CHARACTERISING THE PATHOGENICITY DIVERSITY OF *USTILAGINOIDEA VIRENS* IN HYBRID RICE IN CHINA

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SUMMARY

Fifty nine isolates of *Ustilaginoidea virens*, the cause of false smut of rice (*Oryza sativa* L.), were obtained from 46 rice hybrids in 14 counties in Sichuan, China during a survey conducted in 2006. Their pathogenicity was tested by inoculation on 3 rice hybrids, Gangyou182, Gangyou94-11 and Yixiangyou2292, 6-9 days before heading in 2007. False smut was assessed 3 weeks after inoculation using a disease index (*DI*) based on symptom frequency on the panicles. The sporulation capacity of these isolates was also measured. The results showed that: (i) *DIs* were significantly different (P<0.01) both among pathogen isolates and rice hybrids, ranging from 0 to 98.52; (ii) there was a significant interaction between isolates and hybrids (P<0.01); (iii) significant differences in sporulation among the 59 isolates were found (P<0.01), but no relationship between sporulation and virulence on the 3 hybrids; (iv) variation in sporulation was observed among isolates originating from different counties and from the same county (P<0.05); (v) there were significant differences (P<0.01) between isolate groups from different host origin, female parents and male parents. Our results indicated a linkage between the pathogenicity of *U. virens* isolates and the resistance of rice hybrids. The 59 isolates could be classified into 6 groups based on their virulence to the tested rice hybrids. While variation in sporulation did not indicate host genotype-pathogen isolate interaction, pathogenicity data suggest specialisation, which is dependent on the site of origin of the isolates, the original host (rice hybrids) and the parentage of the original host.

Key words: *Ustilaginoidea virens*, rice false smut, rice hybrids, plant-pathogen interaction, disease assessment.

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INTRODUCTION

False smut (green smut) of rice caused by the fungal pathogen, *Ustilaginoidea virens* (Cke.) Tak., was first reported from Tinnevelly in Tamil Nadu State of India by Cooke in 1878 (Ou, 1972). Since then, it has been reported from most rice-growing countries worldwide (Rush *et al.*, 2000; Abbas *et al.*, 2002; Biswas, 2001). Rice false smut has long been considered a minor disease (Ou, 1972, 1985; Dodan *et al.*, 1996). However, in recent years, it has become a serious problem, possibly due to high input cultivation for high-yield, increased use of hybrid varieties, and climate change. In 1988, *U. virens* severely affected rice production in northern Japan (Yaegashi *et al.*, 1989). In China, false smut has become one of the most important fungal diseases in rice since the 1980s. False smut affected approximately 200,000 to 330,000 ha in Liaoning from 1984 to 1996. In 2005, in Hubei, it was estimated that about 633,000 ha and 1.37 billion tons yield were lost due to this disease (Ji *et al.*, 1995; Wang *et al.*, 2005). In Sichuan, disease incidence has reached 75% in some areas, with about 330,000 ha and 20-40% of the panicles affected in 2005 (He, 2006). Yield loss estimates ranged from 0.2 to 49% in different regions with different rice varieties (Biswas, 2002; Singh *et al.*, 1992; Baruah *et al.*, 1992).

The morphology of chlamydospores, ascospores, conidia, hyphae, fungal colonies and the cultural conditions of *U. virens* have been well documented (Ou, 1985; Wang *et al.*, 1993; Zhou *et al.*, 1999; Chen *et al.*, 2007). However, studies on natural resistance, or breeding for resistant rice varieties are few (Miao *et al.*, 1994; Bhagat, 1993). We also lack information on the variation in pathogenicity among isolates from different regions, and different cultivar-origin combinations. In this report, pathogenicity differences among isolates from different rice areas in Sichuan and in different rice hybrids were examined using the injection method of inoculation (Zhang *et al.*, 2004) at the booting stage. Conidium-producing ability of the isolates of *U. virens* and the relationship with pathogenicity of isolates was also analyzed.

MATERIALS AND METHODS

Sample collection. Samples of smut balls were collected from 46 infected rice hybrid cultivars in 14 different areas of Sichuan province in 2006 (Table 1).

