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Biology and artificial inoculation of *Ustilaginoidea virens* (Cooke) Takahashi in rice

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False smut of rice (*Oryza sativa* L.) caused by *Ustilaginoidea virens* (Cooke) Takahashi (teleomorph *Villosiclava virens*) is one of the most common fungal diseases of rice in China. The objectives of the research were: 1) to study the biology of *Ustilaginoidea virens,* 2) to develop suitable media for inoculum production of *U. virens,* and 3) to develop an artificial inoculation technique for the study of false smut resistance in rice. Seventy diseased rice samples were collected from seven districts of Guizhou province, China during 2007-2008 and 138 single-spore isolates of *U. virens* were isolated. Among two inoculation methods applied at 3 rice growth stages of rice variety Gangxiang 707 using spore suspension of *U. virens* isolate 2008-33-1, conidia injection at late booting stage produced the highest false smut incidence of 50.43%, while conidia spraying during the same stage gave only 34.75% disease incidence. Pathogenicity test of selected eight *U. virens* isolates on rice variety Zhongyou 177 using the conidia injection at late booting stage, showed that the isolates were different in their aggressiveness in which isolate 2008-11-1 gave the highest virulence score of 9 with the disease incidence of 81.66%, while isolate 2007-48-1 gave the lowest score of 5 with only 15.57% disease incidence. These eight *U. virens* isolates were further tested on six rice varieties using the same inoculation technique. Most of the rice varieties were either susceptible or moderately susceptible except Fengyouxiangzhan that was highly susceptible to *U. virens* isolate 2007 -79-1 and Nongfengyou 256 was moderately resistant. When the evaluation was done across all the *U. virens* isolates, Gangyou 827, Jixiangyou 830 and Fengyouxiangzhan were among the most susceptible varieties with disease incidence of 55.06, 54.79 and 53.50%, respectively, while Nongfengyou 256 varieties showed the lowest disease severity as 23.85%. For the pathogen strain, 2008-33-1 was the most aggressive giving 58.09% disease incidence while the fungal pathogen strain 2008-2-2 gave the lowest of 28.17%.

Key words: False smut, rice, colony morphology, fungal characterization, *Ustilaginoidea virens.*

INTRODUCTION

The rice false smut, caused by *Ustilaginoidea virens,* is a worldwide disease and is a minor rice disease throughout major rice-growing countries in the world before 1970's (Deng, 1989; Yaegashi et al., 1989; Sugha et al., 1992). It has been found in many countries, such as China, India, Japan, Italy, Australia, Philippines, Brazil and Mayanma (Ou, 1972; Dodan and Singh, 1996). With the change of weather condition, large application of nitrogen

fertilizer and large-scale planting of hybrid rice, the rice false smut has become more and more serious. It has already changed from a minor disease to a major disease in all rice growing areas in China, and many rice-growing countries in Asia since 1970. In 1982, the disease had spread more than 666,000 hectares in Hunan, China. The epidemic area had increased from 200,000 to 330,000 hectares from 1984-1996 at Liaoning, China. In 1993, the disease was reported to increase from 60,000 to 100,000 hectares in Yunnan, China (Liao and Li, 1994), and respect as 13.7% of the total rice production. The disease incidence was 10-30%, but in some serious fields it could be as high as 50-60%. Up to 39 false smut balls could be found on each rice plant. The rice yield was reduced 5-30% as a result of false smut infection in Guizhou. The false smut balls have toxin including ergot alkaloid toxin that can cause rumination stopping in cows, suppress the tubulin of mammals and cause necroses of liver, kidney, and bladder tissues in mice (Dhindsa et al., 1991; Nakamura and Izumiyama, 1992; Chib et al., 1992; Iwasaki, 1992; Yukiko et al., 1994; Nakamura et al., 1994; Li et al., 1995; Sinha et al., 2003). Therefore, the rice false smut not only threatens rice production in yield and quality, but also produces toxins that are dangerous to the health of human and livestocks.

Lu et al. (1996) reported artificial culture conditions of temperature, carbon source, and pH value. However, there have been few reports on optimal sporulation culture conditions and component of culture media, single spore isolation and conservation methods. There have been some studies on disease resistance using artificial inoculations (Zhang et al., 2003), but there were different results of disease incidence and lower reproducibility. At present there have not been an established artificial inoculation technique and evaluation criteria for disease resistance to rice false smut in China.

