Original Research Article

Techniques for Isolation, Conidiation and Pathogenicity of *Ustilaginoidea virens* Isolates (Uv3, Uv4, and Uv15) Causing False Smut Disease in Rice

Abstract

Rice false smut, caused by *Ustilaginoidea virens* (Uv3, Uv4 and Uv15) showed small mycelial growth during isolation from chlamydospores and mycelial discs of smut balls, and exhibited varied in density of growth at 7 days of incubation. Twenty seven days after incubation, isolate Uv3 exhibited the highest growth at 77.00 mm, characterized by raised elevation and white-yellow surface color with yellow-green pigmentation. Isolate Uv4 showed circular growth with a mycelial growth of 64.00 mm, while isolate Uv15 displayed the lowest growth at 55.66 mm, with flat elevation and white-yellow-green appearance. In BPT 5204 rice leaf extract (BRLE), conidia counts increase significantly with higher concentrations: Uv3 reaches 31.38×10^6 conidia/ml, Uv4 27.71×10^6 conidia/ml, and U15 20.11×10^6 conidia/ml. TN1 rice leaf extract (TRLE) media yields lower counts but shows similar concentration-dependent trends. PSB yields notably fewer conidia. Pathogenicity assessments on TN1 and BPT 5204 rice varieties revealed Uv3 as the most aggressive isolate, causing higher numbers of smut balls per panicle and greater grain infection percentages compared to Uv4 and Uv15.

Keywords: false smut of rice; *U. virens* isolation; rice leaf extract; pathogenicity test; Microscopic observation

1. INTRODUCTION

Rice is the world's second most vital cereal crop, serving as a primary food source for 60% of the global population. Annually, the global rice production stands at 503 million metric tons [14]. In India, during the 2021-22 season, rice cultivation spanned 46.38 million hectares, yielding 130.29 million tons with an average productivity of 2.809 tons per hectare [1]. Nonetheless, rice production is hindered by biotic stresses (such as pests and diseases) and abiotic stresses (such as environmental factors), leading to yield losses between 10% and 30% [10].

False smut, caused by the fungus *Ustilaginoidea virens*, is among the most prevalent and damaging diseases affecting rice fields globally. The first recorded instance of this disease was in the Thirunelveli district of Tamil Nadu, India [4]. Symptoms of false smut manifest on the spikelets of mature rice crops, appearing as green smut balls covered in powdery dark green chlamydospores. Historically, false smut was known as 'Lakshmi disease' and was considered a sign of a good harvest [5]. However, it has recently emerged as one of the most destructive diseases impacting rice grains. Since 2001, the severity of false smut has intensified in major rice-growing states in India, causing notable yield reductions and deterioration in rice grain quality.

Furthermore, pathogen produces large amount of mycotoxins (ustiloxin and ustilaginoidins), which have carcinogenic properties and pose a significant risk to both human and animal health when contaminated rice grains and straw are consumed [6, 15]. False smut balls are associated with many saprophytes, making the isolation of the pathogen very difficult. This experiment details the methods for isolating the false smut pathogen, conidia production using rice leaf extracts, and conducting pathogenicity tests for *Ustilaginoidea virens* isolates (Uv3, Uv4 and Uv5).

2. MATERIALS AND METHODS

2.1 Collection of smut balls

In this study, three smut ball samples collected from Telangana State were used: Uv3 from Morthad Village, Nizamabad District; Uv4 from RS & RRS, Rudrur, Nizamabad; and Uv15 from the Institute of Rice Research, ARI, Rajendranagar, Hyderabad. The experiment was conducted from April to December 2022. Laboratory experiments were performed in the Department of Plant Pathology, College of Agriculture, Rajendranagar. The pathogenicity test was conducted in a polyhouse at the Institute of Rice Research (IRR), Agricultural Research Institute (ARI), Rajendranagar, Hyderabad.

