Incidence and epidemiology of rice (*Oryza sativa*) false smut caused by *Ustilaginoidea virens* in northern India

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Received: 12 May 2023; Accepted: 20 September 2023

ABSTRACT

False smut of rice (*Oryza sativa* L.) caused by *Ustilaginoidea virens* (Cooke) Takah., 1896 is an important emerging disease of rice. An experiment was conducted during 2018–19 and 2019–20 at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh to study the *U. virens* prevalence and morphological variability in northern India with the effect of weather variables on the percentage disease incidence (PDI) of false smut of rice. The study revealed that the false smut incidence was higher in Varanasi district of Uttar Pradesh (53.64%). The lowest percentage (20.68–27.55%) of infected tillers was found in Kangra district of Himachal Pradesh, and Tehri Garhwal district of Uttarakhand. The grain replacement percentage was highest in the Moti cultivar (51.17%), while the highest yield loss percentage was in the Pusa Basmati (57.11 %) variety. The majority of the isolates in our investigation had fluffy, raised, and irregular colony morphology. The correlation between the false smut disease incidence and weather has been evaluated in the Varanasi district, and found that the temperature of 20.36°C with an RH of 85.13% and low rainfall of 8.92 mm was conducive to disease initiation and positively correlated with the PDI. Other epiphytotic factors like sunshine hours, and wind speed were all found to be negatively linked with PDI. In this investigation, for the first time, the viability of spores was also checked. It was found that 2-year-old spores were not viable because they did not retain any Brilliant Cresyl blue colour dye, which stains the stored food material in spores, while 1-month-old spores were found to be 100% viable. All the isolates were further confirmed with the specific ITS marker.

Keywords: False smut, ITS markers, Prevalence, Varieties, Weather

Rice (*Oryza sativa* L.) is one of the leading cereal crops in the world, including maize and wheat (Husson *et al.* 2022). India and China are the leading rice-producing countries in the world and they are facing the issue of false smut disease in their fields. Nevertheless, the incidence of false rice smut accounted for 2–85% yield losses in India (Ladhalaxmi *et al.* 2012). In addition to yield loss, this disease also causes deterioration of grain quality, viz, grain length, grain breadth, length-to-breadth ratio, cooking quality, total soluble protein and antioxidants (Bag *et al.* 2021). The pathogen belongs to the family Clavicipataceae with its biotrophic nature (Fan *et al.* 2016). The infected plants can be identified only during the panicle emergence stage with white mycelium on the spikelets, which later turns to yellow to black smutted balls (Bag *et al.* 2017).

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The causes of the vulnerability of commercial cultivars against the false smut are high inputs of fertilizers, a narrow genetic base, and changing climatic scenario. Thus, resistant varieties show susceptible reactions to this disease (Singh et al. 2021). The survey and epidemiological studies are less studied fields in false smut incidence. The survey and incidence have been seen in different regions of Tamil Nadu, Punjab, and some regions of Odisha (Ladhalaxmi et al. 2012, Baite et al. 2017), but other areas of India are less explored. Another important field that has had an impact on the false smut occurrence is weather correlation. The studies were insufficient and not clear in epidemiological aspects as some says cloudy weather and wet days during the time of flowering favour disease development (Sanghera et al. 2012, Raji et al. 2016). While other made emphasis on less rain (Alase et al. 2021).

Owing to the huge economic losses caused by false smut and its irrational occurence, a thrust for detailed study of pathogen epidemiology, transmission, characterization, and pathogenesis is required (Bag *et al.* 2021, Masurkar *et al.* 2022). Thus, in the present investigation, we have shed some light on the false smut epidemiology and its status in various central and north Indian rice-growing states.

MATERIALS AND METHODS

Survey and disease occurrence: Observations were taken during the zig-zag marking of a 1 to 5 m² area in the farmer's field during the grain filling stage of the crop in 2018–19 and 2019–20. The surveyed regions were districts and villages of the states, viz. Uttar Pradesh (UP), Madhya Pradesh (MP), Himachal Pradesh (HP), and Uttarakhand (UK) (Table 1).