Selecting and using resistant varieties are the most cost-effective measures to control plant diseases. In recent years, some investigations have been done in Guizhou. Results show that the disease incidence of rice false smut was significantly different among rice varieties. For example, in 2008, the disease incidence and disease index of various rice varieties were 1.07 to 39.19% and 0.32 to 16.77%, respectively. The differences observed among the varieties were 36.6 times and 52.4 times. In order to effectively control rice false smut by using resistant varieties, the study of biological characteristics leading to the successful artificial inoculation is most important. Therefore, this study aims to examine the biology of *U. virens* and to develop suitable medium for inoculum production of *U. virens* and moreover, to develop an artificial inoculation techniques suitable for the study of false smut resistance in rice plant.

MATERIALS AND METHODS

Sample collection and isolation of *Ustilaginoidea virens*

Samples of the rice false smut balls were collected from 7 districts (Zunyi, Guiyang, Tongren, Qiandongnan, Qiannan, Anshun and Xingyi) in Guizhou in 2007-2008. To isolate the causal agent, the diseased samples were washed thoroughly under distilled water and dried under a lamina flow in the laboratory. Subsequently, the smut balls from each location were separately surface sterilized with 75% ethanol (EtOH) for 25 s. The excess traces of EtOH on the balls were removed by washing 2 times in sterile distilled water and then transferred aseptically into a flask containing sterile distilled water and 10 glass beads. The cultured flasks were then incubated and shaked at 28±2°C in the dark for 2 min after that 2 ml of the culture suspension was spread on surface of potato sucrose agar (PSA: potato 200 g, sucrose 20 g, agar 17 g, distilled water 1000 ml) (Liu et al., 2009). The PSA plates were incubated at 28±2°C for few hours and were examined frequently under a microscope for germinating single spores which were then marked with ink on the plate surface. These single spores were aseptically transferred with a sterile cork borer to fresh PSA and incubated at 28±2°C for 10 days. The pure single spores were then transferred into PSA slants and maintained for the future experiments. To maintain the culture, the fungus that was isolated from respective geographical region and maintained as a separate isolate, was sub-cultured on PSA slants and allowed to grow at 28±2°C for two weeks. Subsequently, the slants were preserved in a refrigerator and renewed once every two months.

Pathogenicity test of the *Ustilaginoidea virens* isolates

To confirm and prove pathogenicity of the eight *U. virens* isolates, spore suspension of each isolate at 1×10^6 spore/ml were inoculated into rice cultivar Zhongyou 177 at the late booting stage using the conidia injection technique. In another set, instead of spore suspension, only sterile distilled water was injected to serve as a negative control. The experiment was conducted as factorial in CRD with 4 replications. Observations were made at regular intervals for symptom development within 3 days. The virulence level of each isolate was evaluated based on the percentage of disease incidence classified into different scores as indicated in Table 1.

Study on suitable inoculation techniques

Inoculum preparation

The randomly selected *U. virens* isolates were used in this experiment. Details of the isolates are as shown in Table 2. Spore suspensions of the respective fungal isolates were prepared at approximately 1×10^6 conidia/ml. For the injecting inoculation, tween 80 was added into the spore suspension prior to use. For the spraying inoculation, 0.5% gelatin was added prior to use to prevent spore desiccation.

Rice plant preparation

Seeds of Gangxiang 707 were soaked in warm water at 60 °C for

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Table 1. Scoring criteria for categorizing virulence level of *Ustilaginoidea virens.*

Scoring	Disease incidence (%)	Smut ball density (No/panicle)
0	≤1%	0
1	＞1 but≤5%	1
3	＞5 but≤10%	＞1 but≤5
5	＞10 but≤25%	＞5 but≤10
7	＞25 but≤50%	＞11 but≤15
9	＞50%	＞16

Table 2. *Ustilaginoidea virens* single spore isolates used in the experiment.

Isolate	Origin (District)
2008-1-3	Zunyi
2007-79-1	Zunyi
2008-2-2	Guiyang
2007-11-1	Guiyang
2007-48-1	Tongren
2007-6-1	Qiandongnan
2007-66-1	Qiandongnan
2008-11-1	Qiannan
2008-33-1	Anshun
2008-36-1	Xinyi

1 h to kill seed-borne pathogens and left overnight to absorb water. After that, the seeds were pre-germinated at 30°C for 36 h before planting in a small field plot. At 25 days after planting, seedlings were transplanted into cement plots, 90×80 cm in size, in the greenhouse, 25 seedlings per plot.

