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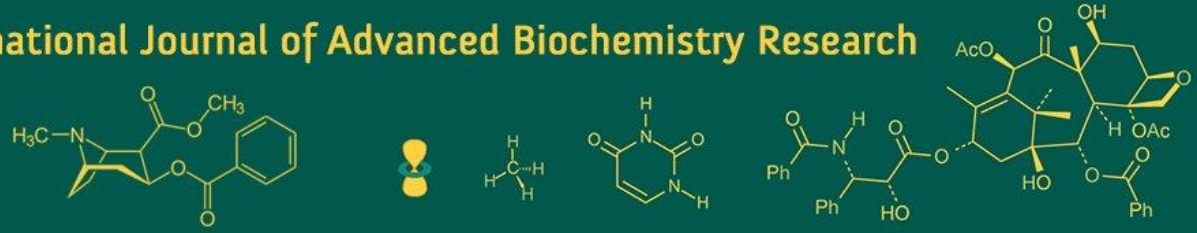

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Studies on false smut of rice caused by *Ustilaginoidea virens* and its' mitigation strategies

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Abstract
Among all the important food grain crops, rice (O*ryza sativa*) is one of the most important worldwide. It is of the Gramineae or Poaceae family. Numerous diseases manifest in rice nurseries and primary field diseases. Among all the diseases, False Smut of Rice (*Ustilaginoidea virens*) will be the subject of our discussion. It is also referred to as "Laxmi" disease, is one of the main diseases affecting rice and is getting more common. It affects most of the rice-growing regions of India and all over the world. Morphological identification and surveying were also done in this study. During survey it was seen that the Punjab state was most affected than the Manipur. Colony morphology showed regular, fluffy, and whitish-yellow with an average growth of about three cm in PDA media. The screening of different media for the best growth media was also seen and PDA was the most suitable for growth. The natural screening of RFS is done in 43 rice elite varieties and we found only three varieties are resistant. The resistant was found in the only one variety i.e. IR 64 DUB1 against the false smut. This study primary focus on the fundamental basic breeding approach i.e. screening and also provide the path for the evolution of disease immune variety.

Keywords: Gramineae, varieties, smut ball, rice false smut, *Ustilaginoidea virens*

Introduction
Rice is of the Gramineae or Poaceae grass family, it's scientifically known as *Oryza sativa*. It is the most remarkable major cereal crop in developing continents like Africa, Asia, South America, and the United States. It is cultivated in warm climates or during the warm summer months. There are a lot of carbohydrates in rice. In rice there are so many diseases shown in nursery and main field diseases (Sindhu *et al*., 2014) [18]. The total production of rice was reduced due to the attack of pests and diseases. Nearly 1.01 to 10.91% yield loss is caused due to fungal infection.

Rice false smut (RFS), also referred to as rice cancer, is one of the diseases affecting rice and is getting more common. In the majority of the major rice-producing regions, develops on rice grains and causes significant yield losses. RFS was once thought to be a sign of a healthy harvest because it was previously described as a minor disease of rice (Chen *et al*., 2022) [6]. Among many diseases, the most harmful one is the RFS. It can also be called Pseudo smut or green smut. The Disease incidence (DI) of False Smut (FS) has the highest amount of infection rate that was found in India, Tamil Nadu having 85% (Dodan and Singh, 1996) [8]. It is triggered by fungal ascomycete phytopathogen *U. virens* (teleomorph: *Villosiclava virens*). Due to this, the quality of the grains gets decreased and even the yield will be less compared to normal crop. As the quality of the grains gets decreased it also affects the health of animals and humans. This is due to the presence of mycotoxins which are found in the infected grains and straws which are poisonous.

RFS do have different management methods like cultural, biological, chemical and even genetic resistance also. The most effective for this fungus pathogen is the *Trichoderma* sp. These biocontrol agents can stop the growth of the pathogen RFS. Many researchers have tried the different management agents but mostly like *Pseudomonas* sp., *Bacillus* sp., *Trichoderma* sp., and *Dendryphiella* sp. Among all these *Trichoderma* sp., *and Bacillus* sp., is most effective for controlling the *U. virens* pathogen in the field and even in the lab condition.

In this paper morphological identification and surveying at Punjab and Manipur, screening of different media for the best growth and in last the natural screening of RFS is done in 43 rice elite varieties and we found only three varieties are resistant.

Materials and Methods

The experiment is conducted at Lovely Professional University which is located between 31.25^0 N and 75.70^0 E longitude at an elevation of 300 m above the sea level.

The Survey was conducted in Punjab which is located between $31^0$04'18.48 N and $75^0$24'16.92 E at an elevation of 300 m above the sea level, with a range from 180 m in the south-west to more than 500 m around the north-east border and in Manipur is lies between $23^0$83'N to $25^0$68'N and $93^0$03E to $94^0$78'E at an elevation of 790 m above the sea level.