Observations were recorded for the percentage of infected tillers/m², the number of smut balls/panicle, 1000-grain weight reduction percentage, and the grain replacement percentage. Percentage infected tillers were estimated using the formula, and the number of percentage infected balls was calculated as:

Grain replacement = Number smutted grains per panicle –
Number of grains per diseased panicle
Percentage Number of grains (Healthy + diseased)

Apart from these, collected isolates were purified for the characteristics of pure growth culture, a colour pattern of the colony, mycelia growth (diameter mm), average growth rate per day (mm), and *sclerotia* formation in the Department of Plant Pathology, Banaras Hindu University, Varanasi, Uttar Pradesh.

Isolation, purification and maintenance of isolates: The collected samples were taken in a plant pathology laboratory, Banaras Hindu University, where each sample was grown onto potato dextrose agar (PDA) media. The individual balls were sterilized using a 0.1% concentration of HgCl₂ and rinsed 3 times with distilled water. Individual balls were cut into small pieces with a sterilized scalpel, and the middle part of the smutted ball, having yellow colour, was used for inoculation on PDA due to the presence of live mycelia having maximum viable spores. The spores were taken for further purification (Bag et al. 2021).

DNA isolation and purification: A 5 mm bit of the purified culture was put into a 100 ml conical flask with 50 ml of PSB (Potato Sucrose Broth) and shaken at 150 rpm for 20 days at 26°C. Mycelial growth was collected, dried on Whatman filter paper, frozen to liquid nitrogen, and then ground with a mortar and pestle into a fine powder. The genomic DNA was extracted from 200 mg of powdered mycelia in a sterile Eppendorf tube using

Table 1 Occurrence and incidence of rice false smut disease in selected districts of North India

Location	Varieties	Percentage infected tillers (Mean)			Infected balls/ panicle (Mean)		Pooled
		2018	2019	Pooled	2018	2019	_
Uttar Pradesh							
Varanasi	Nati Mansuri, Sonam, Moti, Kaveri Sampurna	57.28	50	53.64	17.5	15.5	16.5
Chandauli	Kaveri Sampurna, HPR2143, BPT 5204, Chintu, Moti Gold	44.28	37	40.64	10.5	5	7.75
Mirzapur	HUR-917, Moti Gold, Dhanrekha, Krishna, JRH 4, Sonam	48.71	57	52.855	6	11.5	8.75
Deoria	PB 1, Moti, Pusa RH-10, BPT 5204, Moti, Lakhamandal, JRH 19, HPR 2612	48.28	37.5	42.89	15.5	7.5	11.5
Lucknow	DSN-11	31.28	35	33.14	6.5	3	4.75
Faizabad	PB 1	29.6	23	26.3	11	4	7.5
Madhya Pradesh							
Maharajpur	Moti	45.63	34.5	40.065	3.12	4	3.56
Umaria	JR-201	29.51	30	29.755	4.52	4.5	4.51
Jabalpur	Moti	37.28	47.5	42.39	11.1	7	9.05
Sehora	Sonam	35.58	54	44.79	4	4	4
Balaghat	HMT	35	43	39	4.51	2	3.255
Himachal Pradesh							
Palampur	Palam dhan 957	26.28	48.5	37.39	6.1	3	4.55
Kasauli	Ram jwan	31.14	34	32.57	5.5	3.5	4.5
Banuri	HPR 1068	31.21	26.5	28.855	5.5	3.5	4.5
Una	HPR 2143	25.26	33	29.13	3.5	3	3.25
Kangra	Sukara Dhan 1	15.86	25.5	20.68	4.36	4	4.18
Uttarakhand							
Pantnagar	BPT 5204	24.85	44	34.425	3.5	4	3.75
Nainital	PD-6	16.28	39.5	27.89	4.5	4.5	4.5
Dehradun	VL Dhan-39	53.8	52.5	53.15	17.5	3	10.25
Champawat	Narendra Dhan 2	48.28	34.5	41.39	15.5	3.5	9.5
Tehri Garhwal	KRH 2	29.6	25.5	27.55	11	5	8