2.2 Isolation of rice false smut pathogen (*U. virens*)

Two methods were used for the isolation of rice false smut pathogen, in first method pathogen isolated from chlamydospores of smut balls (Fig 1A). Initially, the collected smut balls were washed with tap water and then surface-sterilized using 4% sodium hypochlorite (NaOCl) solution for 30 seconds, followed by thorough rinsing with sterilized distilled water three times. Chlamydospore suspension was prepared from the sterilized smut balls and used for pathogen isolation. Potato Sucrose Agar (PSA) was used as the growth medium, added with streptomycin sulphate (100 ppm) to prevent bacterial contamination. The spore suspension was carefully streaked onto Petri dishes containing PSA and these plates placed in BOD incubator at a controlled temperature of 26 ± 1°C for seven days. In second method, pathogen isolated from the central mycelial portion of the smut ball (Fig 1B), for that inner part of smut ball cut into small pieces and subjected to surface sterilization using 4% sodium hypochlorite (NaOCl) solution for 30 seconds, followed by thorough rinsing with sterilized distilled water for three times. The smut ball hard mycelial pieces were placed on sterilized blotter papers to remove excess water from the surface, then placed onto PSA plates and incubated similarly as described in first method. After 7 days of incubation mycelial growth was measured using digital colony meter. The pure culture was prepared using the hyphal tip method for subsequent analysis [2, 3]. Mycelium, chlamydspores and conidia was observed under the compound microscope at 10 and 40x. SEM imaging was done for the chlamydopsores (5000 and 10000x).

2.3 Preparation of rice leaf extract media for the *U. virens* conidiation

Rice leaves collected at the early booting stage were used for media preparation. Specifically, the media included BPT 5204 rice leaf extract (BRLE) and TN1 rice leaf extract (TRLE) at various concentrations (0.02 g/ml, 0.04 g/ml, 0.06 g/ml, 0.08 g/ml, and 0.10 g/ml), along with a control medium of potato sucrose broth (2% sucrose in potato extract). For media preparation, the respective quantity of fresh leaves was taken and washed with tap water, followed by rinsing with distilled water. Subsequently, the leaves were chopped into small pieces, which were then blended in a blender with an appropriate amount of distilled water added. The resulting leaf extract was strained and sterilized in an autoclave at 15 psi (121°C) for 20 minutes. Mycelial discs (10 to 15) from actively growing pathogens (Uv3, Uv4, and Uv15) were added to the autoclaved leaf extract media under laminar air flow, and the inoculated flasks were placed in a rotary incubator at 26 ± 1°C for 7 days at 120 rpm. Conidia were counted using a hemocytometer.

2.4 Pathogenicity test for the U3, Uv4 and Uv15 on rice plants

To confirm the pathogenicity of thee isolates (Uv3, Uv4 and Uv15) and to prove the Koch's postulates, polyhouse experiment was conducted from June to October 2022 at Institute of Rice Research, Agriculture Research Station, Rajendranagar, Hyderabad.

In this studies, the false smut susceptible rice cultivars TN 1 and BPT 5204 were used. The seeds were surface-sterilized by soaking them in 4% sodium hypochlorite for 30 seconds and then washed thoroughly with sterilized distilled water three times to remove chemical residues. Pots with a diameter of 12 inches and a height of 10 inches were filled with a mixture of sterilized farmyard manure and soil in 1:3 ratio. The nursery was established and transplantation was performed after 30 days in pots under polyhouse conditions. Inoculation done with surgical syringe loaded with 2 ml of conidial suspension of *U. virens* (2×10^5 conidia ml^{-1}) was injected into leaf sheaths covering the developing panicle of BPT 5204 and TN 1 plant at late booting stage (2 to 3 days before heading). After injection of inoculum, the plants were kept in polyhouse at a temperature of 26 ± 1°C for 10 days and 95% relative humidity was maintained using fogging system. Subsequently, they were maintained under normal room temperature until the appearance of false smut balls from the emerging spikelets. [7]. Data on number of smut balls per panicle (NSBPP) and percent infected grains (PIG) was calculated as given below [13, 9].

$$\text{Number of smut balls per panicle (NSBPP)} = \frac{\text{Total number of observed smut balls/hill}}{\text{Total number of infected panicles/hill}}$$

$$\text{Per cent infected grains (PIG)} = \frac{\text{Number smut balls/panicle}}{\text{Tolal number of grains/panicle}} \text{ x } 100$$

2.5 Statistical Analysis

The experiments were carried out using a completely randomized design (CRD), and the data were analyzed through a one-way analysis of variance (ANOVA) at a 5% significance level ($P \leq 0.05$) in OPSTAT. Each experiment included three replications.