Surveying locations of false smut of rice in the Punjab and Manipur

The survey was done in the district-wise villages of Punjab and Manipur. This survey was done between March – June 2023. In this, a small area of about 100-meter square was taken for each field, and starting from the southeast corner of the field, the Zig-zag method will follow. As every 10 meters along with the zig zag path, the survey was done. The survey has been conducted dated on 12th May – 29th of June, in locations of Punjab and during 17th March – 17th April, 2023 in locations of Manipur table 1.

Table 1: Survey data of Punjab and Manipur region

State	District	Subdivision	Teshil	Village name	Lat.	Long.
Punjab	Jalandhar	Jalandhar	Jalandhar I	Gazipur	31.42	75.71
				Jalandhar I	31.41	75.66
	Kaputhala	Phagwara	Phagwara	Chachoki	31.20	75.77
				Jasdil Nagar	31.22	75.78
			Lovely Professional University	Field 1	31.25	75.70
				Field 2	31.25	75.70
				Field 3	31.25	75.70
				Field 4	31.25	75.70
				Field 5	31.24	75.70
				Field 6	31.24	75.60
				Field 7	31.24	75.70
				Field 8	31.24	75.69
				Field 9	31.24	75.69
				Field 10	31.24	75.70
			Kapurthala	Maheru field 1	31.26	75.69
				Maheru field 2	31.26	75.69
				Nanak Nagri field 1	31.26	75.69
				Nanak Nagri field 2	31.25	75.69
			Hardaspur	Near Shiv mandir Hardaspur Field1	31.24	75.71
				Near Shiv mandir Hardaspur Field2	31.24	75.71
				Near Shiv mandir Hardaspur Field3	31.24	75.71
	Ludhiana	Ludhiana	Ludhiana west	Sundar Nagar	30.92	75.88
Manipur	Imphal west	Lamphelpat	Lamphelpat	Maibam Leikai	24.75	93.92
				Keishamthong	24.8	93.9
				Chingamakha	24.77	93.92
		Patsoi	Patsoi	Yurembum	24.76	93.86
				Awang Khunou	24.76	93.84
			Konthoujam	Khumbong	24.76	93.83
				Konthoujam	24.74	93.84
		Lamsang	Sekmai	Khurkhul Makha Leikai	24.92	93.86
			Lamsang	Awang Lairenkabi	24.82	93.82
		Wangoi	Wangoi	Leiphrakpam	24.66	93.92
			Mayang Imphal	Mayang Imphal	24.59	93.88
	Imphal East	Porompat	Heingang	Khabam	24.84	93.95
				Lairik Yengbam Makha Leikai	24.83	93.96
		Keirao Bitra	Keirao Bitra	Keirao Langdum	24.71	93.97
	Bhisnupur	Bhishnupur	Nambol	Nambol Makha Leikai	24.72	93.84
	Kakching	Kakching	Waikhong	Waikhong	24.43	93.93

Preparation of Media

The PDA media was prepared by peeling and slicing 200 grams of potatoes and boiling them in 1 liter of distilled water for about 30 minutes. The effluent part is then saved by filtering the solution with muslin cloth and make the volume up to 1000 ml. To this infusion, add 20 grams of dextrose and 15- 20 grams of Agar, then heat the mixture while stirring continuously until the agar is fully dissolved. Then, make a cotton pluck to covered the conical flask along with the aluminium foil and sterilized the mixture by autoclaving at 121 °C (15psi) for 15- 20 min. Once cooled to around 45- 50 °C, pour the PDA into sterile Petri- dishes under the aseptic conditions, ensuring each dish is covered with about 20-25 mL of the medium. Allow the media to solidify, then put the samples to isolate and then store the plates upside down in a BOD chamber at 27 °C – 32 °C. But the petri plates should seal properly.

Isolation of Pathogen

Isolation of *Ustilaginoidea virens*

The pathogen *U. virens* was isolated from the infected panicles of rice. Infected panicles showing typically symptoms were collected from the field of Lovely Professional University and were taken in the lab. The infected spikelet underwent the serial dilution with 1% of sodium hypo-chloride for 1mins and rice with sterile distilled water thrice was allowed to dry on sterile blotting paper to get rid of excess moisture and cut the spikelet into small pieces and transferred on Petri plates with PDA (Potato dextrose Agar) media supplemented with streptomycin and incubated at 27 °C ± 1 °C for 7 days. The entire procedure was accomplished under laminar airflow (LAF). The cultures were identified with the microscopic studies and pure cultures were obtained from growth for further studies.

Purification and Identification of the pathogens.

Singles-pore isolation or hyphal tip transfer method were used to further purify the pathogen from grown petri plates. With help of sterilized inoculating needles, a tip of sample was taken from the resulting colony and placed in Petri plates with PDA media. The sample was aseptically placed on the center of the Petri-plate and was allowed to grow in the incubator at 27 °C ± 1 °C until fully grown. The pure cultures of *Ustilaginoidea virens* were transferred at 4^0 C in the refrigerator and subculturing of the isolates were periodically done at interval od 30 days.