a DNA isolation kit (Zymo Research, USA). During the DNA isolation from kit, genome lysis buffer 800 µl was used for mixing the mycelium by vortexing, and this material was transferred into the Zymospin column II. Than Zymospin column II was treated with DNApre buffer 200 µl and centrifugation (>10000 rpm) was done. After this process, the samples were transferred into the clean microcentrifuge tube. After this the 50 µl DNA elution buffer was added and again centrifugation was done so that the DNA dissolved in the elusion buffer. After the isolation of DNA, it was quantified on 1.8% of agarose gel electrophoresis and a nanodrop of 260/280 nm absorbance to ensure purity. A pure form of DNA was obtained using the treatment of RNAse. The isolated DNA samples were checked for specificity of the pathogen i.e. U. virens by ITS, US1-5 (CCGGAGGATACAACCAAAAAAACTCT) and US 3-3 (GCTCCAAGTGCGAGGA-TAACTGAAT).

Correlation of weather parameters to false smut disease: Field trials were conducted at the Agriculture Farm, Banaras Hindu University, Varanasi (Latitute: 25.2557; Longitude:

82.9877), Uttar Pradesh. Sowing was done on June 15 of each year i.e. 2018–19 and 2019–20 during the rainy (*kharif*) season and the lowland rice field was puddled with the rotavator. After 21 days of sowing, seedlings were transplanted in the prepared field. The close spacing of 15 cm × 15 cm was kept and a higher recommended dose of nitrogenous fertilizer i.e. 100 kg/acre was used. Randomized block designs for weather correlation with the false smut disease incidence were used. The data were laid out from the month of September to November. In this study, the HUR-105 variety was selected for sowing in the field.

Spore viability test: A brilliant cresyl blue stain (CI51010) was used to check the viability of the *U. virens* spores. The spores were taken from the smut balls and fresh culture. The CI51010 were mixed in water for assessment then the spores were stained from the dye and kept for 1 h so the dye could be absorbed by the spores. The dye retention percentage was calculated as:

Dye retention percentage =
$$\frac{\text{Number of spores retained dye}}{\text{Total number of spore assessed}} \times 100$$

Statistical analysis: The statistical analysis, correlogram, heat map and chart correlation diagram was done to determine the weather variants' correlation with the per cent disease incidence (R Core Team 2013).

RESULTS AND DISCUSSION

Incidence of false smut of rice: The surveys were done in the 4 states of India, viz. Uttar Pradesh, Madhya Pradesh, Himachal Pradesh, and Uttarakhand, for the percent disease incidence. The percent of infected tillers

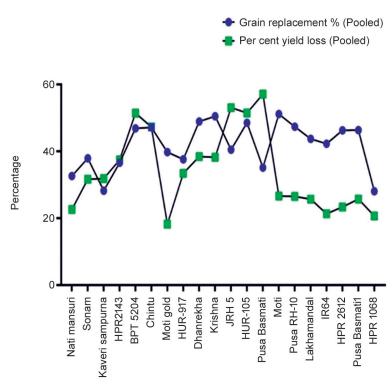


Fig 1 Grain replacement and yield loss per cent in 20 cultivars of rice.

was seen highest (53.64%) in Varanasi (UP) followed by Dehradoon (UK) (53.15%). A minimum percentage of infected tillers were observed in Kangra (HP), and Faizabad (UP) with per cent infected tillers of 20.68% and 26.3%, respectively (Table 1). The maximum number of infected balls/panicles was found in the cultivars of Varanasi (UP) (16.5%), followed by Deoria (UP) (11.5%). The lowest number of infected balls were recorded in Una (HP) (3.25%), followed by cultivars of Balaghat (MP) (3.255%) (Table 1). Some researchers done survey in various regions of India, Ladhalaxmi et al. (2012) for districts of Punjab and Tamil Nadu and Baite et al. (2017) for Cuttack, Odisha. High incidence false smut was also found in the districts of Punjab and Tamil Nadu (Ladhalaxmi et al. 2012). The Raigarh district of Chhattisgarh also severely infected by false smut pathogen (Singh and Pophaly 2010). Recently, Ladhalaxmi et al. (2019) reported the average number of smut balls/panicles varied from 1 to 26 in rice genotypes. The grain replacement percentage and yield loss due to false smut of rice were also checked in the surveyed varieties. The highest grain replacement due to smutted balls was found in the Moti variety (51.17%), followed by the Krishna variety. However, the lowest grain replacement percentage was found in the HPR1068 variety, followed by 28.25% in the Kaveri Sampurna variety (Fig 1). Baite et al. (2017) also screened various varieties against similar trait and reported Swarna Sub-1 as the most replaced by Ustilaginoidea virens smut balls.