Isolation and purification. The XBZ agar medium (Zhou *et al.*, 1999), i.e potato 300 g, peptone 5 g, sugar 15 g, $Ca(NO_3)_2 \cdot 4H_2O$ 0.5 g, $Na_2HPO_4 \cdot 12H_2O$ 2 g, agar 16 g, and distilled water 1000 ml, was used for isolation and culture of *U. virens*. The PS liquid medium (Fang, 1996) (potato 200 g, sugar 20 g, and distilled water 1000 ml) was used for purification and conidial production of *U. virens* and also for the inoculum culture.

Following the method developed by Fang (1996), smut balls were sterilized with 75% ethanol for 2 to 3 min followed by 0.1% mercuric chloride for 1 to 3 min and then washed three times with sterile distilled water. The innermost layer of these sterilized smut balls was cut into pieces (2-3 mm) and 5 pieces were transferred to each of the XBZ medium plates, and incubated at 28°C until the appearance of mycelium and conidial production. Colonies with characteristics of *U. virens* were then examined under the light microscope, and were transferred to XBZ agar slants in a test tube.

To obtain single conidial cultures, fungal isolates were transferred individually to flasks containing PS medium and incubated in a rotary incubator (HZQ, F160, China) at 130 rpm at 28°C for 7 days. The contents of the flasks were then filtered through two layers of sterile gauze and the conidia suspension was streaked onto XBZ agar plates. Five days later, single colonies were transferred to XBZ plates. After incubation for five days, each individual isolate was transferred to XBZ plates for incubation at 28°C. One month later, conidial isolates were kept on XBZ agar slants at 4°C. These steps produced 59 single-spore cultures of *U. virens* (Table 1).

Pathogenicity analysis. Three rice hybrids, Gangyou182, Gang you94-11 and Yixiang2292, were used to test the pathogenicity of the isolates by using a split-plot design with three levels (hybrids) as main units. Each hybrid was sown on April 10 to raise the seedlings, which were transplanted on May 18 into a split-plot design field experiment with three replications. Each replication consisted of 118 rows (2 rows per isolate), with 10 plants per row, plant spacing 16 cm, row spacing 23 cm. The normal rice production practice used in this province by the farmers was followed for irrigation management and fertilizer application. No fungicide was applied, while insecticides and herbicides were applied as needed.

Inocula of the single-spore isolates were prepared by transfer from the stock culture slopes to XBZ plates incubated at 28°C for 10 days. For each isolate, five agar discs 7 mm in diameter were transferred to 500 ml flasks containing 150 ml PS medium, and the flasks were incubated as described above. After 7 days, the mycelium was broken up with a high-speed blender (DS-1, China) and the suspension of hyphal fragments and conidia was used for inoculation.

For field inoculation**,** twenty young rice spikelets still in the leaf sheath at booting stage were selected in the middle row of each rice hybrid. Following the split-plot experimental design with 60 levels for isolates (including 59 isolates and blank check) as sub-units, each isolate was replicated three times. Two ml of inoculum suspension for each isolate were injected in each panicle between 4 and 6 p.m. Each hybrid was inoculated with each fungal isolate, with 20 panicles for each row of each replication. Inoculated plants were tagged with a plastic band and the two rows surrounding the inoculated plants were left as non-inoculated controls.

Three weeks after inoculation, panicles were examined for false smut development and the infection was assessed. The disease assessment scale was as follows (Tang *et al.*, 2000): class 0, no smut ball; class 1, one smut ball or grain infected on inoculated panicle; class 2, two smut balls or infected grains on inoculated panicle; class 3, three to five smut balls or infected grains on inoculated panicle; class 4, six to nine smut balls or infected grains on inoculated panicle; class 5, more than 10 smut balls or infected grains on innoculated panicle.

The following formula was used to calculate the *DI* (disease index) used to differentiate the pathogenicity of *U. virens* isolates and the resistance of rice hybrids.

$$DI = \frac{\sum (N_i \times V_i)}{\sum N_i \times V_{max}} \times 100$$

where N_i is the number of panicles in the different classes, V_i = class value, V_{max} = max class. Value.

Conidial production of the isolates. Agar discs containing the mycelium grown on XBZ agar plates were prepared as described in the previous section. Five discs of each isolate were transferred to 100 ml PS medium for conidial production. The flasks were incubated in a rotary incubator for 7 days at 28°C. The number of conidia in the suspension was counted using a haemocytometer under the microscope.

Data analysis. Variation in *DI* values were analyzed using the DPS system, version 7.05 (Tang *et al.*, 2007), after arcsine conversion. The two-factor ANOVA on a split-plot design, with three levels (hybrids) as main units, and 60 levels for isolates as sub-units, was performed. A cluster analysis of isolates (with check CK) was carried out, and a dendrogram was developed, using an unweighted pair group method with arithmetic mean algorithm (UPGMA).