3. RESULTS

3.1 Growth of *Ustilaginoidea virens* from smut balls

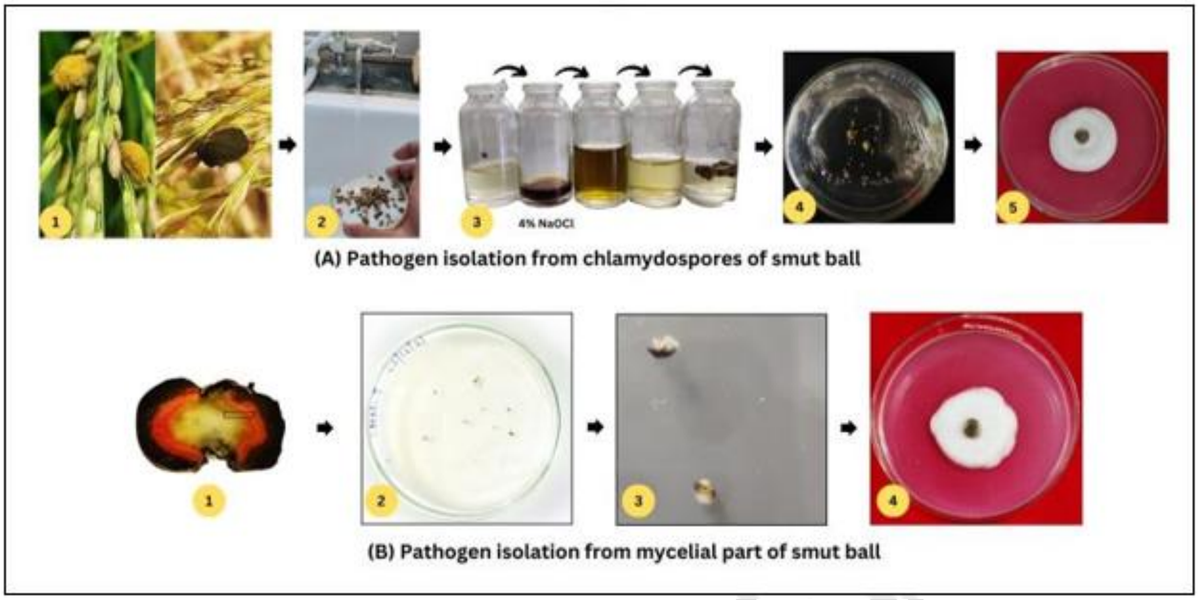
After 7 days of incubation, small colonies with white or green-colored mycelia formed from germinating chlamydospores and central mycelial pieces of smut balls (Fig. 1). The maximum mycelial growth was observed in growth from the mycelial discs of smut balls: 7.20 mm (Uv3), 5.31 mm (Uv4), and 4.43 mm (Uv15), compared to growth from chlamydospore germination: 5.56 mm (Uv3), 3.94 mm (Uv4), and 3.31 mm (Uv15). The density of the mycelia was higher in growth from chlamydospore germination and lower in mycelial growth from mycelial discs of smut balls (Table 1).

After obtaining the pure culture, growth was normalized for the pathogen isolated from both methods. At 27 days after incubationIsolate Uv3 displays raised elevation with irregular growth form and entire mycelial margins. It shows zonation and has a white-yellow surface color with yellow-green reverse pigmentation. Chlamydospores are produced, and it has the highest mycelial growth among the three isolates, measuring 77.00 mm. Isolate Uv4 is characterized by a flat elevation, circular growth form, and filiform mycelial margins. It lacks zonation and sectoring and exhibits a white-green surface color with yellow-green reverse pigmentation. It produces chlamydospores and shows a mycelial growth of 64.00 mm. Isolate Uv15 has a flat elevation, circular growth form, and filiform mycelial margins. It does not exhibit zonation or sectoring. The surface color is white-yellow-green, and the reverse pigmentation is yellow-green. Chlamydospores are produced, and the mycelial growth is 55.66 mm, the lowest among the three isolates.

Table 1. Mycelial growth from germinating chlamydospores, smut ball mycelial discs and pure cultures