Identification of pathogen

Based on culture and morphological characteristics the pathogen was identified based on colony color, texture, growth Rate, conidia, and chlamydospores. Microscopic Observation was also done for confirmation of the pathogen.

Screening of germplasm against false smut disease

The observation is done in the LPU Agriculture field, Latitude: 31.249469 and Longitude: 75.698386. The natural screening of RFS has been done in 43 rice elite varieties and we found total 13 varieties are highly susceptible and 3 are highly resistance.

In observations, we do the following process: Number of infected plants, Number of infected panicles, Disease severity, Disease incidence, Varieties name, Height of the plant and Number of tillers, spikelets (Standard Evolution System, IRRI, 2002).

Table 2: Scorecard for Disease reaction

Index	% of infected panicles	Response
0	0%	Highly resistant
1	≤1%	Resistant
3	1.1-5%	Moderate Resistant
5	5.1-25%	Moderate susceptible
7	25.1-50%	Susceptible
9	≥ 50%	Highly Susceptible

Results

Survey

The survey results indicate a wide range of false smut disease incidences across Punjab and Manipur locations. During the survey, the latitude and longitude of the location were also observed.

In Punjab, the highest incidence was recorded in Kapurthala Field 4 (58%), followed by Lovely Professional University Field 3 (56%) and Hardaspur Field 1 (55%). The lowest incidence was observed in Gazipur, Kapurthala (17%). Although, in Manipur, the highest incidence was recorded in Waikhong (25%), followed by Leiphrakpam and Lairik Yengbam Makha Leikai (both 24%). The lowest incidence was observed in Khurkhul Makha Leikai (7%) followed by Yurembum (8%) and Awang Lairenkabi along with the Maibam leikai (10%).

Visual symptoms and Morphology of *U. virens*

Visual Symptoms

Infected spikelet's are converted into conspicuous yellowish-orange to olive-green smut balls. These smut balls are spherical to oval in shape and significantly larger than normal rice grains. The smut balls initially appear yellowish and gradually turn into an olive-green or dark green colour as the disease progresses. Infected spikelet's become chaffy, which means they are hollow and lightweight, often containing no grain at all.

In severe infections, entire panicles can become chaffy and filled with numerous smut balls, giving a black, smoky appearance from a distance due to the mass discharge of spores. (Figure 2.).

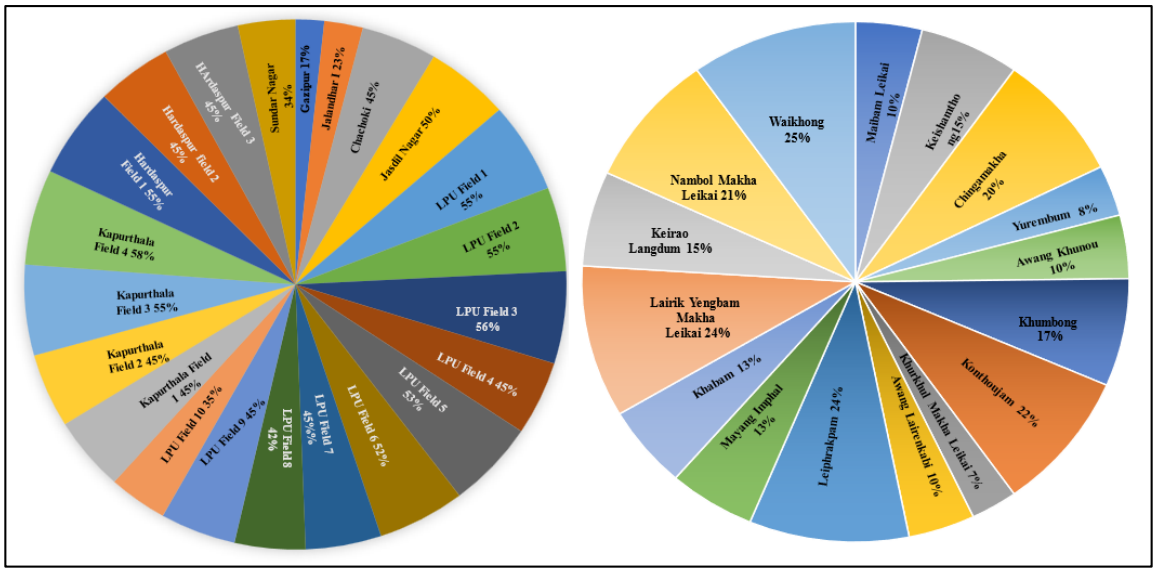
Morphological identification of *Ustilaginoidea virens* (False smut)

The morphological analysis of *U. virens* is identifiable through visual field observations with microscopic and laboratory examination of the pathogen. The disease primarily affects the spikelets, leading to distinctive morphological changes.

The data was collected from the petri-plate culture for a duration of 7^{th}, $14^{th,}$ and 21^{st} days after inoculation (DAI). The result of the pathogen culture is shown in Table 6: The result of the growth diameter of the pathogen in the petri plate on the 7^{th}, $14^{th,}$ and 21^{st} day.

