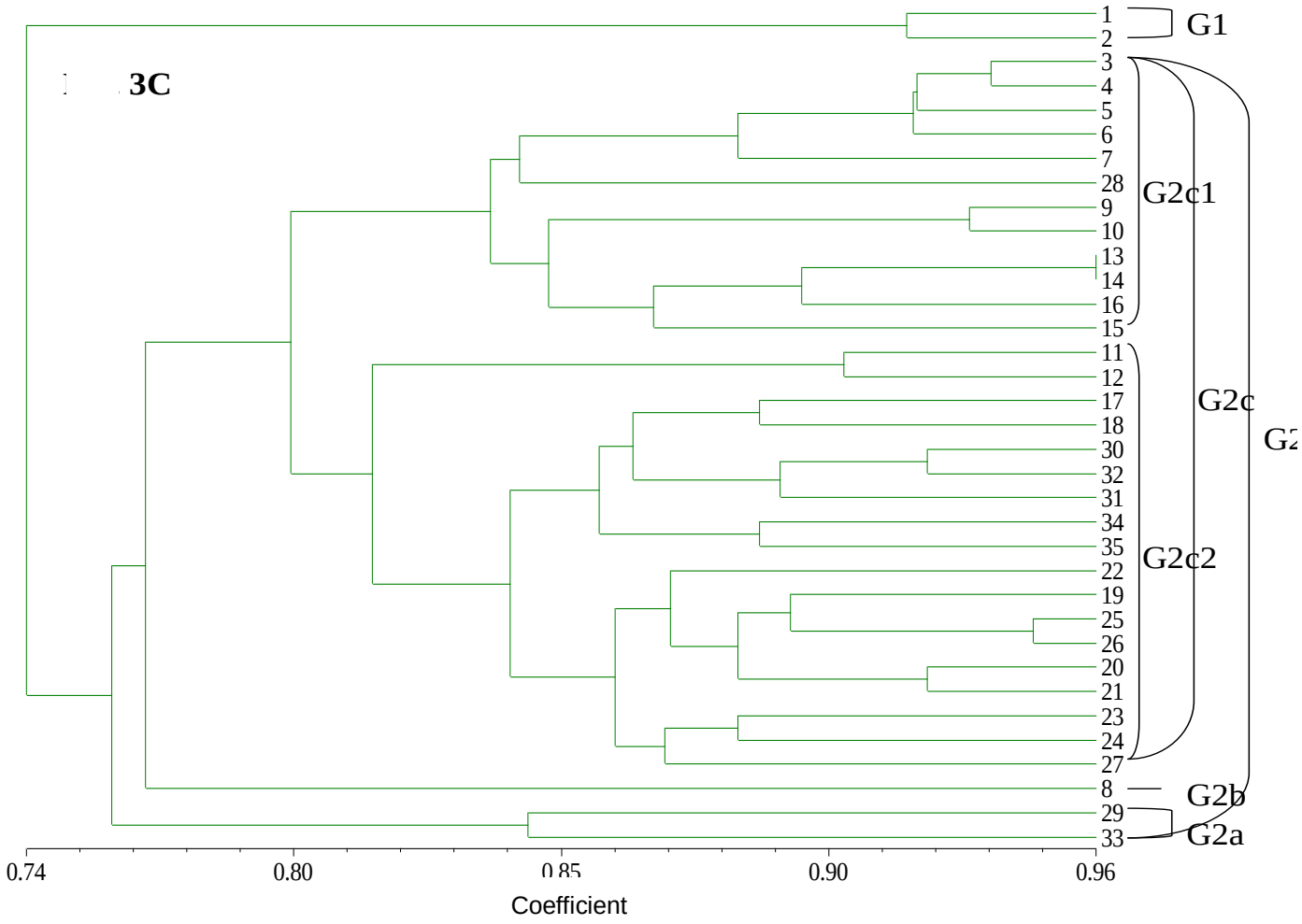

Figure 3. Contd.

Subsequent analysis using the combined data of ISSR and RAPID showed that the Jaccard's genetic similarity coefficients varied from 0.61 to 0.96 (Table 4). The highest genetic similarity (0.96) was found in the isolates No. 13 and 14, which were more or less expected as they were isolated from different plants of the same sugarcane variety (ROC16) at the same geographic location (Table 1), whereas the lowest similarity (0.61) was observed between the sugarcane isolates No. 1 and 29. While the isolate No. 1 was collected from sugarcane variety F134 in Guangzhou city, the isolate No. 29 was from sugarcane variety Yin0518 in Zhanjiang district of Guangdong Province (Table 1).