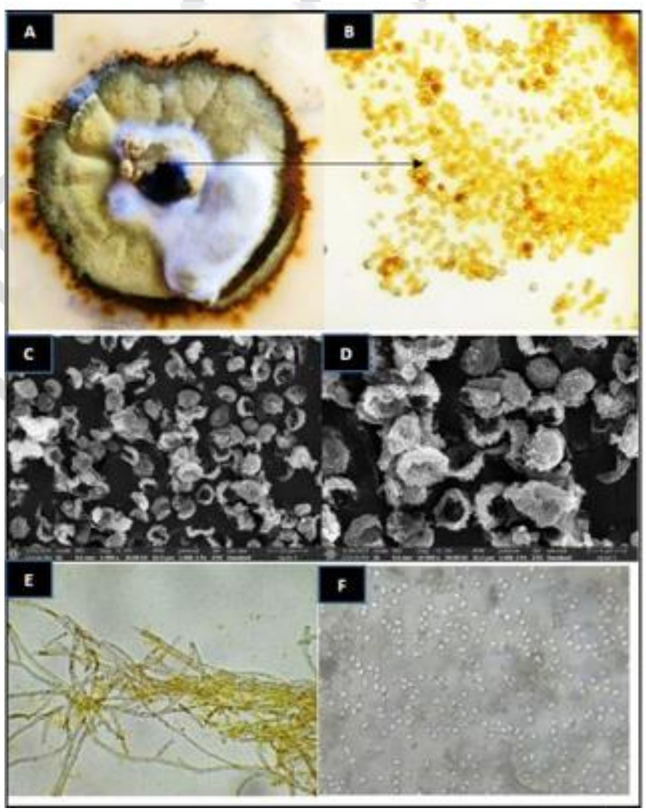
Isolate	**Mycelial growth (mm) at 7 days after incubation**		**Mycelial growth (mm) from pure cultures at 27 days after incubation**
	Chlamydospores of smut balls	**Mycelial disc of smut balls**	
Uv3	5.56±1.03*	7.20±1.52	77.00 ±3.33
Uv4	3.94±0.52	5.31±0.57	64.00 ±2.65
Uv15	3.31±0.51	4.43±0.37	55.66 ±2.46
C.D. (p<0.05)	0.374	0.407	3.265
SEm±	0.106	0.115	0.925

*Mean standard error



A1- collection of smutted rice grains from field, **A2-** washing of smut balls under running tap water, **A3-** surface sterilization of smut balls at 4% NaOCl, thoroughly washing of smut balls using sterilized water for three times and spore suspension preparation, **A4**- small mycelial colonies developed from chlamydospore suspension streaked of on PSA media at one week after incubation, **A5**- 15 days old pure culture of *U. virens*. **B1**- small mycial pieces were cut from inner portion of smut balls, **B2**- surface sterilized mycelial pieces of smut balls placed on PSA media, **B3**- mycelial growth from mycelial pieces of smut balls at one week after incubation, **B4**- pure culture of 15 days old *U. virens*.

Fig 1. Isolation of rice false smut pathogen (*U. virens*) from smutted grain



A- Chlamydospores (Uv3) formed on PSA media, **B-** Microscopic observation of chlamydospores (40x), **C-D-** SEM images of Chlamydopsores (5000x and 10000x), **E-** Geniculate mycelium of *U. virens* (40x), **F-** Oval shaped conidia formation in rice leaf extract media (40x).

Fig 2. Microscopic images of rice false smut pathogen (*U. virens*)

3.2 Conidiation in rice leaf extract media (RLEM)

The Table 2 provided, the number of conidia produced by three strains of *Ustilaginoidea virens* (Uv3, Uv4, and U15) at 7 days after incubation in media include BPT 5204 rice leaf extract (BRLE) and TN1 rice leaf extract (TRLE) at various concentrations (0.02 g/ml, 0.04 g/ml, 0.06 g/ml, 0.08 g/ml, and 0.10 g/ml), along with a control medium (PSB). In the BRLE medium, the number of conidia for Uv3 starts at $5.32x10^6$ conidia/ml at a concentration of 0.02 g/ml and increases significantly with higher concentrations, reaching $31.38x10^6$ conidia/ml at 0.10 g/ml. Similarly, Uv4 produces $4.11x10^6$ conidia/ml at the lowest concentration, increasing to $27.71x10^6$ conidia/ml at the highest concentration. U15 shows the lowest conidia counts among the three strains, starting at 1.64 $x10^6$ conidia/ml at 0.02 g/ml and rising to 20.11 $x10^6$ conidia/ml at 0.10 g/ml. These results indicate a positive correlation between the concentration of BRLE and the number of conidia produced for all three strains. In the TRLE medium, the conidia counts are generally lower compared to the BRLE medium. For Uv3, the conidia count is $3.67x10^6$ at 0.02 g/ml and increases to $25.56x10^6$ at 0.10 g/ml. Uv4 follows a similar trend, with conidia counts starting at $1.45x10^6$ at the lowest concentration and reaching $21.48x10^6$ at the highest concentration. U15 again shows the lowest numbers, with 0.57±0.01 conidia at 0.02 g/ml and increasing to $22.16x10^6$ at 0.10 g/ml. Despite the lower overall conidia counts in TRLE, the increase in conidia with higher concentrations is still evident.