Table 3: Survey of false smut disease of rice for its incidence in Punjab and Manipur Districts

State	District	Subdivision	Teshil	Village name	Diseases incidence = total no. of Plant infected / total no. of plant X 100
Punjab	Jalandhar	Jalandhar	Jalandhar I	Gazipur	17%
				Jalandhar I	23%
	Kaputhala	Phagwara	Phagwara	Chachoki	45%
				Jasdil Nagar	50%
			Lovely Professional University	Field 1	55%
				Field 2	51%
				Field 3	56%
				Field 4	45%
				Field 5	53%
				Field 6	52%
				Field 7	45%
				Field 8	42%
				Field 9	45%
				Field 10	35%
			Kapurthala	Maheru field 1	45%
				Maheru Field 2	45%
				Nasnak Nagri Field 1	55%
				Nanak Nagri Field 2	58%
			Hardaspur	Near Shiv Mandir Hardaspur Field1	55%
				Near Shiv Mandir Hardaspur Field2	45%
				Near Shiv Mandir Hardaspur Field3	45%
	Ludhiana	Ludhiana	Ludhiana west	Sundar Nagar	34%
Manipur	Imphal west	Lamphelpat	Lamphelpat	Maibam Leikai	10%
				Keishamthong	15%
				Chingamakha	20%
		Patsoi	Patsoi	Yurembum	8%
				Awang Khunou	10%
			Konthoujam	Khumbong	17%
				Konthoujam	22%
		Lamsang	Sekmai	Khurkhul Makha Leikai	7%
			Lamsang	Awang Lairenkabi	10%
		Wangoi	Wangoi	Leiphrakpam	24%
			Mayang Imphal	Mayang Imphal	13%
	Imphal East	Porompat	Heingang	Khabam	13%
				Lairik Yengbam Makha Leikai	24%
		Keirao Bitra	Keirao Bitra	Keirao Langdum	15%
	Bhisnupur	Bhishnupur	Nambol	Nambol Makha Leikai	21%
	Kakching	Kakching	Waikhong	Waikhong	25%



A **B**

Fig 1: Disease Severity shown in Villages of a. Punjab and b. Manipur

Fig 2: *U. virens* found in field of Lovely Professional University

Table 4: Data of the Pathogen Culture Diameter

PDA				
Isolates	**DAI (in cm)**			**Colony Diameter (Average mean) (in cm)**
	7	14	21	
I-1	1.5	3	5	3.17
I-2	1.6	2.9	4.8	3.10
I-3	1.3	3.3	5.4	3.33
I- 4	1.2	2.5	4	2.57
I-5	1.7	3.3	4.1	3.03
CD	3.44(5% level)			
Cv	20.02			
Sem±	1.05			

Comparison of different media in growth parameter of false smut of rice

In this study, we found that the maximum colony growth rate was found in PDA media *viz*., I-3 with an average diameter of 3.33 cm, followed by an I-1 and I-2 with an average diameter of 3.17 and 3.10 cm each, and the slowest growth was shown in the I-4 with 2.57 cm. Similarly, the second highest medium was PSA which shows I-5 at 2.23cm followed by I-3 at 2.17 cm and I-2 with an average diameter of 2.13 cm and the lowest is I-4 at 1.7 cm. is PSA media. The third highest is RYDA media *viz*., I-5 at an average of 2.13 cm followed by I-1 at 1.9 and I-4 at 1.73 and the lowest is I-2 at 1.37. The slowest growth was shown in OMA and PDB media showing the minuium growth rate between the 1.26 – 1.7 cm diameter (Table 5).

Table 5: Comparison of different media on the growth of *U. virens*.

Media								
Isolates	**PDA**	**RYDA**	**PSA**	**Oatmeal potato agar**	**PDB**	**Czapek Dox agar**	**Rice Meal Agar**	**Colony Diameter (Average mean) (in cm)**
I-1	3.17	1.9	1.97	1.43	1.5	1.83	1.83	1.69875
I-2	3.1	1.37	2.13	1.43	1.57	1.53	1.63	1.5375
I-3	3.33	1.63	2.17	1.7	1.27	1.7	1.9	1.7075
I- 4	2.57	1.73	1.70	1.7	1.37	1.73	1.97	1.59625
I-5	3.03	2.13	2.23	1.33	1.7	1.67	1.8	1.73625
CD (5% level)	3.44	1.80	2.26	1.36	1.26	1.58	1.68	
Cv	20.02	18.14	19.61	15.89	15.07	16.57	16.24	
Sem±	1.05	0.55	0.69	0.42	0.39	0.49	0.51	