UPGMA dendrogram analysis showed that the 35 isolates of *U. scitaminea* can be divided into 2 main groups based on the Jaccard's similarity coefficient of 0.74 (Figure 3C). Group 1 (G1) includes the *U. scitanminea* isolates No. 1 and 2, which are respectively the plus and minus mating-type haploid sporidia originnated from the same single-teliospore of the sugarcane variety F134 cultivated in Guangzhou city, Guangdong Province. The group 2 (G2) consisted of the remaining 33 isolates of *U. scataminea*. This is similar to the results based on RAPD data, but the combination of ISSR data with those of RAPD allowed further dividing group G2 into three sub-groups which are related to geographic origins (Figure 3C). The first sub-group (G2a) included two isolates (No. 29 and 33) collected from Zhanjiang district, Guangdong. The second sub-group (G2b) included only one isolate (No. 8), also collected from Zhanjiang, Guangdong. The third sub-group (G2c) contained 30 isolates derived from Guangdong (Zhanjiang, Guangzhou and Shaoguan), Yunnan Province and Guangxi Zhuang autonomous region.

The sub-group G2c can be further divided into two small sub-groups, that is G2c1 and G2c2 (Figure 3C). G2c1 composed of 12 isolates collected from Guangdong, and G2c2 was consisted of 18 isolates, including all the isolates from Yunnan and Guangxi, as well as some isolates of the Zhanjiang and Shaoguan

Table 4. Jaccards similarity coefficient of 35 isolates of *U. scitaminea* based on ISSR and RAPD combined data.

Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.92	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	0.82	0.85	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	0.83	0.85	0.94	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	0.81	0.83	0.92	0.92	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0.81	0.82	0.92	0.92	0.92	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	0.78	0.80	0.89	0.88	0.89	0.88	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	0.68	0.70	0.74	0.75	0.75	0.74	0.81	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	0.75	0.78	0.83	0.83	0.87	0.84	0.81	0.75	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
10	0.77	0.79	0.86	0.85	0.89	0.85	0.84	0.75	0.93	1.00	-	-	-	-		-	-	-	-	-	-	-
11	0.79	0.80	0.81	0.80	0.81	0.77	0.84	0.82	0.79	0.83	1.00	-	-	-	-	-	-	-	-	-	-	-
12	0.78	0.78	0.78	0.76	0.77	0.74	0.81	0.79	0.74	0.77	0.91	1.00	-	-	-	-	-	-	-	-	-	-
13	0.72	0.74	0.81	0.82	0.84	0.82	0.86	0.80	0.83	0.84	0.84	0.83	1.00	-	-	-	-	-	-	-	-	-
14	0.74	0.76	0.84	0.84	0.88	0.85	0.88	0.79	0.85	0.89	0.87	0.83	0.96	1.00	-	-	-	-	-	-		-
15	0.69	0.73	0.80	0.78	0.81	0.77	0.81	0.80	0.83	0.82	0.83	0.80	0.87	0.88	1.00	-	-	-	-	-	-	-
16	0.77	0.80	0.87	0.87	0.87	0.85	0.86	0.78	0.86	0.87	0.86	0.82	0.88	0.92	0.86	1.00	-	-	-	-	-	-
17	0.74	0.74	0.78	0.77	0.78	0.78	0.78	0.76	0.77	0.79	0.82	0.80	0.77	0.78	0.75	0.83	1.00	-	-	-	-	-
18	0.76	0.77	0.82	0.81	0.82	0.80	0.82	0.78	0.79	0.81	0.85	0.81	0.78	0.82	0.76	0.86	0.89	1.00	-	-	-	-
19	0.70	0.72	0.77	0.76	0.79	0.76	0.82	0.78	0.79	0.82	0.86	0.80	0.82	0.86	0.82	0.82	0.83	0.86	1.00	-	-	-
20	0.71	0.72	0.74	0.74	0.73	0.74	0.79	0.77	0.74	0.76	0.82	0.80	0.79	0.78	0.76	0.77	0.86	0.81	0.88	1.00	-	-
21	0.68	0.69	0.74	0.72	0.74	0.73	0.76	0.75	0.74	0.76	0.81	0.81	0.81	0.81	0.78	0.77	0.84	0.82	0.89	0.92	1.00	-
22	0.70	0.73	0.77	0.76	0.78	0.76	0.77	0.74	0.74	0.79	0.81	0.78	0.81	0.82	0.76	0.79	0.83	0.82	0.89	0.86	0.87	1.00

districts of Guangdong Province. The results of cluster analysis also showed that the plus and minus mating-type isolates from the same single-teliospore of *U. scataminea* had an extremely high genetic similarity coefficient (Figure 3). For example, the genetic similarity coefficient was over 0.96 between plus and minus mating-type isolates (No. 13 and 14) from the same single-teliospore of *U. scataminea* collected from the sugarcane variety ROC16 in Zhanjiang, Guangdong (Figure 3). The findings are highly rational as the plus and minus mating-type isolates from the same single-teliospore should be closely related in genetics, which provide a strong validation for the genetic diversity analysis conducted in this study.