Artificial inoculation of rice

The spore suspension of *U. virens* isolate 2008-33-1 (1×10^6 spore/ml) was used for inoculating Gangxiang 707. Two different artificial inoculation methods, conidia suspension spraying and conidia injection were investigated at tillering, booting and late booting stages. For conidia spraying, the suspension was sprayed on leaf surface until run–off by an air compressor. For conidia injection, 1 ml of the suspension was injected into the upper part of leaf sheath covering the developing panicle of each test plant using a syringe. In another set, instead of spore suspension only sterile distilled water was sprayed or injected to serve as a negative control. The experiment was conducted as factorial in CRD with four replications. Subsequently, the inoculated rice plants were kept in moist condition and observations were made at regular intervals for symptom development within 3 days after inoculation.

Reaction of rice varieties to *Ustilaginoidea virens*

Eight rice varieties of different resistant levels and from different areas of origin (Table 3) were used. The plants were prepared as described above. The inoculation was done by conidia injection technique at late booting stage (5~7 days before flowering). Selected eight virulent fungal isolates, including 2008-1-3, 2008-2-2, 2007-11-1, 2007-6-1, 2008-33-1, 2008-11-1, 2008-36-1 and 2007-48-1, were prepared in sterile distilled water as spore suspensions.

The experiment was conducted as factorial in CRD with four replications. Observations were made at regular intervals for symptom development within 3 days. Number of infected rice plants and grains were counted at 40~50 days after the inoculation (maturity stage). The disease incidence, disease index and density of rice smut balls were calculated and evaluated as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Total infected rice panicles}}{\text{Total inoculated rice plants}} \text{x } 100$$

The rice false smut incidence and density of smut ball were classified into six scoring scales based on Zhang et al. (2006) (Table 1). The disease index was calculated according to the amount of rice smut balls of each score and corresponding value of scale (Table 1) as follows:

$$\text{Disease index} = \frac{\sum(\text{infected panicles of each rating} \times \text{rating value})}{\text{Total panicles} \times \text{The highest rating value}} \times 100$$

The comprehensive evaluation index (CEI) was calculated as follows:

$$\text{CEI} = [(\text{score of disease incidence} \times 60) + (\text{score of smut ball density} \times 40)]/100$$

(Ministry of Agriculture of P. R. China, 2006). Subsequently, the CEI was used for the resistance evaluation of the rice varieties using the criteria in Table 4.

Statistical analysis

Treatment effects on most experiments were analyzed using

Table 3. Rice varieties and their observed reaction to *U. virens* employed in the study.

Rice variety	Area of origin	Reaction to false smut
Gangxiang 707	Sichuan	S
Zhongyou 177	Sichuan	R
Gangyou 827	Sichuan	S
Heyou 6	Sichuan	R
Suaiyoulianhe 2	Guizhou	R
Jinxiangyou 830	Guizhou	S
Fengyouxiangzhan	Jiangshu	S
Nongfengyou 256	Anhui	R

R=resistant; S=susceptible.

Table 4. Scoring of the comprehensive evaluation index (CEI) of reaction to rice varieties of false smut disease.

Scoring	CEI	Resistant level
0	0	Highly resistant (HR)
1	≤1	Resistant (R)
3	＞1 but≤3	Moderately resistant (MR)
5	＞3 but≤5	Moderately susceptible (MS)
7	＞5 but≤7	Susceptible (S)
9	＞7	Highly susceptible (HS)

ANOVA by the SPSS program. In some experiments, the data were arcsine transformed before the analysis. Duncan's multiple range test (DMRT) at p≤0.05 was used to separate treatment means.

RESULTS

Sample collection and single spore isolation of *Ustilaginoidea virens*

To obtain *U. virens* in the test, 70 rice diseased panicle samples (Figure 1) were collected in Guizhou province during 2007- 2008. From the samples, 138 single spore isolates were isolated from different regions (Table 5). There were 10 samples and 16 single spore isolates from Zunyi, 13 and 26 from Guiyang, 6 and 14 from Tongren, 11 and 24 from Qiandongnan, 10 and 21 from Qiannan, 8 and 23 from Anshun, and 8 and 14 from Xinyi, respectively.