The control medium, PSB, showed significantly lower conidia counts for all strains. Uv3 produces $0.84x10^6$ conidia/ml, Uv4 produces $0.57x10^6$ conidia/ml, and U15 produces only 0.15 $x10^6$ conidia/ml. This stark contrast underscores the influence of the rice leaf extracts on conidia production, highlighting the effectiveness of BRLE and TRLE in promoting conidia formation in *Ustilaginoidea virens*.

Table 2. Effect of different concentrations of rice leaf extract on conidia formation

	Conidiation (x10^6 Conidia/ml) at 7 days after incubation		
Media	**Uv3**	**Uv4**	**U15**
0.02 g/ml BRLE	5.32±0.05	4.11±0.04	1.64±0.02
0.04 g/ml BRLE	9.91±0.13	8.63±0.04	4.37±0.05
0.06 g/ml BRLE	15.67±1.21	13.87±0.26	8.72±0.16
0.08 g/ml BRLE	23.28±1.16	20.16±0.83	14.49±1.13
0.10 g/ml BRLE	31.38±1.91	27.71±1.24	20.11±0.71
0.02 g/ml TRLE	3.67±0.07	1.45±0.02	0.57±0.01
0.04 g/ml TRLE	7.14±0.06	3.16±0.04	2.14±0.04
0.06 g/ml TRLE	12.94±0.62	7.22±0.01	5.31±0.03
0.08 g/ml TRLE	19.48±0.01	13.93±0.86	11.75±0.61
0.10 g/ml TRLE	25.56±1.17	21.48±0.66	22.16±0.77
PSB	0.84±0.02	0.57±0.01	0.15±0.00
C.D. ($p<0.05$)	2.56	1.66	1.48
SEm±	0.87	0.56	0.50

*Mean standard error, BRLE- BPT 5204 rice leaf extract, TRLE- TN1 rice leaf extract

3.3 Microscopic observations of *U. virens*

Microscopic observation (Fig. 2) revealed that the mycelium of the fungi was septate and hyaline in color. On PSA media, chlamydospores formed from pseudomorphs started as smooth spheres with an orange-yellow hue, maturing into dark brown echinulate structures with a rough surface. On leaf extract media, oval-shaped conidia formed for all three isolates.

3.4 Pathogenicity test for *U. virens*

After 20 days of inoculation, the inoculated plants have shown the symptoms of smut balls (Fig. 3e). Again, the *U. virens* have been successfully isolated from these smut balls on PSA medium and Koch's postulates was proved for *U. virens*. The fungus infected the young ovary of the individual kernels and transformed them into large, velvety green balls. Smut balls were initially white in colour visible between glumes, grew gradually and covered with a mycilial membrane which bursted in the later stage and released the chlamydopspores which gave the yellow to orange colour appearance to the smut balls, later turned to olive green to black (Fig 4a).

When smut balls were young, they were fleshy inside and hardened over time. The smut ball consisted of a central hard mycelial tissue consisting of thin, hyaline septate hyphae (Fig 4b).

The results of the study on the number of smut balls per panicle (NSBPP) and the percent infected grains (PIG) by *Ustilaginoidea virens* isolates Uv3, Uv4, and Uv15 on two rice varieties, TN1 and BPT 5204, demonstrate significant differences in pathogenicity (Table 3). For the TN1 variety, isolate Uv3 showed the highest NSBPP at 15.55 and PIG at 23.32%, indicating its aggressive nature in causing false smut disease. This was followed by Uv4, which had an NSBPP of 8.41 and a PIG of 12.62%, and Uv15, with an NSBPP of 5.02 and a PIG of 7.53%. Similarly, for the BPT 5204 variety, Uv3 again exhibited the highest levels of infection, with an NSBPP of 9.56 and a PIG of 21.03%. Uv4 and Uv15 followed, with NSBPPs of 3.71 and 2.75, and PIGs of 8.16% and 6.05%, respectively.