One-way ANOVAs (among 14 original counties) were also performed, using average *DIs* of the isolates from the same county (taking *DIs* of three rice hybrids

Table 1. Isolates of *U. virens* used in the present study, rice hybrids from which the isolates were collected and the areas where the cultivars were grown in Sichuan Province.

Isolates	Rice hybrids (female×male)	Cultivation area
UV1	IIyou363(II-32A×Shuhui363)	Pujiang
UV2	Xieyou57(Xieqingzao A×2DZ057)	Pujiang
UV3	Zhongyou177(Zhong9×Chenghui 177)	Pujiang
UV4	IIyou95-18(II-32A×Neihui95-18)	Pujiang
UV5	Luyou502(LujiuA×Mianhui502)	Pujiang
UV6	Jinyou18(Jin23A×R18)	Pujiang
UV7	Liuyou105(613A×Mingdian105)	Pujiang
UV8	Yixiang725(Yixiang1A×Mianhui725)	Pujiang
UV9	Nuoyou2(N2A×D091)	Pujiang
UV10	Fuyou802(Fu74A×Chuanhui802)	Pujiang
UV11	Gangyou827(G2480A×Shuhui527)	Pujiang
UV12	Kyou3(K19A×Minghui63)	Pujiang
UV13	Zhong936(Zhong9A×Dahui936)	Pujiang
UV14	Chuanxiang9(Chuanxiang29A×Chenghui425)	Pujiang
UV15	Mian2you151(Mian2A×Jianghui151)	Pujiang
UV16	Kyou527(K17A×Shuhui527)	Pujiang
UV17	IIyou501(II-32-8A×Mianhui501)	Pujiang
UV18	Gangyou26(Gang46A×6326)	Pujiang
UV19	Gangyou22(Gang46A×CDR22)	Ya'an
UV20	Jinyou182(Jin23A×Neihui182)	Ya'an
UV21	Gangyou151(Gang46A×Jianghui151)	Ya'an
UV22	IIyou95-18(II-32A×Neihui95-18)	Ya'an
UV23	Gangyou94-4(Gang46A×Neihui94-4)	Ya'an
UV24	IIyou746(II-32A×Kehui746)	Ya'an
UV25	Chuanxiang9(Chuanxiang29A×Chenghui425)	Ya'an
UV26	Taixiang5(Chuanxiang29A×Luxianghui425)	Ya'an
UV27	Shuofeng2(II-32A×THR-2-1)	Jintang
UV28	Chuanxiang5(Chuanxiang29A×Chenghui761)	Jintang
UV29	Chuanxiang9(Chuanxiang29A×Chenghui425)	Jintang
UV30	Yixiang2292(Yixiang1A×Yihui2292)	Shuangliu
UV31	Fuyou1(II-32A×R21)	Shuangliu
UV32	Jinyou18(Jin23A×R18)	Shuangliu
UV33	Gangyou527(Gang46A×Shuhui527)	Chongzhou
UV34	Fuyou838(Fu74A×Fuhui838)	Chongzhou
UV35	Fuyou63(Fu74A×63-1)	Chongzhou
UV36	Gangyou94-4(Gang46A×94-4)	Dazhou
UV37	Gangyou725(Gang46A×Mianhui725)	Dazhou
UV38	Gangyou363(Gang46A×Shuhui363)	Dazhou
UV39	Gangyou527(Gang46A×Shuhui527)	Nanchong
UV40	Gangyou151(Gang46A×Jianghui151)	Nanchong
UV41	Jinyou188(Jin23A×Lehui188)	Nanchong
UV42	Chuanxiangyou6(Chuanxiang29A×Chenghui425)	Luzhou
UV43	Wangyou8(Yixiang1A×wanhui481)	Luzhou
UV44	Jinyou527(Jin23A×Shuhui527)	Luzhou
UV45	Gangyou336(Gang46A×Yuhui336)	Yibin
UV46	Dyou63(DshanA×Minghui63)	Yibin
UV47	IIyou7(II-32A×Luhui17)	Yibin
UV48	Gangyou151(Gang46A×Jianghui151)	Jianyang
UV49	Yixiang1577(Yixiang1A×Yihui1577)	Jianyang
UV50	Shanyou63(Zhenshan97A×Minghui63)	Mianyang
UV51	Chuanxiangyou2(Chuanxiang29A×Chenghui177)	Mianyang
UV52	Gangyou151(Gang46A×Jianghui151)	Mianyang
UV53	Tianxiang017(400A×Chenghui177)	Guanghan
UV54	Tianyou116(TianfengA×Guanghui116)	Guanghan
UV55	Tianyou2(Tianlong1A×Tianlonghui140)	Guanghan
UV56	Gangyou725(Gangyou46A×Mianhui725)	Shehong
UV57	Gangyou19(Gang46A×Chenghui19)	Hanyuan
UV58	Jinyou527(Jin23A×Shuhui527)	Hanyuan
UV59	Gangyou527(Gang46A×Shuhui527)	Hanyuan