Pathogenicity test of the *Ustilaginoidea virens* isolates

The results are shown in Table 6. Most of the *U. virens* isolates selected for the test could infect the rice variety Zhongyou 177, indicating that they all were pathogenic fungi. Among of them, the isolate 2008-11-1 revealed the highest rice panicle disease incidence with 81.66% which is the maximum according to the grading of disease incidence at score 9.

Study on suitable inoculation technique

In the experiment, 40 rice panicles were used per replication. The average disease incidences of spraying inoculation were 0.00, 21.18, and 34.75% at tillering, early booting and late booting stages respectively. The disease incidence was much higher when rice plants were inoculated by conidia injection (Figure 2), which had 0.00, 38.50 and 50.43% at tillering, early booting and late booting stages respectively (Table 7). Result from combined analysis showed clearly that conidia injection was the suitable inoculation technique in this experiment (Table 8) and it should be done at the late booting stage (Table 9).

Reaction of rice varieties to *Ustilaginoidea virens* isolates

The results were as shown in Table 10. Most of the rice varieties were susceptible or moderately susceptible to the *U. virens* isolates except variety Fengyouxiangzhan which was highly susceptible to the 2007-79-1 isolate while variety Nongfengyou 256 showed moderately resistant reaction to the same isolate. Combined analysis of all factors contributed to the rice panicle disease reaction was presented in Tables 11, 12, and 13. Among the six rice varieties tested, Gangyou 827 appeared to have the highest disease incidence (55.06%) while Nongfengyou 256 seem to have the lowest (23.85%) (Table 11). Differences in virulence among the *U. virens* isolates were also observed in that isolate; 2008-33-1 showed the highest disease incidence (58.09%) while isolate 2008-2-2 gave the lowest incidence (28.17%) (Table 12). When the overall disease reactions were analyzed by combining all disease parameters, it appeared that Nongfengyou 256 performed the best by being moderately resistant to 12.5% of the *U. virens*