DISCUSSION

Singh et al. (2005) showed that genetic diversity among geographically separated strains of *U. scitaminea* was limited, with no difference in the major banding pattern following RAPD amplify-cation using a set of primers. However, significant molecular diversity was observed among 18 isolates of *U. scitaminea* collected from six different provinces of mainland China by RAPD (Xu et al., 2004). In this study, ISSR and RAPD were conducted to analyze 35 isolates of *U. scataminea* collected from 16 sugarcane cultivars with varied levels of smut disease susceptibility. Among these cultivars, F134 is resistant to the physiological race 1 but susceptible to race 2 of *U. scataminea*, and N:Co376 is immune to both races. RAPD-based UPGMA dendrogram could establish a significant correlation between molecular

Table 4. Contd.

Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
23	0.65	0.67	0.72	0.72	0.75	0.73	0.78	0.78	0.73	0.76	0.79	0.76	0.77	0.80	0.77	0.78	0.81	0.81
24	0.69	0.71	0.77	0.77	0.79	0.77	0.81	0.77	0.76	0.79	0.81	0.79	0.83	0.84	0.81	0.80	0.81	0.82
25	0.73	0.73	0.81	0.79	0.81	0.79	0.85	0.77	0.79	0.82	0.84	0.81	0.86	0.86	0.83	0.82	0.79	0.81
26	0.71	0.71	0.77	0.76	0.78	0.77	0.82	0.77	0.79	0.81	0.82	0.80	0.87	0.87	0.84	0.82	0.81	0.79
27	0.70	0.72	0.77	0.76	0.79	0.78	0.78	0.77	0.79	0.83	0.82	0.78	0.82	0.83	0.79	0.82	0.86	0.83
28	0.77	0.78	0.85	0.84	0.86	0.82	0.85	0.72	0.82	0.84	0.81	0.78	0.82	0.84	0.80	0.81	0.80	0.80
29	0.61	0.65	0.70	0.69	0.73	0.71	0.72	0.66	0.69	0.74	0.75	0.71	0.77	0.80	0.75	0.74	0.71	0.72
30	0.74	0.76	0.82	0.80	0.82	0.79	0.84	0.78	0.75	0.78	0.87	0.86	0.84	0.85	0.81	0.84	0.84	0.87
31	0.76	0.78	0.82	0.82	0.82	0.80	0.81	0.75	0.76	0.79	0.82	0.80	0.80	0.81	0.75	0.81	0.87	0.89
32	0.72	0.74	0.81	0.79	0.82	0.79	0.83	0.77	0.77	0.80	0.84	0.84	0.84	0.85	0.81	0.82	0.84	0.88
33	0.67	0.70	0.72	0.74	0.74	0.75	0.77	0.68	0.71	0.75	0.77	0.76	0.84	0.82	0.78	0.78	0.74	0.73
34	0.78	0.79	0.84	0.83	0.86	0.82	0.84	0.76	0.79	0.83	0.84	0.82	0.84	0.87	0.83	0.85	0.85	0.87
35	0.71	0.73	0.77	0.76	0.79	0.77	0.80	0.76	0.76	0.79	0.80	0.78	0.81	0.82	0.79	0.79	0.84	0.84