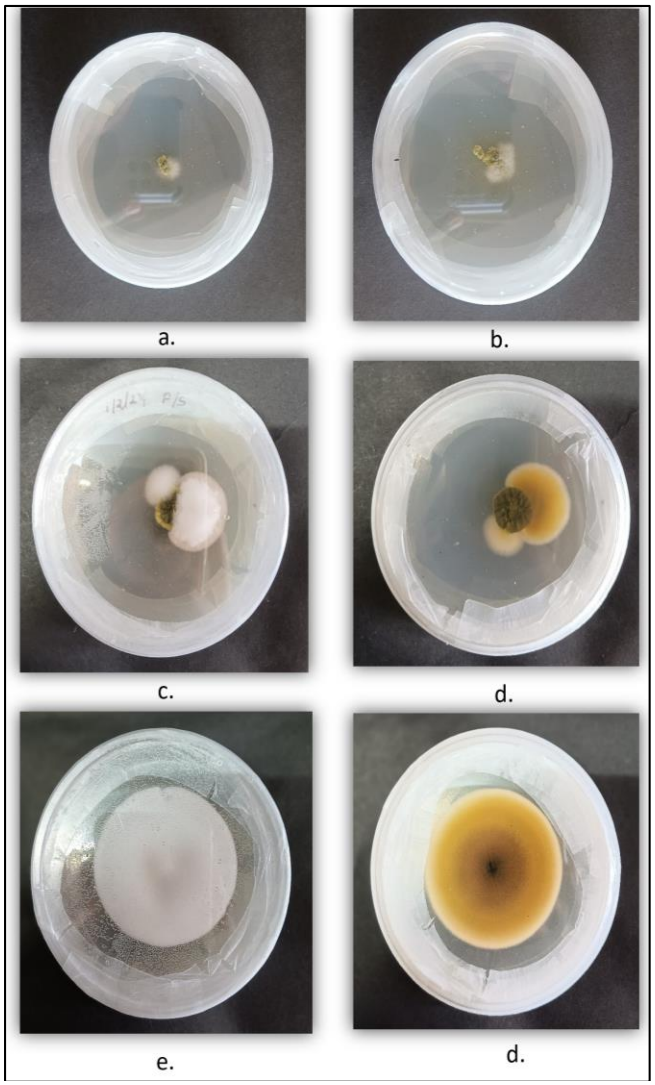


Fig 3: Growth of Pathogen *U. virens*: a. 7th day, b. 10th day, c. and d. 15th day; e and f. 21st Day Growth of Pathogen *U.virens*

Microscopic Characteristics

The smut balls are composed of fungal sclerotia, which are hardened masses of mycelium. These sclerotia can be observed under a microscope, revealing their dense and compact structure. The surface of the smut balls is covered with a powdery mass of fungal spores. The spores are typically globose to sub-globose, measuring about 3-5 µm in diameter. They are smooth, olive-green in color, and contain a single large oil droplet (Fig. 3).

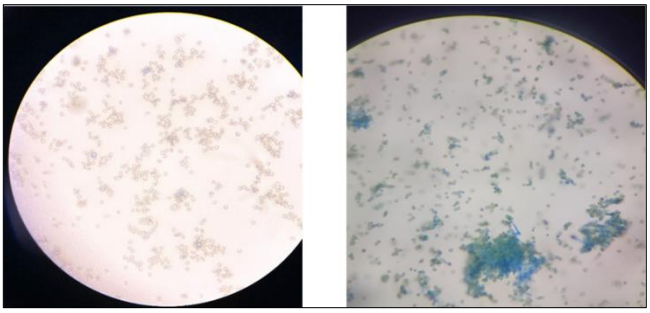


Fig 4: Microscopic structure observation

Screening

The screening for 43 rice varieties *viz*., IR 64 DUB-1, IR- 64 (DRT), BIRDPUR KALANAMAK, PR-121, ASGST-39, DRR50, SAHBHAGI, NAVEEN, ASGST-16, SAMBA SUB-1, PUSA SL -03, CR DHAN 800, HUR-1304, MTU-1010, IR 167 1662, IR 9578-15-1-1-4, HUR -36, ASGST-34, ASGST-36, SAMBA MASURI, GOPAL BHOG, PUSA 1638, SURJOO- 52, LALAAT, BIRSAMATI, CRD202, HUR- 1309, RAJENDREA KASTURI, JGL-384, PUSA SAMBA 1850, HUR-917, PUSA 1776, SGST- 11, SWARNA, ASGST-26, PUSA-1509, HUR-105, IR 82635-B-B- 75-2, HUR 1322, RAJENDRA MAHSURI, CO-51, HUR-2-1, SWARNA SUB – 1 were done.