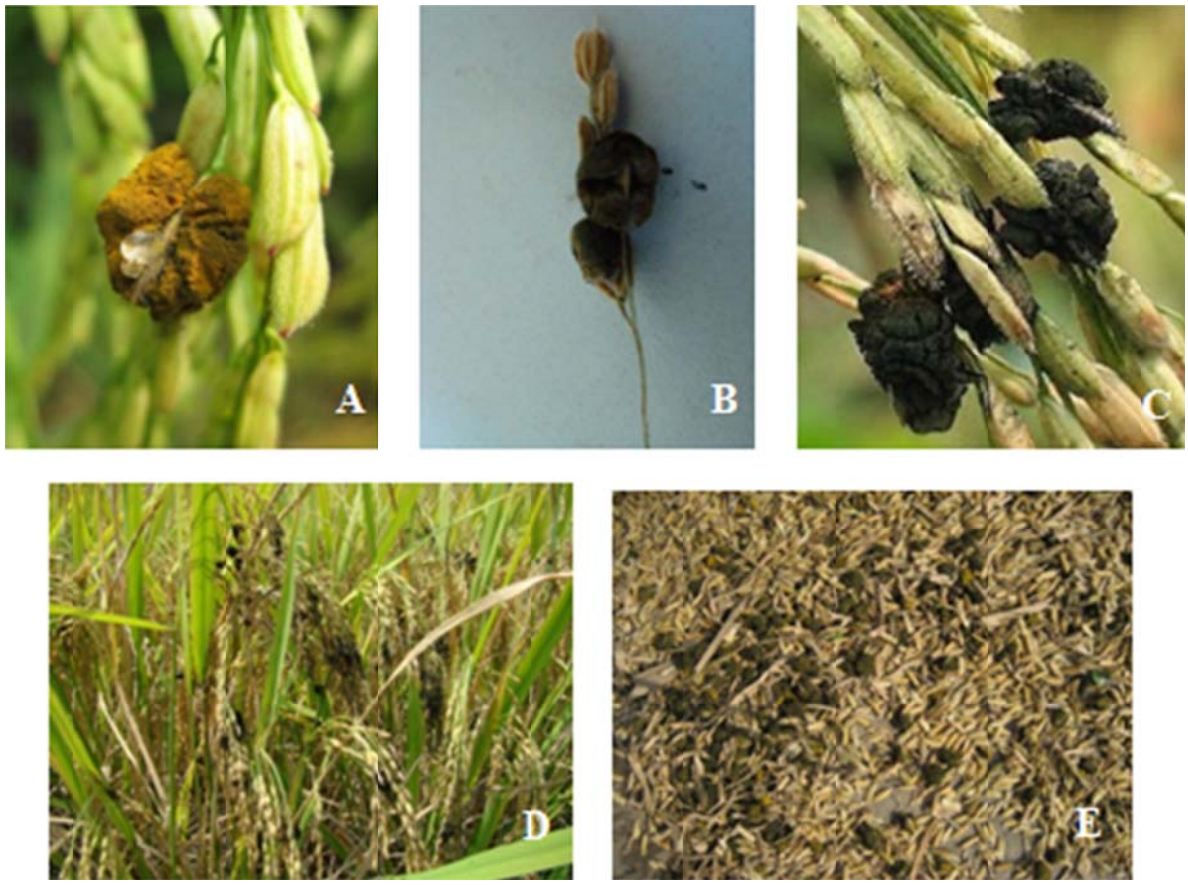

Figure 1. Symptoms of false smut on rice panicles. (A) orange stage; (B) brown stage; (C) green black stage; (D) field symptoms; (E) smut balls on harvested grains.

Table 5. Single spore isolates of *U. virens* collected from various districts of Guizhou Province, China.

District	Year of collection	Number of sample	Number of single spore isolate
Zunyi	2007-2008	10	16
Guiyang	2007-2008	13	26
Tongren	2008	6	13
Qiandongnan (Southeast)	2008	11	24
Qian nan (South)	2008	10	21
Anshun	2007-2008	12	23
Xinyi	2008	8	15
Total	—	70	138

isolates and only moderately susceptible to the left 87.5% (Table 13).

DISCUSSION

Seventy diseased samples were collected from seven districts of Guizhou province. 138 single-spore isolates of *U. virens* were successfully isolated. Among the two inoculation methods applied at three rice growth stages, conidia injection at late booting appeared to be the most effective giving the smut incidence of 50.43% while conidia spraying at the same growth stage gave only 34.75% incidence. Both methods at early booting gave

Table 6. The virulence of *U. virens* isolates on Zhongyou 177 rice cultivar.

U. virens isolate	Disease incidence (%) [1]	Virulence score
2008-1-3	33.05^{d}	7
2008-2-2	33.72^{cd}	7
2007-11-1	41.85^{c}	7
2007-6-1	51.26^{b}	9
2008-33-1	58.76^{b}	9
2008-11-1	81.66^{a}	9
2008-36-1	23.97^{e}	5
2007-48-1	15.57^{f}	5
CK	0.00^{g}	
F-test	**	
CV(%)	8.98	

[1]The data were arcsine transformed before analysis. Means in the same column followed by different letter are significantly different at P≤0.05 by DMRT technique.

