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Culture condition and variation in the pathogenicity of *Ustilagonoidea virens* isolates causing false smut disease of rice

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Abstract

False smut of rice caused by *Ustilagonoidea virens* was previously considered a minor disease of rice or rather a sign of bumper harvest, hence the disease was also known by the name Lakshmi disease. However, the disease is gaining much importance at present and is reported to occur in most of the rice growing areas of world in moderate to severe forms. Rice which is cultivated throughout the year in West Bengal is also observed to produce the symptoms of the disease every year in varying degree of severity. Effect of light and different temperature on mycelial growth of *Ustilagonoidea virens* in BOD incubator was tested. It was found that temperature of 28°C and dark condition favors mycelial growth. Twelve different isolates of *Ustilagonoidea virens* collected from different districts of West Bengal were isolated in laboratory and tested for their variability in pathogenicity using the susceptible rice variety Swarna (MTU 7029). 2 ml of inoculum suspension of each isolate was injected to rice plant at booting stage with three replications, whereas in control plants 2 ml of sterilized water was injected and *percent* grain infection was calculated and analyzed. All the inoculated plants were observed to produce typical false disease symptom with varying degree of severity indicating variation in pathogenicity among the isolates. *Percent* grain infection ranged from 1.461% to 7.936%. Isolate Uv5 from Alipudwar district was found to be significantly more virulent as compared to other isolates with 7.936% grain infection followed by isolate Uv4 (Jalpaiguri district) and Uv3(Nadia district) with 5.050% and 4.557% grain infection respectively. Significantly lower infection was observed in isolate Uv9 collected from Coochbehar district with 1.461% grain infection followed by isolate Uv2 (North 24 Parganas district) and Uv12 (Hooghly district) with 1.912 and 3.279% grain infection.

Keywords: Pathogenicity, *Ustilaginoidea virens*, False smut.

1. Introduction

Rice (*Oryza sativa*) is considered as one of the most important cereal crops in the world especially in Asia. India is the second largest producer of rice in the world after China. State of West Bengal occupies largest area under rice and is the highest producer among the states (NFSM 2016). Rice is grown in all the six agro-climatic zones of West Bengal and is the richest reservoir of rice bio-diversity for which it is also known as the rice bowl of the country (Chatterjee and Gupta, 2013). Due to the very diverse ecotypes of rice in West Bengal that scientists at one time coined them as *Oryza sativa* var. *benghalensis* (Chatterjee *et al.,* 2008). False smut of rice caused by *Ustilagonoidea virens* (Cooke) Takahashi, was first reported from Tirunelveli district of Tamil Nadu (Cooke 1878). The

disease at first was recognized as minor disease and was also known by the name Lakshmi disease as believed by some as an indication of bumper harvest. However, at present the disease has been reported from different rice growing areas of the world such as such as China, India, Philippines, Indonesia, Bangladesh, Myanmar, Thailand, Vietnam, Brazil and Japan (Ou, 1972; Dodan, 1996) and yield reduction of upto 75% has been reported (Rashmi *et al.,* 2014). Changes in the weather condition throughout the world in recent times, faulty use of nitrogenous fertilizers and increasing adoption of hybrid rice varieties are the reasons for the disease progress (Zhou *et al* 2008, Haiyong 2012).

False smut of rice caused by *Ustilagonoidea virens* is a flower infecting pathogen which initiates its infection during booting to flowering stage by infecting the ovaries of the rice kernel (Lee and Gunnell 1992) which later transforms into large velvety green, orange or yellow when young and black smut balls at maturity that are usually twice the size of the normal grains (Biswas 2001a). The spore balls contain the chlamydospore, conidia and mycelium and sometimes sclerotium may also appear (Ou 1972).

2. Materials and Methods

False smut balls from 12 development blocks of 6 different districts of West Bengal were collected (**Table 1). S**mut balls were thoroughly washed by dipping the balls in sterile distilled water in petri plates and surface sterilized with 0.1% mercuric chloride solution for 1 minute and subsequently washed three times with sterile distilled water followed by 70% ethanol wash for 1 min and again samples were washed 3 times with distilled water. Using a sterilized needle, the mass of chlamydospores was streaked onto petri dishes containing potato sucrose agar (PSA) medium, under complete sterile and aseptic conditions. After the isolation, pure cultures of the isolates were maintained in culture tubes containing potato sucrose agar.