as replications), and average *DIs* on three rice hybrids (taking *DIs* of different isolates as replications), respectively.

One-way ANOVAs (among 8 original hosts, 21 female parents or 38 male parents of original hosts) were performed respectively, using average *DIs* of the isolates from the same hosts (female parents, male parents), taking *DIs* of three rice hybrids as replications.

A one-way ANOVA of conidial numbers produced by isolates on PS medium, and correlation analyses between conidial numbers and *DIs* of three rice hybrids were also carried out.

RESULTS

Pathogenicity analyses of *U. virens* isolates. The averaged *DIs* isolates-hybrids, varied from 0 to 98.52 (Table 2). The responses of the three rice hybrids to the 59 isolates showed that there were very large and significant, differences among the isolates. Isolate UV 56 was most viruent to all rice hybrids tested.

The pathogenicity of UV 25 on Gangyou182 was significantly different from other isolates except UV24 while its pathogenicity to Gangyou94-11 significantly differed from the other isolates except for UV28, UV29

Table 2. Disease index (*DI*) of the three rice hybrids and their responses to inoculation with different isolates of *U. virens* collected from different rice hybrids grown in Sichuan province.

Isolates	*DI*			No.of conidia (10^4)	Isolates	*DI*			No.of conidia (10^4)
	Gangyou 182	Gangyou 94-11	Yixiang 2292			Gangyou 182	Gangyou 94-11	Yixiang 2292	
UV1	1.48ghi	0.74j	36.30ab	1.0c	UV31	1.85ghi	0.00j	1.48de	33.7abc
UV2	0.00i	0.00j	0.00e	14.3bc	UV32	0.37hi	0.00j	0.00e	2.3c
UV3	0.00i	0.00j	0.00e	14.7bc	UV33	0.00i	0.37j	18.89bcd	73.0abc
UV4	0.00i	0.00j	0.00e	4.3c	UV34	0.00i	0.37j	0.00e	26.7abc
UV5	21.11efgh	21.48efg	1.48de	135.0ab	UV35	0.00i	1.11j	14.44bcde	70.0abc
UV6	0.74ghi	0.00j	0.00e	10.7bc	UV36	27.41def	51.48bcd	35.19ab	7.3bc
UV7	5.19fghi	0.74j	8.89bcde	81.0abc	UV37	4.07ghi	0.74j	14.44bcde	63.7abc
UV8	0.37hi	0.00j	0.00e	39.7abc	UV38	0.37hi	0.00j	0.37e	7.3bc
UV9	7.41fghi	5.19hij	1.11de	50.0abc	UV39	0.00i	0.00j	0.00e	6.3c
UV10	0.00i	0.37j	0.00e	19.7abc	UV40	0.00i	0.00j	0.00e	3.7c
UV11	0.37hi	0.00j	5.56cde	120.7abc	UV41	0.00i	0.00j	5.93cde	80.0abc
UV12	0.00i	0.00j	0.00e	6.0c	UV42	5.93fghi	41.85cde	3.33cde	57.3abc
UV13	0.00i	0.00j	0.00e	5.7c	UV43	1.11ghi	0.00j	0.00e	5.7c
UV14	0.37hi	0.37j	0.00e	4.0c	UV44	0.00i	0.00j	0.00e	7.0bc
UV15	10.37fghi	11.85ghi	2.59de	54.7abc	UV45	0.37hi	0.00j	10.37bcde	43.0abc
UV16	0.00i	0.00j	60.37a	4.7c	UV46	38.15cde	19.63fgh	0.00e	23.0abc
UV17	51.85cd	44.81bcd	16.30bcde	142.7a	UV47	7.41fghi	23.33efg	0.00e	58.0abc
UV18	40.37cde	21.11fgh	15.93bcde	11.7bc	UV48	16.30efgh	8.15hij	0.00e	72.0abc
UV19	0.37hi	0.00j	1.48de	30.7abc	UV49	0.00i	0.00j	0.00e	20.3abc
UV20	0.00i	0.00j	0.00e	41.0abc	UV50	0.00i	0.00j	0.37e	6.7bc
UV21	0.00i	0.00j	38.15ab	9.3bc	UV51	0.00i	0.00j	1.11de	7.3bc
UV22	0.00i	0.00j	1.85de	3.3c	UV52	0.00i	0.37j	0.00e	4.7c
UV23	0.37hi	0.00j	0.00e	2.7c	UV53	0.00i	0.00j	0.00e	5.0c
UV24	62.59bc	52.96bcd	11.11bcde	62.7abc	UV54	20.00efg	1.85ij	7.78bcde	143.3a
UV25	76.67b	65.19b	0.00e	8.0bc	UV55	41.85cd	35.56def	12.96bcde	16.7a
UV26	10.74fghi	4.81ij	0.00e	3.7c	UV56	98.52a	91.11a	19.63bcde	75.0abc
UV27	4.07fghi	5.19ij	1.48de	64.3abc	UV57	0.00i	0.00j	0.00e	13.3abc
UV28	52.22cd	55.56bcd	0.00e	1.7c	UV58	4.44fghi	0.74j	12.22bcde	5.0bc
UV29	57.78bcd	62.59bc	29.63abc	74.3abc	UV59	9.26fghi	3.70ij	0.00e	58.7abc
UV30	5.93fghi	5.19ij	18.15bcde	142.3a	ck	0.00i	0.00j	0.00e	0.0c