Among the 43 elite varieties, the data has been categorized into three disease reaction groups: Resistant (1 variety), highly susceptible (10 varieties), moderately resistant (2), susceptible (3), and no reaction (27 varieties). The resistant variety was IR 64 DUB-1, which showed the 15.78% percentage of infected panicles. Moderately resistant were IR-64 (DRT), and BIRDPUR KALANAMAK having the reaction between 26.31- 30%. Highly susceptible varieties were PR-121, ASGST-39, DRR50, SAHBHAGI, NAVEEN, ASGST-16, SAMBA SUB-1, PUSA SL -03, CR DHAN 800, HUR-1304, have infection rates between 66% and 89%. And MTU- 1010, IR 167 1662 and IR 9578-15-1-1-4 shows the susceptible with the range of 54 – 60%. Varieties showing no reaction, such as HUR -36, ASGST-34, ASGST-36, SAMBA MASURI, GOPAL BHOG, PUSA 1638, SURJOO- 52, LALAAT, BIRSAMATI, CRD202, HUR- 1309, RAJENDREA KASTURI, JGL-384, PUSA SAMBA 1850, HUR-917, PUSA 1776, SGST- 11, SWARNA ASGST-26, PUSA-1509, HUR-105, IR 82635-B-B- 75-2, HUR 1322, RAJENDRA MAHSURI, CO-51, HUR-2-1, and SWARNA SUB – 1., exhibit 0% infected panicles.

Table 5: Screening of 43 elite varieties against false smut of rice at natural condition

Varieties	Total no. of panicles	Height of the plant	No. of tillers	No. of spikelets	No. of seeds	No. of Infected panicles	Percent of infected panicles	Disease reaction
IR 64 DUB-1	22	87	14	10	77	0.2	0.909	Resistant
IR- 64 (DRT)	25	119	8	20	130	1	4	Moderately resistant
BIRDPUR KALANAMAK	20	96	15	20	150	1	5	Moderately resistant
PR-121	9	89	14	16	150	6	66.66	Highly Susceptible
ASGST-39	10	91	11	13	105	7	70	Highly Susceptible
DRR50	9	92	9	11	79	8	88.88	Highly Susceptible
SAHBHAGI	10	78	11	12	70	6	66.66	Highly Susceptible
NAVEEN	10	98	20	10	87	7	70	Highly Susceptible
ASGST-16	9	122	7	13	110	6	66.66	Highly Susceptible
SAMBA SUB-1	9	92	12	10	100	7	77.77	Highly Susceptible
PUSA SL -03	9	74	6	11	110	8	88.88	Highly Susceptible
CR DHAN 800	10	91	13	19	85	7	70	Highly Susceptible
HUR-1304	8	76	11	10	99	6	75	Highly Susceptible
MTU- 1010	5	79	7	17	140	2	40	Susceptible
IR 167 1662	10	90	11	15	100	3	30	Susceptible
IR 9578-15-1-1-4	11	77.5	15	19	100	5	45.45	Susceptible
HUR -36	19	84	11	10	99	0	0	No reaction
ASGST-34	25	147	19	15	110	0	0	No reaction
ASGST-36	15	107	19	15	115	0	0	No reaction
SAMBA MASURI	10	113	13	11	130	0	0	No reaction
GOPAL BHOG	13	79	19	17	112	0	0	No reaction
PUSA 1638	5	90	9	19	122	0	0	No reaction
SURJOO- 52	11	128	15	19	125	0	0	No reaction
LALAAT	4	70	5	19	140	0	0	No reaction
BIRSAMATI	9	82	10	11	100	0	0	No reaction
CRD202	9	95	11	11	100	0	0	No reaction
HUR- 1309	7	92	10	10	87	0	0	No reaction
RAJENDREA KASTURI	9	65	7	9	78	0	0	No reaction
JGL-384	9	88	11	10	100	0	0	No reaction
PUSA SAMBA 1850	10	103	22	11	120	0	0	No reaction
HUR-917	8	107	9	14	140	0	0	No reaction
PUSA 1776	10	92	17	13	100	0	0	No reaction
ASGST- 11	9	91	11	9	110	0	0	No reaction
SWARNA	12	107	4	14	120	0	0	No reaction
ASGST-26	8	77.5	11	15	120	0	0	No reaction
PUSA-1509	5	94	9	13	90	0	0	No reaction
HUR-105	10	64.5	12	14	95	0	0	No reaction
IR 82635-B-B- 75-2	10	67	6	5	70	0	0	No reaction
HUR 1322	15	92	11	8	80	0	0	No reaction
RAJENDRA MAHSURI	9	54	10	11	87	0	0	No reaction
CO-51	5	69	7	11	100	0	0	No reaction
HUR-2-1	5	65	7	14	110	0	0	No reaction
SWARNA SUB - 1	5	110	6	13	65	0	0	No reaction

Plant heights range from 54 cm (RAJENDRA MAHSURI) to 147 cm (ASGST-34), with tiller counts from 4 (SWARNA) to 22 (PUSA SAMBA 1850), spikelet numbers from 5 (IR 82635-B-B-75-2) to 20 (IR-64 (DRT) and BIRDPUR KALANAMAK), and seed counts from 65 (SWARNA SUB-1) to 150 (PR-121 and BIRDPUR KALANAMAK).