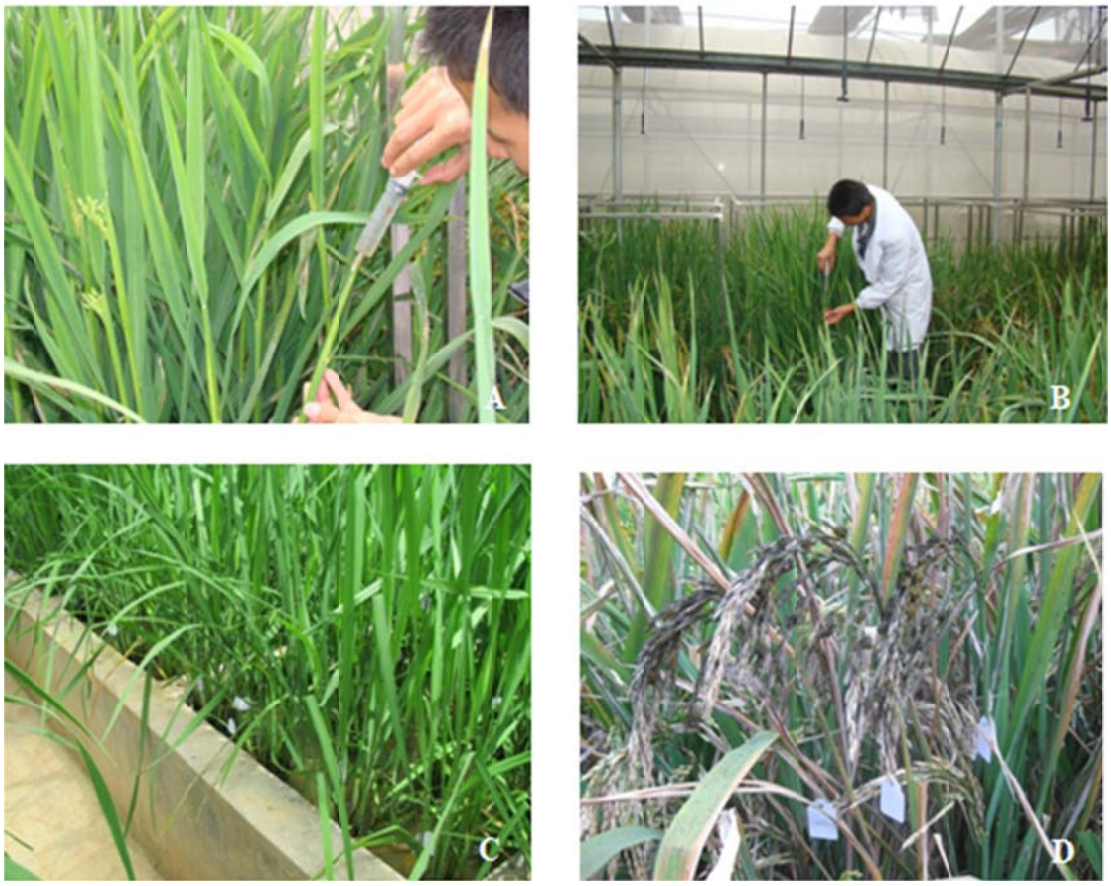

Figure 2. Artificial inoculation and symptom of rice false smut (A) and conidia injection inoculation (B), labeling of the inoculated plants (C), symptoms of rice false smut developed from artificial inoculation (D).

lower smut incidence, while applying inoculum at tillering failed to cause any infection. This finding indicates that growth stage is an important factor contributing to the success or failure of the inoculation. Similar finding has been reported by Wang et al. (1996) who found that the smut incidence was higher when rice plants were injected than when they were sprayed with conidia suspension and the critical susceptible stage was at booting.

Table 7. False smut incidence on Gangxiang 707 rice variety inoculated by 2 different methods at 3 growth stages of rice.

Inoculation method	Inoculation stage	Disease incidence (%) [1]
Spraying inoculation	Tillering stage	0.00[e]
	Early booting stage	21.18[d]
	Late booting stage	34.75[c]
	Blank control	0.00[e]
Injecting inoculation	Tillering stage	0.00[e]
	Early booting stage	38.50[b]
	Late booting stage	50.43[a]
	Blank control	0.00[e]
F-test		**
CV (%)		12.86

[1]The data were arcsine transformed before the analysis. Means in the same column followed by different letters are significantly different at P≤0.05 by DMRT.

Table 8. Combined analysis of false smut incidence on Gangxiang 707 inoculated by 2 different methods at 3 growing stages of rice.

Inoculation method	Disease incidence (%) [1]
Spraying	13.98[b]
Injection	22.23[a]
F-test	**
CV(%)	8.12

[1] The data were arcsine transformed before the analysis. Means in the same column followed by different letters are significantly different at P≤0.05 by DMRT.

Table 9. Combined analysis of false smut incidence on Gangxiang 707 inoculated at 3 growth stages of rice by different methods.

Inoculation stage	Disease incidence (%) [1]
Tillering	0.00[c]
Early booting	29.84[b]
Late booting	42.59[a]
Blank control	0.00[c]
F-test	**
CV(%)	8.12

[1] The data were arcsine transformed before the analysis. Means in the same column followed by different letters are significantly different at P≤0.05 by DMRT.