*Figures are the mean of three replications. Those followed by the same letter are not significant

Table 3. ANOVA of *DIs* between isolates and rice hybrids.

Variable	df	Mean square	F
Main Unit (Hybrids)	2	653.8819	1.519
Error (a) – For Main Unit	4	430.3893	
Sub Unit (Isolates)	59	1728.173	28.022**
Hybrid x Isolate interaction	118	382.802	6.207**
Error (b) – For Sub-Unit	354	61.6725	
Total	539		

**Significant difference at P<0.01.

and UV36. There were no significant differences among other isolates (Table 2).

Twenty-eight isolates (47.46% of the totality) could infect one or two hybrids, eighteen isolates (30.51%) could infect all three hybrids, the remaining thirteen isolates (22.03%) could not infect any of the three hybrids. The results also showed that *DI* of the same isolate infecting the different hybrids was significantly different. For example, the *DI* of UV24 to Gangyou182, Gangyou94-11 and Yixiang2292 was 62.95, 52.96 and 11.11, respectively; the *DI* of UV25 to the three cultivars was 76.67, 65.19 and 0, respectively.

Isolate and rice hybrid two-way ANOVA is shown in Table 3. The differences among isolates and the interactions between isolates x hybrids were significant (P<0.01), but that of hybrids were not significant.

The 59 isolates could be classified into 6 groups at 25.89 Euclidean distance as shown in the dendrogram (Fig. 1) developed by UPGMA. Group I included only isolate UV56 which was highly virulent on Gangyou182 and Gangyou94-11 but less virulent on Yixiang2292. Group II isolates composed of UV25 and UV28 were virulent to Gangyou182 and Gangyou94-11 with a *DI* of more than 50, but avirulent to Yixiang2292 with a *DI* of 0. Group VI consisting of three isolates (UV1, UV21 and UV16) was virulent on Yixiang2292 and less virulent on Gangyou182 and Gangyou94-11. Group III included 6 isolates (UV17, UV18, UV29, UV24, VU36 and UV55) with a *DI* of more than 21.11 on Gangyou182 and Gangyou94-11 and with a *DI* of more than 11.11 on Yixiang2292. Group IV included 11 isolates (UV5, UV9, UV15, UV26-27, UV42, UV46-48, UV54 and UV59) with the minimal averaged *DI* of 5.19 for UV27 on Yixiang2292. Group V consisted of the other 36 isolates (UV2-4, UV6-8, UV10-14, UV20, UV22-23, UV30-35, UV37-39, UV40-41, UV44-45, UV49, UV50-53 and UV57-58) which were virulent or avirulent on the three hybrids. Isolate UV42 of Group IV for instance, was uniquely virulent to hybrid Gangyou 94-11 but avirulent on both Gangyou 182 and Yixiang 2292, while isolate UV1 of group VI was virulent on Yixiang 2292 but avirulent on Gangyou 192 and Gangyou 94-

Table 4. The *DI* of isolates from different geographical origins.