To understand the better culture condition for mycelial growth, 5mm discs from the pure culture were transferred to petri dishes containing potato sucrose agar with three replications and incubated in BOD incubator for 30 days at different temperature of 22°C, 25°C, 28°C and 31°C under both light and dark condition. After the study of culture condition, inoculum of each isolate for pathogenicity test was prepared. 5mm discs from pure culture of *Ustilaginoidea virens* isolates were transferred into conical flasks containing 100ml of potato sucrose broth and incubated in an incubator at 28°± 1°C under dark condition for a month and the conidia were harvested and suspended in sterile distilled water.

Each of 12 different isolates was tested for variation in their virulence against rice variety (MTU 7021, Swarna) which is a susceptible rice variety to rice false smut disease. The experiment was conducted in net house, dept of plant pathology, B-C-K-V- Kalyani. Seedling were prepared in the seed beds and transplanted to the black pots with three replications. Plants at booting stage were injected with 2 ml conidial suspension of concentration of $4x10^5$ spore/ml and observed for symptom expression after 15 days of inoculations. In control pots 2 ml of distilled water was injected. Different disease variables such as number of smut balls per infected panicle and total number of grains per panicle was recorded. From the recorded data percent grain infection was calculated based on formula given by Singh and Dube (1978). Mean of the data recorded were analyzed in OPSTAT with one factor analysis.

Percent grain infection:

$$\frac{\text{Number of smut balls/panicle}}{\text{Total number of grains/panicel}} X100$$

Table 1: Place of collection of isolates from different blocks of different districts

Sl. No	Isolates	Place of collection/Blocks	District
1.	UV1	Barasat 1	N24 Parganas
2.	UV2	Aamdanga	N24 Parganas
3.	UV3	Chakdah	Nadia
4.	UV4	Dhupguri	Jalpaiguri
5.	UV5	Falakata	Alipurdwar
6.	UV6	Mainaguri	Jalpaiguri

7.	UV7	Coochbehar II	Coochbehar
8.	UV8	Ranaghat	Nadia
9.	UV9	Coochbehar I	Coochbehar
10.	UV10	Shantipur	Nadia
11.	UV11	Alipurdwar I	Alipurdwar
12.	UV12	Chinsurah-Mogra	Hooghly

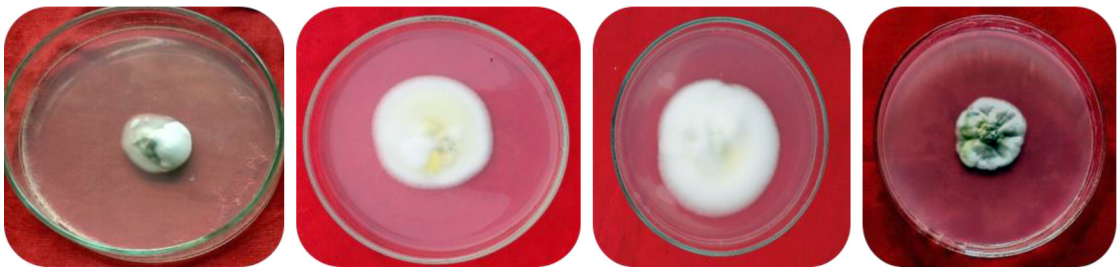
Fig 1: a) Growth of *U. virens* on PSB, b) Inoculated plants with three replication, c) Inoculated plants showing typical false smut symptom

3. Results and Discussion

Study on culture conditions for better mycelial growth revealed that, temperature of 25°C to 28°C favours the mycelial growth of *U. virens* as compared to other temperature tested. Highest mycelial growth was observed at temperature of 28°C with a colony diameter of 4.70cm whereas at temperature of 22°C restriction of mycelial growth was observed with a colony diameter of 2.267cm. Culture of *U. virens* incubated under dark condition was found to have significantly higher mycelial growth as compared to cultures under light condition (**Table 2**). Present findings have been found in agreement with previous reports. Baite *et al.,* (2015) have observed that the cultures incubated in dark condition showed better growth than the one in light condition. He has also suggested that among the different temperature tested 27°C was found best for the mycelial growth of *Ustilaginoidea virens.* Ruiling *et al.,* (2009) from their experiment have also found the alike results with the maximum growth of the fungus at 27°C as compared to 25°C and 29°C.