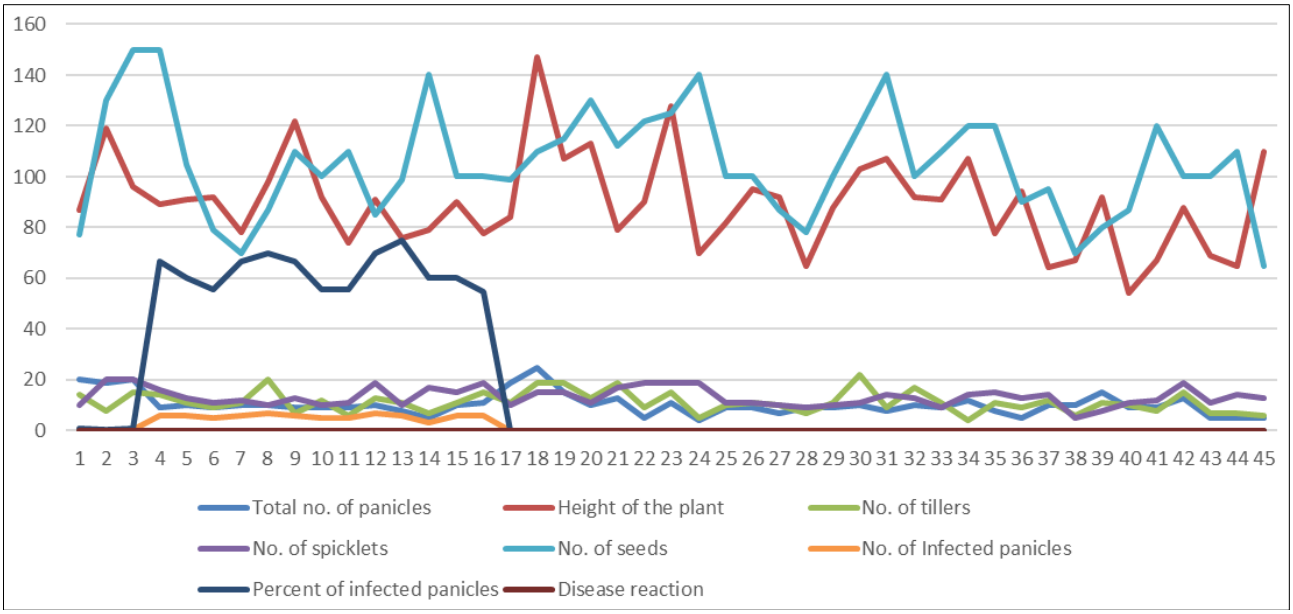


Fig 5: Screening of 43 rice elite germplasms

Discussion

In this paper, we have done basic but important research on various aspects of false smut of rice through surveys, morphological studies, and the identification of resistant sources.

The survey focused on the prevalence and distribution of false smut disease caused by *U. virens* in rice crops. We found that Punjab state has the highest incidence than Manipur. At Punjabs Kapurthala Field 4 (58%) showed highest incidence while, in Manipur region, Waikhong has the highest incidence which was 25%. These results raised the question of why Punjab shows a higher incidence than Manipur. The possible reason behind it is that the state of Punjab more fertilizers were used by the farmers than the Manipur. At Manipur, there is more preference for organic agriculture and they are following it. The increase in the incidence of false smut of rice due to the high use of nitrogenous fertilizers was Rani *et al*., 2015 [17] and Barnwal *et al*., 2009 [2]. Rani *et al*., 2015 [17] worked on this aspect extensively and found that 100 kg urea/acre increases 55% false smut incidence while 40 kg/acre application reduces the false smut incidence by up to 40%.

The comparative study of various media *viz*., PDA, RYDA, PSA, Oatmeal potato agar, PDB, Czapek Dox agar, and Rice Bran Agar have been done for colony growth of *U. virens*. Among all the media it was seen that the PDA showed highest growth of colony i.e. 3.04 cm followed by PSA media with 2.04 cm growth. While the lowest growth of *U. virens* showed in Czapek Dox agar. With 1.482 cm growth. According to Baite and Sharma, 2015 [1], the growth of *U. virens* in their different media *viz*., PDA, RYDA, and PSA. The best growth was achieved in PSA medium 30 days after incubation for most isolates, with diameters ranging from 2.5 cm to 4 cm, followed by PDA and RYDA media, colony diameters ranged from 2.3 to 3.6cm and 2.1 to 3.7 cm. respectively across different isolated. Overall results shows that PDA and RYDA media were also effective in promoting *U. virens* growth.

According to the Bashyal, *et al*., 2021 [5], the PSA is the most suitable media for optimizing the single spore isolation of a pathogen. The *U. virens* were isolated from the rice variety Samba Mahsuri from the Uttar Pradesh region. The PSA medium plates were incubated at 27 ° C ± 1 ° C for 7 days after which white fungal colonies appeared and transferred into the PSA plates for further pure culture. The development of the methodology is comparatively faster with 80-85% success. According to the research, the colony will be an evenly spread creamiest white *viz*., *U. virens* grows after 7-10 days. After 20 days, the growth will be fluffy mycelia and will turn into velvety white very dense leathery growth with a simultaneous change in media from yellow to dark brown.