Cultivation area	No. of isolates	*DI*									Mean
		Gangyou 182			Gangyou 94-11			Yixiang 2292			
		Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	
Pujiang	18	51.85	0	7.76	44.81	0	5.93	60.37	0	8.25	7.31
Ya'an	8	76.67	0	18.84	65.19	0	15.37	38.15	0	6.57	13.60
Jintang	3	57.78	4.07	38.02	62.59	5.19	41.11	29.63	0	10.37	9.75
Shuangliu	3	5.93	0.37	2.72	5.19	0	1.73	18.15	0	6.54	3.91
Chongzhou	3	0	0	0	1.11	0.37	0.62	18.89	0	11.11	3.91
Dazhou	3	27.41	0.37	10.62	51.48	0	17.41	35.19	0	16.67	14.90
Nanchong	3	0	0	0	0	0	0	5.93	0	1.98	0.66
Luzhou	3	5.93	0	2.35	41.85	0	13.95	3.33	0	1.11	5.80
Yibin	3	38.15	0.37	15.31	23.33	0	14.32	10.37	0	3.46	11.03
Jianyang	3	16.30	0	8.15	8.15	0	4.07	0	0	0	4.07
Mianyang	3	0	0	0	0.37	0	0.12	1.11	0	0.49	0.21
Guanghan	3	41.85	0	20.62	35.56	0	12.47	12.96	0	6.91	13.33
Shehong	1	100.00	95.56	98.52	100.00	82.22	91.11	37.78	1.11	19.63	69.75
Hanyuan	3	9.26	0	4.57	3.70	0	1.48	12.22	0	4.07	3.37

Table 5. ANOVAs of isolate groups with different host origins.

Variable	df	Mean square	F
Host	7	534.5239	10.42**
Error	16	51.2988	
Total	23		
Female parents	20	346.5902	3.532**
Error	42	98.1389	
Total	62		
Male parents	37	458.6561	5.482**
Error	76	83.6688	
Total	113		

** Significant difference at P<0.01.

11; as isolate UV56 of Group I was virulent on both Gangyou 182 and Gangyou 94-11 but avirulent on Yixiang 2292.

Pathogenicity analysis of the isolates of different geographical origin. The results showed that the range of pathogenicity of isolates from the same location was very wide. For example, the *DI* values of 18 isolates from Pujiang ranged from 0 to 60.37; the *DI* values of 8 isolates from Ya'an ranged from 0 to 76.67, and the *DI* values of 3 isolates from Hanyuan ranged from 0 to 12.22. In spite of strong local variation, ANOVA indicated that there were significant differences (P<0.05) among groups of different geographical origin. The pathogenicity of isolates from Shehong was the highest with an averaged *DI* of 69.75 (the maximum *DI* and disease panicle rate was 98.52 and 100% respectively) while the pathogenicity of isolates from Mianyang was the lowest with an averaged *DI* of 0.21 ranging from 0 to 1.11. The pathogenicity of isolates from Nanchong was also lower, with an averaged *DI* of 0.66 ranging from 0 to 5.93. In addition, the averaged *DI* of more than 10 included isolates from Ya'an, Dazhou, Yibin and Guanghan while that of less than 10 included isolates from Pujiang, Jintang and Shuangliu etc. (Table 4).

Pathogenicity difference analysis of the isolates from different host genotypes. Twenty of 59 isolates were identified from 8 hosts from which two isolates or more were recovered. Analysis of variance showed that there were large and significant differences in pathogenicity among these 20 isolates, when tested on the three hybrids as replications (the average *DI* of several isolates from the same host origin) (Table 5).

The results of multiple comparisons of the 8 hosts showed that there were significant differences among the isolates of different host origin. For example, there were large and significant differences in pathogenicity among isolates from Gangyou725 compared to those from Gangyou151, Gangyou527, Jinyou527, Jinyou18 and IIyou95-18 (Table 6).