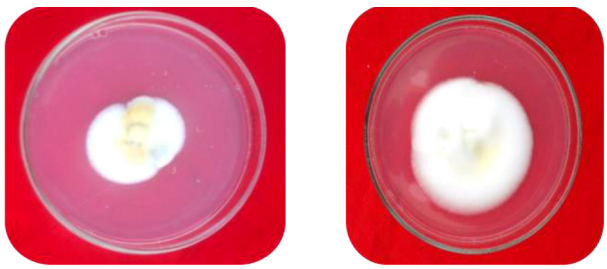
Table 2: Effect of temperature and light on mycelial growth of *U. virens*

Temperature	**Mycelial growth (cm)**		**Light**	**Mycelial growth (cm)**	
	Mean	**S.E.**		**Mean**	**S.E.**
22 °C	2.267	0.088	Light	3.500	0.058
25 °C	4.200	0.058			
28 °C	4.700	0.058	Dark	4.700	0.115
31 °C	2.967	0.067			
C.D.	0.228		C.D.	0.368	
SE(m)	0.069		SE(m)	0.091	



22 °C 25 °C 28 °C 31 °C

Fig 2: Mycellial growth of *U. virens* at different temperatures after 30 Days of inoculation.



Light Dark

Fig 2: Mycellial growth of *U. virens* under dark and light conditions after 30 Days of inoculation

All the inoculated plant were found to develop characteristic false smut symptoms i.e., large dark green powdery spore balls in infected panicle of rice which were initially yellowish green to yellowish orange in colour. There was much variation in number of development of false smut balls in tillers inoculated with different isolates which ranged from 1.462% to 7.936% grain infection. Isolate Uv5 was found to be significantly more virulent as compared to other isolates with 7.936% grain infection, followed by isolates Uv4 (5.050%), Uv3 (4.557%), Uv1 (4.468%), Uv11 (4.068%), Uv7 (4.00%), Uv6 (3.576%), Uv8 (3.372%) and Uv10 (3.320%). Lowest percent grain infection was observed with isolate Uv9 with 1.462% grain infection, followed by isolate Uv2 (1.912 %) and Uv12 (3.279 %) (**Table 3**). In control plants no false smut symptom was observed.

Table 3: Variation in pathogenicity of different isolates of *U. virens*

Isolates	% Grain infection	
	Mean	S.E.
UV1	4.468	0.604
UV2	1.912	0.137
UV3	4.557	0.521
UV4	5.050	0.253
UV5	7.936	0.855
UV6	3.576	0.128
UV7	4.000	0.800
UV8	3.372	0.130
UV9	1.461	0.531
UV10	3.320	0.266
UV11	4.068	0.263
UV12	3.279	1.229
Control	0.000	0.000
C.D.	1.125	
SE(m)	0.383	

Development of the characteristic symptom in the inoculated plants confirmed the isolated fungi as *Ustilaginoidea virens* and also suggests that inoculation of *Ustilaginoidea virens* by injecting at booting stage of rice is a very efficient method of inoculation. The same has also been reported by the work of Wang *et al.,* (2008) who have tested different method for inoculation of *Ustilaginoidea virens* in rice plants and found that injection of inoculum at booting stage produce a greater number of false smut spore balls than all the other method tested. Variations in the disease severity in rice plants developed by the inoculated isolates suggest the variation in specific interaction of different isolates with the rice variety. Zhang *et al.,* (2003b) in his study for pathogenicity test with different isolates of *Ustilaginoidea virens* have reported significant variation in the disease severity suggesting the specific interaction between the different strain and variety. Lu *et al.,* (2009) from their study have also revealed differences in the ability to infect rice hybrids in all the 59 isolates collected from different places. They reported that same isolates had caused different level of disease severity in different rice varieties tested. They found that among the 59 isolates only 18 (30.51%) isolates could infect all the three rice hybrids, 28 (47.46%) isolates were able to infect one or two rice hybrids and the remaining isolates (22.03%) were not able to infect any of the three varieties suggesting variation in interaction between the isolates and the varieties. Zang *et al.,*(2004) from their study have suggested that inoculation of *Ustilaginoidea virens* by injecting at booting stage can cause infection of panicle upto 100%.

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Author's contribution

Conceptualization of research (ST, SD); Collection of samples (ST, DB, PS) ; Execution of lab experiments and data collection (ST); Analysis of data and interpretation (ST, DB, PS, SD); Preparation of the manuscript (ST, DB, PS, SD).

Declaration

The authors declare no conflict of interest.

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