According to Ladhalakshmi *et al*., 2012 [14], the PDA medium and PDB medium are suitable for simple isolation, The PDA medium was incorporated with the streptomycin to check bacterial contamination. After the successful growth of the pathogen *viz*., *U. virens*, the pure culture was incubated in PDB for 2 weeks and used as the conidial suspension in a plant at a booting stage and incubated for one week at 95%r.h. and observed the symptoms expression about 15 days and rice panicles were used as a control.

A screening of 43 rice varieties was conducted to assess their resistance to false smut disease. Researchers from India, *viz*., Singh *et al*., 2019 [19]; Bag *et al*., 2021 [4], Ladhalakshmi *et al*., 2012 [14] and Lore *et al*., 2021 [13], have examined various features of pathogens, including genetic diversity, disease resistance, and fungal variability. However, the precise mechanism underlying disease incidence remains undetermined.

Natural screening process out of 43 varieties only one variety IR 64 DUB-1 showed resistant disease reaction although Birdpur Kalanamak and IR-64 (DRT) showed moderately resistant reaction. Susceptible MTU-1010, IR 167 1662, IR 9578-15-1-1-4 While highly susceptible was found in PR-121, ASGST-39, DRR50, SAHBHAGI, NAVEEN, ASGST-16, SAMBA SUB-1, PUSA SL-03, CR DHAN 800, HUR-1304, with infection rates between 50% to 75%. Despite this, no cultivars have demonstrated a highly resistant or moderately sensitive disease response in 27 varieties. IR28 (Li *et al*., 2014; Lv *et al*., 2012; Zheng *et al*., 2013) [12, 15, 22], IR36 (Guo and Jiang, 2010) [9], and Rajeet (Bag *et al*., 2021) [4], are some of the varieties that have been proven to be resistant against the false smut based on natural infection in India.

Under natural epiphytotic conditions, 102 genotypes were evaluated in AHRS, Ponnampet, for resistance to FS disease. Of them, 11 genotypes exhibited a highly resistant reaction, while only one variety, IR- 64, displayed a moderately resistant reaction. None of the genotypes were totally resistant. Additionally, 34 genotypes were vulnerable out of 53 that showed a somewhat susceptible reaction. Notably, IET 26219, IET 24518, and ITE 25191 genotypes demonstrated a very vulnerable response to False Smut disease (Banasode and Hosagoudar, 2021) [3].

To assess the resistance of different rice genotypes to False smut under artificial inoculation circumstances, 61 genotypes were evaluated at the Rice and Wheat Research Centre in Malam, 17 showed a highly resistant *viz*., HPR 3236, HPR 3239, HPR 3243, HPR 3248, HPR 3250, HPR 3251, HPR 3253, HPR 3254, HPR 3260, HPR 3261, HPR 3262 PB 1121, Kasturi, HPR 2612, HPR 3228, HPR 3218, HPR 3226, 7 were exhibited resistance *viz*., HPR 3256, HPR 3259, HPR 2929, HPR 3213, HPR 2703, HPR 2696, 23 were moderately resistant and 5 were moderately susceptible. Based on the Disease Incidence, it was discovered that Arize 6129, Arize 6444, Arize Swift, AZ 6508, BS 10008, DR 8101, PAC 834, PAC 807 Plus, and Arize 6129 Gold, were found to be susceptible to False smut (Upmanyu *et al*., 2023) [20].

While in the Directorate of Rice Research, Hyderabad, 29 germplasms of rice were found to be unaffected by FS namely: IET 16295, IET 16298, IET 16303, IET 15836, IET 15862,IET 14807,IET 15295,IET 15296, IET 15298, IET 15314, IET, 15337, IET 15399, IET 10664, IET 15358, IET 15266, IET 16434, IET 16435, IET 16437, IET 15163, IET 15924, IET 159163, IET 15924, IET 15928, IET 15941, and IET 15948, and IET 15949. The results of a propagule viability test showed that as soil depth and storage duration increased, and the percentage of propagules that germinated decreased. Additionally, the protocols fared better in a lab setting than in an uncontrolled field setting with temperature fluctuations (Mohiddin *et al*., 2018) [16].

Certain cultivars, such as Nongxiang 21 (Gan *et al*., 2013) [10], Luxiang 90-1 (Fu *et al*., 2016) and Shuangkang 7701 (Fu *et al*., 2016) appeared to be resistant. Additionally, some works has been done to identify QTLs for the creation of resistant cultivars. After being evaluated for disease resistant QTLs, 2 QTLs, namely QFsr 10 and QFsr12, were discovered among the 266 NILs (Near Isogenic Lines) introgressed lines in TequingxLemonat (Jianlong *et al*., 2002) [21]. Li *et al*., 2011 [11], identified four QTLs on the rice chromosome region: qFsr- 6-7, qFsr- 10-5, qFsr-10-2 and qFsr-11-2. Subsequently, Li *et al*., 2014 [12] discovered additional QTLs for disease resistance in the NILs of the Daguandao/IR28 hybrid.

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