Table 3. False smut disease caused by Uv3, Uv4 and Uv15 in TN1 and BPT 5204

Isolate	**TN1**		**BPT 5204**	
	NSBPP	**PIG**	**NSBPP**	**PIG**
Uv3	15.55±1.89*	23.32±1.28	9.56±1.01	21.03±1.32
Uv4	8.41±1.48	12.62±1.22	3.71±0.68	8.16±0.57
Uv15	5.02±0.51	7.53±1.14	2.75±0.50	6.05±0.43
C.D. (p<0.05)	3.972	3.773	2.423	2.873
SEm±	1.126	1.07	0.687	0.815

*Mean standard error, NSBPP- number of smut balls per panicle, PIG- percent infected grains

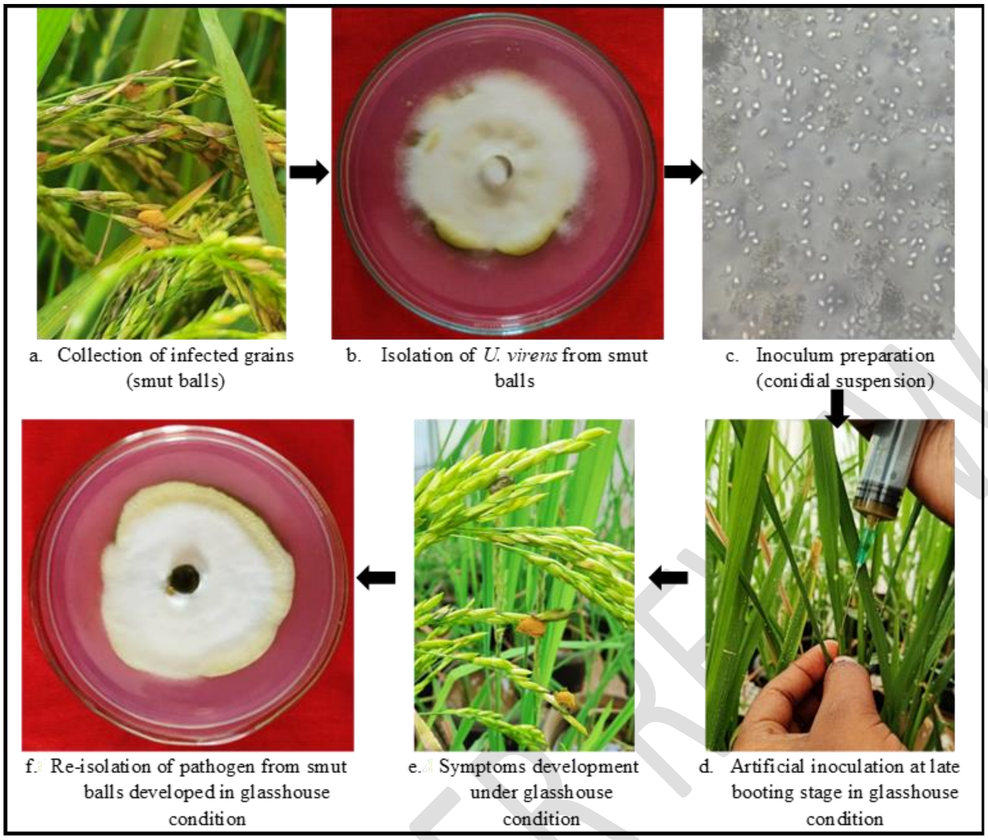


Fig 3. Pathogenicity test for false smut of rice caused by *Ustilaginoidea virens*