When the pathogenicity was tested among eight selected *U. virens* isolates on Zhongyou 177 rice cultivar, the 2008-11-1 isolate showed the highest virulence score of 9 with the disease incidence of 81.66% while the 2007-48-1 isolate performed the lowest score of only 5 with the disease incidence of 15.57%. Such results reflected the diversity in virulence among the *U. virens* isolates. The 2008-11-1 isolate was collected from Qiannan which was the major planting area for hybrid rice with excessive use of nitrogen fertilizer, a very conducive condition for *U. virens* epidemic (personal observation, 2011). Smut incidence of 15.55% has been commonly observed on rice grown in Qiannan. Crop monoculture and excessive use of nitrogen have been known to cause epidemic of many rice diseases (Ou, 1972; Long et al., 2000). The existing of aggressive race in Qiannan should be made known so that the farmers would be more cautious in selecting rice cultivars and applying nitrogen fertilizer.

The eight selected *U. virens* isolates were further tested on six rice varieties using the conidia injection at late booting stage. Most of the rice varieties tested showed different resistant level from those that had been observed in the field prior to the experiment. Most varieties were either susceptible or moderately susceptible except Fengyouxiangzhan that was highly susceptible to *U. virens* isolate 2007-79-1 and Nongfengyou 256 was moderately resistant to the same *U. virens* isolate. The different reaction observed in the field could have come from the existing of different *U. virens* races in different locations, the phenomenon that has been noted earlier. After combined analysis, such diversity could be seen again in that among the 8 *U. virens* isolates tested, 2008-33-1 isolate was found to be most aggressive giving disease incidence as high as 58.09% while 2008-2-2 isolate gave only 28.17%. All cultivars tested, Gangxiang 827, Jixiangyou 830 and Fengyouxiangzhan had highs smut incidence of 55.06, 54.79 and 53.50%, respectively while Nongfengyou 256 had the lowest of only 23.85%. This cultivar appeared to perform best being moderately resistant to 12.5% of the

Table 10. Reaction of rice varieties to infection by *U. virens* isolates evaluated as smut ball density scoring (SDS), disease incidence (DI), disease incidence score (DIS), comprehensive evaluation index (CEI) and CEI scores (CEIS).

Rice variety	*U. virenes* isolates	SDS (ball/panicle)	DI(%) [1]	DIS	CEI	CEIS	Reaction
	2008-2-2	1	34.72^{d}	7	4.6	5	MS
	2008-33-1	3	80.78^{a}	9	6.6	7	S
	2007-11-1	3	55.44^{c}	9	6.6	7	S
	2007-6-1	3	69.53^{ab}	9	6.6	7	S
Gangyou 827	2008-1-3	3	56.95^{c}	9	6.6	7	S
	2008-36-1	1	42.05^{d}	7	4.6	5	MS
	2007-66-1	3	60.27^{bc}	9	6.6	7	S
	2007-79-1	1	40.77^{d}	7	4.6	5	MS
F-test			**				
CV(%)			18.49				
	2008-2-2	3	39.11^{d}	7	5.4	7	S
	2008-33-1	3	74.14^{a}	9	6.6	7	S
	2007-11-1	3	53.78^{c}	9	6.6	7	S
	2007-6-1	3	74.14^{ab}	9	6.6	7	S
Jinxiangyou 830	2008-1-3	3	57.17^{c}	9	6.6	7	S
	2008-36-1	1	40.67^{d}	7	4.6	5	MS
	2007-66-1	3	60.11^{bc}	9	6.6	7	S
	2007-79-1	1	39.23^{d}	7	4.6	5	MS
F-test			**				
CV(%)			13.04				
	2008-2-2	1	14.94^{c}	5	3.4	5	MS
	2008-33-1	3	42.12^{a}	7	5.4	7	S
	2007-11-1	1	36.22^{a}b	7	4.6	5	MS
	2007-6-1	1	42.05^{a}	7	4.6	5	MS
Heyou 6	2008-1-3	1	32.90^{ab}	7	4.6	5	MS
	2008-36-1	1	26.57^{b}	5	3.4	5	MS
	2007-66-1	1	29.89^{ab}	5	3.4	5	MS
	2007-79-1	1	31.55^{ab}	7	4.6	5	MS
F-test			**				
CV(%)			30.05				

Table 10. contd.