The percentage yield loss estimation was also estimated for the *kharif* 2018–19 and 2019–20. The highest yield loss was observed in Pusa Basmati-6 (57.11%), followed

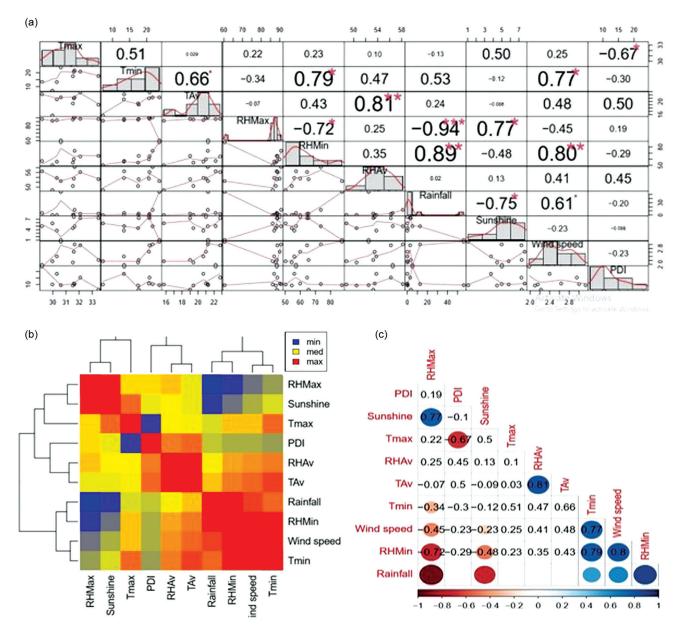


Fig 2 Correlation of weather features with per cent disease incidence (Pooled 2018-19 and 2019-20).

by JRH-5 (53.08%). The varieties with the lowest per cent yield loss were found in Moti Gold (18.23%), followed by HPR1068 with 20.68% (Fig 2). The yield loss parameters were also studied by Sarker *et al.* (2016), Baite *et al.* (2019 and Jaglan *et al.* (2023).

Correlation coefficient analysis of weather and per cent disease incidence: During the 2018–19 and 2019–20 study periods, the correlation plot indicates that three weather parameters, i.e. average temperature (20.36°C), maximum relative humidity (85.31%), and low rainfall (8.92 mm), positively correlate with the PDI (per cent disease incidence), while the maximum (31.16°C) and minimum temperatures (17.33°C), wind speed (2.51 m/s) and sunshine (5.26) showed a negative correlation with the PDI. The average temperature showed a correlation of 0.50, maximum relative humidity (0.19), and rainfall

(0.20). While the minimum temperature (-0.30), wind speed (-0.23), sunshine (-0.098), average relative humidity (-0.19), and minimum relative humidity (-0.29) have a negative correlation. The maximum temperature was also negatively correlated (-0.67), having one star, which indicates a level of significance at 10%. Based on our analysed data, only three parameters were significantly correlated with the disease. In Fig 2 the correlation plot with histograms, density functions, smoothed regression lines and correlation coefficients with the corresponding significance levels were represented; if no stars, the variable is not statistically significant, while one, two and three stars mean that the corresponding variable is significant at 10%, 5% and 1% levels, respectively. The same data were represented with heat map and correlogram. The circle size and colour intensity have a direct relationship with the correlation coefficients. The correlation coefficients and

Table 2 Assessment of viability of spores from various time scales

Spores (month old)	Dye retention (%)	Glycogen	Viability test in petri plates
24 month	0.0	no	no
12 month	16.7	Yes	Less viable
6 month	67.0	Yes	Moderately viable
3 month	91.8	Yes	Viable
1 month	100.0	Yes	Full viable
SEM		0.69	
CD (P=0.05)		2.27	
CV		3.07	

their associated hues are displayed in the legend colour on the correlogram's right side. In the heatmap of the correlation between weather features and the per cent disease incidence indicated and in the correlogram positive correlations are shown in blue, while negative correlations are shown in red. The circle size and colour intensity have a direct relationship with the correlation coefficients. The correlation coefficients and their associated hues are displayed in the legend colour on the correlogram's right side (Table 2).