The 59 isolates considered in this study were further classified according to the 21 female parents and the 38 male parents of the hybrid combinations on which the isolates were collected. The *DI* of isolates was averaged for the same rice hybrids on which they originated. The results showed that there were more significant differences among the different female and male parents compared with those than that of the same female and male parent origin (Table 6).

Table 6. Pathogenicity, measured as *DI*, of isolates from 8 different rice cultivars.

Cultivar of Isolate origin	Gangyou182	Gangyou94-11	Yixiang2292	Average	5%	1%
Gangyou725	47.33	38.79	24.32	36.81	a	A
Chuanxiang9	38.03	36.54	10.99	28.52	ab	A
Gangyou94-4	17.53	22.92	18.19	19.55	bc	AB
Gangyou151	5.95	5.02	9.54	6.84	cd	B
Gangyou527	5.91	4.86	8.59	6.45	cd	B
Jinyou527	6.08	2.47	10.23	6.26	cd	B
Jinyou18	4.21	0.00	0.00	1.40	d	B
IIyou95-18	0.00	0.00	3.91	1.30	d	B

*Figures followed by the same letter do not differ at P<0.05 and P<0.01.

Table 7. ANOVA of sporulation ability of the 59 isolates from different geographical sites and cultivar origins.

Variable	sum of squares	df	Mean square	F
Sporulation ability	279413.209	58	4817.4691	2.082**
Error	273072.6667	118	2314.1751	
Total	552485.8757	176		

**: highly significant difference (P<0.01).

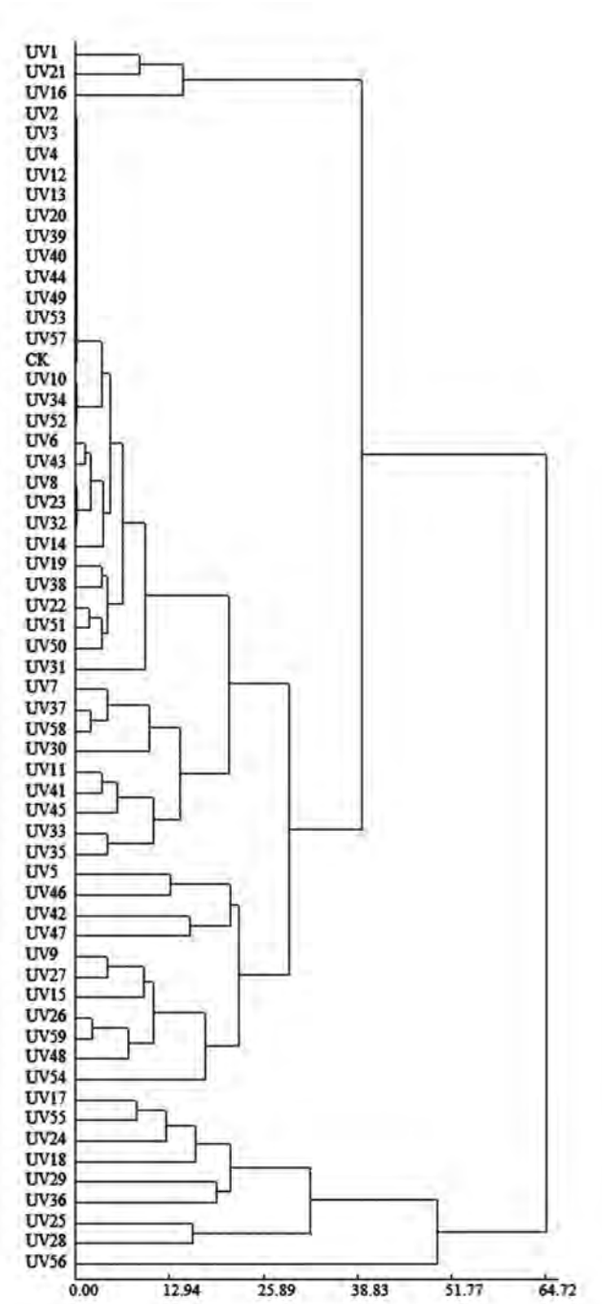

Fig. 1. *DIs* dendrogram of 59 isolates of *U. virens* collected from the different rice hybrids grown in the Sichuan Province, including check CK, developed by an unweighted pair group method with arithmetic mean algorithm (UPGMA).