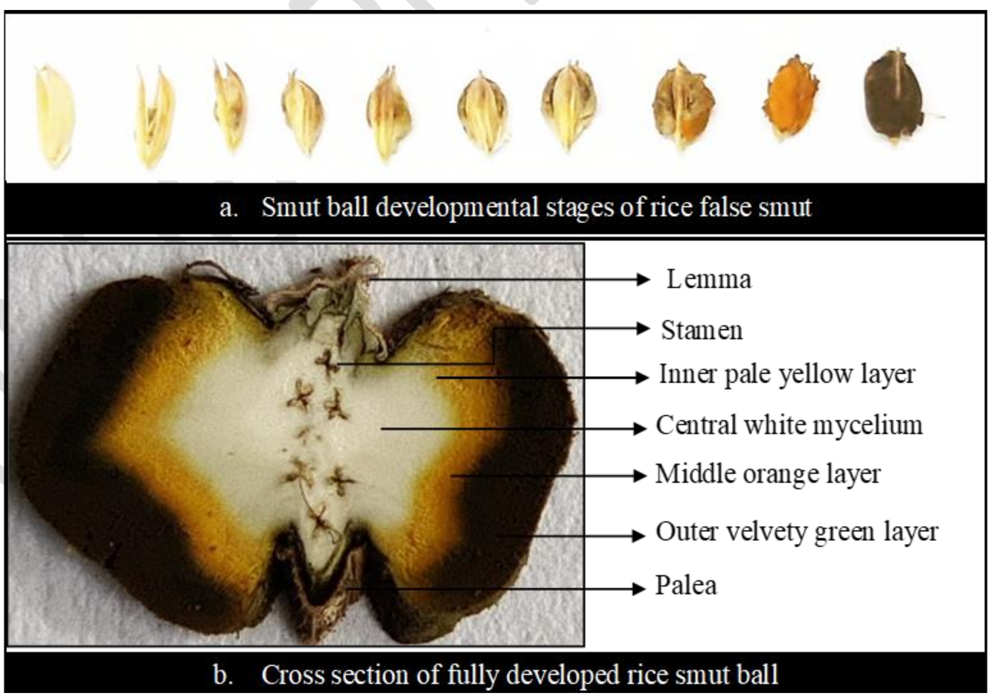


Fig 4. Developmental stages and cross section of rice false smut ball

4. DISCUSSION

Rice false smut pathogen, *Ustilaginoidea virens* (Uv3, Uv4 and Uv15) showed varied mycelial growth from chlamydospores and mycelial discs of smut balls at 7 days after incubation. Maximum growth was observed from smut ball discs: 7.20 mm (Uv3), 5.31 mm (Uv4), and 4.43 mm (Uv15), compared to chlamydospore germination: 5.56 mm (Uv3), 3.94 mm (Uv4), and 3.31 mm (Uv15). Mycelial density was higher from chlamydospores and lower from smut ball discs. Prior studies used chlamydospores for isolating the false smut pathogen [3], noting its slow growth over one to two weeks to form large colonies [8]. After obtaining pure cultures, the growth characteristics of *Ustilaginoidea virens* isolates Uv3, Uv4, and Uv15 were standardized and analyzed. Uv3 exhibited the highest mycelial growth at 77.00 mm, followed by Uv4 at 64.00 mm and Uv15 at 55.66 mm. Additionally, these isolates displayed distinct cultural characteristics. Uv3 showed raised elevation with irregular growth form, a white-yellow surface color, and yellow-green reverse pigmentation, while Uv4 and Uv15 exhibited flat elevation with circular growth forms and varying surface and reverse pigmentation patterns. These findings are consistent with previous studies. Savitha et al. [11] reported colony diameters ranging from 42 mm to 71.75 mm in *U. virens*, reflecting variability similar to what we observed. Similarly, Sekhar et al. [12] documented colony diameters spanning from 10.14 mm to 85.68 mm, with growth rates ranging from 0.33 mm to 2.85 mm per day, indicating significant variation in growth patterns across different isolates. Baite et al. [2] also reported mean colony diameters ranging from 25 mm to 40 mm, further supporting the variability in growth characteristics observed in *U. virens* isolates.

Ustilaginoidea virens produces more conidia in BPT 5204 rice leaf extract (BRLE) compared to TN1 rice leaf extract (TRLE), with higher concentrations of these extracts leading to increased conidia production. Among the strains, Uv3 is the most prolific in conidia production, followed by Uv4 and U15. The control medium, PSB, results in significantly lower conidia counts, highlighting the positive impact of rice leaf extracts on the conidia production of *Ustilaginoidea virens*. These findings are consistent with those of Wang et al. [16] who reported that among the tested media, a 0.10 g/ml panicle medium was most efficient for conidiation. Furthermore, certain rice leaf media, except for the 0.10 g/ml panicle medium, were more effective in increasing conidiation than panicle media. This underscores the potential of rice leaf extracts in enhancing the conidiation of *U. virens*, providing a valuable insight into optimizing media conditions for the study and management of this pathogen.

After 20 days, plants showed smut balls, and *Ustilaginoidea virens* was isolated, confirming Koch's postulates. The fungus infected the young ovary, turning kernels into large, green balls that started white, grew, burst to release spores, and turned from yellow to black over time. The study revealed differences in pathogenicity among isolates Uv3, Uv4, and Uv15 on TN1 and BPT 5204 rice varieties. For TN1, Uv3 had the highest smut balls per panicle (15.55) and the highest infected grains percentage (23.32%), followed by Uv4 and Uv15. For BPT 5204, Uv3 also had the highest infection levels, followed by Uv4 and Uv15. These findings align with previous studies by Fu et al. [17], Yong et al. [19], Ladhalakshmi et al. [20], and Ashizawa et al. [18], who examined the pathogenicity of false smut fungi in rice cultivars.