The correlation coefficient analysis indicates that the high temperature >31° and lower temperature <17° both retard the *U. virens* incidence. The average temperature of 20.36°C positively correlated with the false smut incidence. This result suggests that there might be a temperature range (31.16–17.33°C) favourable for higher disease incidence. The false smut incidence was positively correlated with the optimum temperature of pathogen growth, i.e. 25°C ±2 (Jia et al. 2015). The disease incidence will decrease if the temperature is below or above this range. Alase et al. (2021) found a negative correlation between the minimum temperature and the false smut disease. We discovered a link between maximum RH and disease incidence. Past researchers also found that the higher the relative humidity, the higher the chances of false smut disease incidence (Alase et al. 2021). We also observed that low rainfall was positively correlated with disease incidence. Similarly, most researchers found a positive relationship between low rainfall and disease incidence (Saha et al. 2020), as in our studies.

Other weather parameters like temperature minimum and maximum, relative humidity minimum and average, sunshine hours and wind speed were found to have a negative correlation with false smut disease. According to the studies on sunshine hours, bright sunlight has a negative impact on false smut incidence, implying that cloudy weather favours disease development. We also found that wind speed correlated negatively with disease incidence. From this result, we can speculate that lower wind speeds (<2.5 m/s) during the months of September to November favour the disease's initiation, while more than that fails to disperse fungal spores effectively or disperses more in the air and does not land on rice plants. Aerobiological studies on false smut disease incidence were done by Saha *et al.* (2020). Anbazhagan (2022) investigated the influence of

wind velocity on the disease progression of false smut and reported that high wind velocities of 7.65 to 12.57 m/s favour more disease.

Spore viability test: The experiment was performed to determine the viability of different time-scale spores. This study would give an idea about the life span of the spores in the field. It was found that the spores that were 24 months old did not retain the Brilliant Cresyl dye and remained colourless. While the other samples were found to have retained the dye. It was found that 0% of the spores retained the dye in the 24-month-old spores, while the 1-month-old spores showed 100% dye retention. The spore staining results clearly indicate the status of the viability of spores (Boedijn 1956). The stored food material, which is present in the fungal spores of *U. virens* was highlighted by the dye. The energy material-containing spores retained their blue colour, while the other spores remained colourless. The viable spore identification and new species differentiation were done with cresyl blue dye by Lindemann et al. (2014) for Micropeziza spp. and found three new species of Micropeziza.

Internal Transcribed Spacer (ITS) validation: All the collected isolates were further identified at the molecular level through *U. virens*-specific ITS markers. The isolates showed a sharp band of 380 bp at the 100 bp ladder. Similar studies for confirmation of *U. virens* were also done by Bag *et al.* (2021).

The false smut disease was an underestimated and less studied disease, but now the scenario has changed as the impact of the pathogen on the crop has increased and some progress has been made. In this investigation, the survey results were analyzed and found that all Indian states have some incidence of disease, but Uttar Pradesh showed a higher incidence than other Indian states. The smut ball was also more prevalent in this region. The studies also indicate that the grain weights increased due to disease. Grain replacement percentages due to smutted balls were found to be higher in the Moti variety. The yield loss percentage was estimated to measure the yield loss. The highly aromatic variety Pusa Basmati and the high yielding variety JRH-5 were shown to be more prone to disease. The weather correlation, survey, incidence, and spore viability check were made to dwell more on the pathogen behaviour in the Indian context. The temperature range of 25-28°C found ideal for the spread of disease. Max. RH (more than 85%) and low rainfall was linked to a higher risk of disease during the crop flowering initiation stage. Sunshine hours and wind speed have all been demonstrated to be unfavourably related to the disease. The spore viability studies also helped in identifying the life span of *U. virens* spores in the field. This research will guide new and existing researchers' towards understanding the behaviour and prevalence of pathogens.

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