Conidial production in relation to pathogenicity of isolates. Conidial production (Table 2) ranged from 1 to 143.3×10^4 conidia. Twenty four isolates (40.7%) produced 10×10^4 conidia or less. Sixty isolates (27.1%) produced 10-50×10^4 conidia and 14 isolates (23.7%) yielded 50-100×10^4 conidia. There were isolates (UV5, UV11, UV17, UV30, UV54) that produced more than 100×10^4 conidia. Analysis of variance indicated that differences in the number of conidia produced by the 59 isolates were significant (Table 7).

Multiple comparisons showed that the *DI* among isolates that produced more than 50×10^4 conidia, ranged from 0-98.52 on the three hybrids tested. Thirteen isolates that produced less than 50×10^4 conidia could not infect any of the three rice hybrids. Furthermore, UV56, which was highly virulent on Gangyou182 and Gangyou94-11, produced 75×10^4 conidia; isolate UV11 that had a *DI* of 5.56 produced 121×10^4 conidia; on the contrary, isolate UV54, with the highest conidial production had a *DI* of 20.00.

DISCUSSION

Among the 59 isolates of *U. virens* from 46 *indica* hybrids in fourteen counties of Sichuan, there were 6 isolate groups differing in pathogenicity on the three rice hybrids. Group I isolates were virulent on Gangyou 182 and Gangyou 94-11, but virulent on Yixiang 2292, Group II isolates were virulent on Gangyou 182 and Gangyou 94-11 but avirulent on Yixiang 2292, while Group VI isolates were virulent on Yixiang 2292 but less virulent on Gangyou 182 and Gangyou 94-11. Group III isolates were virulent on the three rice hybrids tested while Group V isolates were less virulent. These results imply the existence of differences in pathogenicity and differential response of *U. virens* isolates on rice hybrids. This appears to be the first report on physiologic specialization of the false smut fungus of rice.

The resistance of the three rice hybrids also differed significantly. The results of multiple comparisons showed that the resistance of Gangyou 94-11 and Gangyou 182 appeared to be close, but distinct from Yixiang 2292, which suggests that there are differences in host plant resistance as well as in pathogen specialization. Our results further suggest that the three rice hy-

brids could be useful as a set to differentiate pathogenicity. Given the considerable pathogenicity variation of *U. virens*, resistance assessment of rice varieties is needed to screen suitable isolate types. The results also demonstrated that there was a significant isolate x cultivar ($P<0.01$) interaction. The relationship between *U. virens* and the rice varieties indicated that isolates of *U. virens* might include different pathogenicity types. Additional work is therefore needed to further refine the differential system, which should include a highly susceptible host, and to understand the genetics of resistance, and identify the genes involved.

The variability and differentiation of pathogenicity is often more apparent in specialized pathogens than unspecialized ones (Li, 1995). The virulence differentiation of strongly specialized pathogens such as *Magnaporthe oryzae, Puccinia striiformis* or *Blumeria graminis*, is related to the host and geographical origin while pathogenicity in the unspecialized pathogens (e.g., *Rhizoctonia solani*) often has no such obvious relationships (Roh *et al.,* 1987). Our results showed that the pathogenicity of *U. virens* varied with different geographical origin. There were also significant differences in the pathogenicity of isolates originating from different rice hybrids. Furthermore, there were also significant differences among the different female and male parents as compared with the isolates of the same female and male parent origin, indicating that the female and male parents of the different hybrid combinations may influence the pathogenicity of different groups of isolates. To summarize, pathogenicity appeared to be influenced by site of isolate origin, the rice hybrid from which the isolate was collected and the parental combinations of the rice hybrids.

There were also significant differences ($P<0.01$) in sporulation of the 59 isolates of *U. virens.* Some workers have suggested that the rice panicle could be infected by conidia produced in water by chlamydospores (Lu *et al*., 1996; Zhang *et al*., 2003). Our results show that sporulation capacity was possibly related to pathogenicity but not significantly ($P>0.05$) as supported by the observation that some isolates with the strongest pathogenicity did not produce the highest number of conidia and vice-versa. Above all, our results show that the interactions between isolates of *U. virens* and hybrids were significantly different. An intensive research is urgently needed to confirm whether the host-pathogen relationship is similar to that of *Magnaporthe oryzae*, and follows the of gene-for-gene theory of Flor (1959), in order to make better use of different hybrids. Additionally the host diversity can influence changes in the pathogen population (Zhu *et al.,* 2000), so the different male and female origins should be utilized to improve the resistance of hybrids.

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