Rice variety	*U. virenes* isolates	SDS (ball/panicle)	DI(%) [1]	DIS	CEI	CEIS	Reaction
	2008-2-2	1	29.89^{ab}	5	3.4	5	MS
	2008-33-1	3	37.66^{ab}	7	5.4	7	S
	2007-11-1	1	37.73^{ab}	7	4.6	5	MS
Suaiyou	2007-6-1	3	40.67^{a}	7	5.4	7	S
lianhe 2	2008-1-3	1	31.39^{ab}	7	4.6	5	MS
	2008-36-1	1	26.57^{bc}	5	3.4	5	MS
	2007-66-1	1	17.89^{c}	5	3.4	5	MS
	2007-79-1	1	17.89^{c}	5	3.4	5	MS

Table 10. contd

F-test			**				
CV(%)			27.91				
Fengyou xiangzhan	2008-2-2	1	22.1[d]	5	3.4	5	MS
	2008-33-1	3	80.78[a]	9	6.6	7	S
	2007-11-1	3	49.33[c]	9	6.6	7	S
	2007-6-1	3	61.77[b]	9	6.6	7	S
	2008-1-3	3	49.33[c]	9	6.6	7	S
	2008-36-1	1	42.05[c]	7	4.6	5	MS
	2007-66-1	3	60.27[b]	9	6.6	7	S
	2007-79-1	5	62.31[b]	9	7.4	9	HS
F-test			**				
CV(%)			14.24				
Nongfengyou 256	2008-2-2	1	28.23[ab]	5	3.4	5	MS
	2008-33-1	1	33.06[a]	7	4.6	5	MS
	2007-11-1	1	24.53[ab]	5	3.4	5	MS
	2007-6-1	1	21.59[ab]	5	3.4	5	MS
	2008-1-3	1	22.50[ab]	5	3.4	5	MS
	2008-36-1	1	24.53[ab]	5	3.4	5	MS
	2007-66-1	1	20.47[b]	5	3.4	5	MS
	2007-79-1	1	15.86[b]	3	2.2	3	MR
F-test			**				
CV(%)			40.92				

[1]The data were arcsine transformed before the analysis. Means in the same column followed by different small letters are significantly different at P≤0.05 by DMRT.

Table 11. Combined disease incidence of rice varieties inoculated with 8 *U. virens* isolates by conidia injection.

Variety	Disease incidence (%) [1]
Gangyou 827	55.06[a]
Jinxiangyou 830	54.79[a]
Fengyouxiangzhan	53.50[a]
Heyou 6	32.01[b]
Suaiyoulianhe 2	29.96[b]
Nongfengyou 256	23.85[c]
F-test	**
CV(%)	16.74

[1]The data were arcsine transformed before the analysis. Means in the same column followed by different letters are significantly different at P≤0.05 by DMRT.

Table 12. Combined disease incidence of 8 *U. virens* isolates inoculated to 6 rice varieties by conidia injection.

Isolates	Disease incidence (%) [1]
2008-33-1	58.09[a]
2007-6-1	51.63[b]
2007-11-1	42.84[c]
2008-1-3	41.71[c]
2007-66-1	41.48[c]
2007-79-1	34.60[d]
2008-36-1	33.74[d]
2008-2-2	28.17[e]
F-test	**
CV(%)	16.74

[1]The data were arcsine transformed before the analysis. Means in the same column followed by different letters are significantly different at P≤0.05 by DMRT.

U. virens isolates and moderately susceptible to 87.5% of them. The consistent reaction of this cultivar to *U. virens* observed in the fields with that observed in this experiment indicates that its resistance could be polygenic. The higher disease score observed in the experiment could result from excessive concentration of the inoculum applied to the rice plant and the unnatural inoculation process. With all these shortcomings of the artificial inoculation, it is therefore necessary to repeat the

Table 13. Reaction of rice varieties to *U. virens* isolates.

Variety	Percentage of *U. virens* isolate with the reaction					
	HR	R	MR	MS	S	HS
Gangyou 827	0	0	0	37.50	62.50	0.00
Jinxiangyou 830	0	0	0	25.00	75.00	0.00
Heyou 6	0	0	0	87.50	12.50	0.00
Suaiyoulianhe 2	0	0	0	75.00	25.00	0.00
Fengyouxiangzhan	0	0	0	25.00	62.50	12.50
Nongfengyou 256	0	0	12.5	87.50	0.00	0.00

HR=highly resistant; R=resistant; MR=moderately resistant; S=susceptible; HS=highly susceptible.

screening under field condition before the varieties could be labeled for their reaction to *U. virens.*

Conflict of interests

The authors did not declare any conflict of interests.

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