Isolate	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
23	0.86	0.85	0.84	0.83	1.00	-	-	-	-	-	-	-	-	-	-	-	-
24	0.87	0.86	0.86	0.86	0.89	1.00	-	-	-	-	-	-	-	-	-	-	-
25	0.89	0.88	0.88	0.87	0.86	0.91	1.00	-	-	-	-	-	-	-	-	-	-
26	0.90	0.88	0.91	0.87	0.84	0.88	0.95	1.00	-	-	-	-	-	-	-	-	-
27	0.87	0.86	0.85	0.87	0.86	0.88	0.88	0.90	1.00	-	-	-	-	-	-	-	-
28	0.84	0.81	0.82	0.84	0.75	0.80	0.87	0.86	0.81	1.00	-	-	-	-	-	-	-
29	0.79	0.73	0.76	0.80	0.74	0.79	0.77	0.77	0.77	0.77	1.00	-	-	-	-	-	-
30	0.87	0.86	0.85	0.86	0.85	0.89	0.89	0.84	0.84	0.83	0.77	1.00	-	-	-	-	-
31	0.84	0.84	0.86	0.87	0.83	0.85	0.85	0.82	0.86	0.86	0.77	0.89	1.00	-	-	-	-
32	0.88	0.85	0.87	0.87	0.85	0.89	0.91	0.87	0.86	0.86	0.76	0.92	0.90	1.00	-	-	-
33	0.79	0.82	0.82	0.84	0.74	0.80	0.82	0.84	0.78	0.81	0.84	0.81	0.78	0.81	1.00	-	-
34	0.84	0.81	0.82	0.82	0.78	0.82	0.84	0.84	0.83	0.84	0.73	0.88	0.85	0.89	0.79	1.00	-
35	0.85	0.85	0.84	0.85	0.81	0.84	0.85	0.84	0.82	0.82	0.75	0.85	0.84	0.89	0.82	0.89	1.00

genetic diversity and the physiological races of *U. scitaminea*, as isolates No. 1 and 2 from the sugarcane variety F134 were significantly distinct from the rest isolates at the molecular level (Figure 3B), whereas ISSR-based UPGMA dendrogram showed a link between the molecular genetic diversity and the geographic origins to some degree (Figure 3A). By combination of RAPD and ISSR data, the UPGMA dendrogram clearly reflect these two correlations (Figure 3C). Our results show that molecular diversity levels among 35 isolates of *U. scitaminea* collected from three main sugarcane-producing regions of Southern China were moderately diversified with the polymorphic rate at 71.8% in RAPD and 72.7% in ISSR, higher than those observed by Singh et al. (2005), but lower than those by Xu et al. (2004). These differences in polymorphic rate may be due to the fact that Xu et al. (2004) used fungal isolates from 6 provinces whereas the isolates used in our study were collected from

three provinces. In addition, Xu et al. (2004) used mixed DNA from the dikaryon and two mating-type haploids of *U. scitaminea* as template DNA for RAPD amplification, while in our study DNA samples were from a single mating-type haploid.

Physiological races of *U. scitaminea* are defined by pathogecity on a set of differential hosts, including N:Co310 (susceptible to race 1, resistant to race 2), F134 (susceptible to race 2, resistant to race 1), F173 (susceptible to both races 1 and 2), and N:Co376 (immune to races 1 and 2).

In Taiwan, three physiological races 1, 2, and 3 were found (Leu et al., 1976; Hsieh and Lee, 1978; Lee et al., 1999). For a long time, it was believed that there are only two physiological races (1 and 2) in mainland China (Xu and Chen, 2000). *U. scitaminea* isolates No. 1 and 2 clusters together and are likely to belong to race 2, as they infect host F134, while the remaining 33 isolates were clustered into the G2 group in which isolate No. 16 infects host N:Co376. Since N:Co376 is immune to races 1 and 2, isolate No.16 may represent a new physiological race. It is not clear at this stage that whether or not all isolates of the G2 group behalf the same as isolate No. 16 in term of pathogenecity. However, similarity in patterns of ISSR and RAPD (Figure 3) suggest that they may do. Considering the high frequency of sugarcane importation from Taiwan (Tan and He, 2004), it is possible that this new race may likely be the race 3 of *U. scitaminea* reported in Taiwan and this race was carried along with the sugarcane germplasm from Taiwan to the mainland of China. This new race also seems to be dominant in the sugarcane-producing regions of Southern China (Figure 3). In this regard, Shen and Deng (2011) reported that a new physiological race of *U. scitaminea* was likely to present in Zhanjiang, Guangdong based on the results of artificial inoculation with mixed teliospores of *U. scitaminea* collected from Zhanjiang Guangdong.

Conclusion

In conclusion, the polymorphism analysis conducted in this study strongly suggests the emergence of a new *U. scitaminea* physiological race, that is, race 3, represented by the isolate No. 16, in Southern China. However, it is not clear at this stage whether race 3 is a dominant race in this region. For this, it is essential to test the virulence of other isolates of Group 2 on sugarcane variety N:Co376. The findings from this study may have significant implications not only for developing sensible sugarcane breeding strategies for control smut disease in Southern China and nearby regions, but also for the study of *U. scitaminea* ecology and epidemiology.

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