5. CONCLUSION

The study demonstrated that *Ustilaginoidea virens* isolates Uv3, Uv4, and Uv15 showed varying growth patterns and conidiation rates. Uv3 exhibited the highest mycelial growth and conidia count, indicating its higher virulence. Pathogenicity tests confirmed Uv3 as the most aggressive isolate, causing the most severe infections on TN1 and BPT 5204 rice varieties. These findings highlight the importance of identifying and managing the most virulent strains to control rice false smut effectively.

6. REFERENCES

1. Anonymous. Annual report, 2022-23. Department of Agriculture, Cooperation and Farmers' Welfare, Government of India, 2023.
   Available: https://agriwelfare.gov.in/en/Annual.
2. Baite MS, Sharma RK, Devi TP, Sharma PR, Kamil D. Morphological and molecular characterization of *Ustilaginoidea virens* isolates causing false smut of rice in India. Indian Phytopathology. 2014; 67(3):222-7.
3. Baite MS, Sharma RK. Isolation technique and culture conditions of false smut pathogen (*Ustilaginoidea virens*) on rice. Indian Phytopathology. 2015; 3; 68(1):50-5.
4. Cooke MC. Some extra-European fungi. Grevillea. 1878; 7(1315):3.

5. Duraisamy L, Madamsetty SP, Vellaichamy P, Donempudi K, Banda S, Singh R, Prasad V, Lore JS, Jain J, Mariappan S, Laha GS. Geographic distribution of false smut disease of rice in India and efficacy of selected fungicides for its management. International journal of pest management. 2019; 65(2):177-85.
Available: https://doi.org/10.1080/09670874.2018.1494865
6. Khanal S, Gaire SP, Zhou XG. Kernel Smut and False smut: The old-emerging diseases of rice—A review. Phytopathology®. 2023; 113(6):931-44.
Available: https://doi.org/10.1094/PHYTO-06-22-0226-RVW
7. Ladhalakshmi D, Laha GS, Singh R, Karthikeyan A, Mangrauthia SK, Sundaram RM, Thukkaiyannan P, Viraktamath BC. Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India. Phytoparasitica. 2012; 40:171-6.
Available: https://doi.org/10.1007/s12600-011-0214-0
8. Lin X, Bian Y, Mou R, Cao Z, Cao Z, Zhu Z, Chen M. Isolation, identification, and characterization of *Ustilaginoidea virens* from rice false smut balls with high ustilotoxin production potential. Journal of basic microbiology. 2018; 58(8):670-8.
Available: https://doi.org/10.1002/jobm.201800167
9. Mandhare VK, Gawade SB, Game BC, Padule DN. Prevalence and incidence of bunt and false smut in paddy (*Oryza sativa* L.) seeds in Maharashtra. Agricultural Science Digest. 2008; 28(4):292-4.
10. Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A. The global burden of pathogens and pests on major food crops. Nature ecology & evolution. 2019; 3(3):430-9.
Available: https://doi.org/10.1038/s41559-018-0793-y
11. Savitha, AS, Nagaraja, A and Ajithkumar, K. Variability studies of *Ustilaginoidea virens* isolates causing false smut and its severity in different rice growing ecosystems of Karnataka. The Pharma Innovation Journal. 2022; 11(2):01-08.
12. Sekhar YC, Kamalakannan A, Gopalakrishnan C, Panneerselvam S, Rajesh S, Ganapati PS. Cultural, molecular and genetic variability of *Ustilaginoidea virens* causing rice false smut disease. International Journal of Plant & Soil Science. 2022; 34(23):608-24.
Available: https://doi.org/10.9734/ijpss/2022/v34i232467
13. Singh, RA, Dube, K. Assessment of loss in seven rice cultivars due to false smut. Indian Phytopathology. 1978; 31:186-188.

14. Statista. Worldwide production of grain in 2022/23.
Available: https://www.statista.com/statistics/263977/world-grain-production-by-type/.
15. Sun W, Fan J, Fang A, Li Y, Tariqjaveed M, Li D, Hu D, Wang WM. *Ustilaginoidea virens*: insights into an emerging rice pathogen. Annual review of phytopathology. 2020 Aug 25; 58(1):363-85.
Available: https://doi.org/10.1146/annurev-phyto-010820-012908
16. Wang Y, Wang F, Xie S, Liu Y, Qu J, Huang J, Yin W, Luo C. Development of rice conidiation media for *Ustilaginoidea virens*. Plos one. 2019; 14(10):e0217667.
17. Fu R, Chen C, Wang J, Liu Y, Zhao L, Lu D. Diversity analysis of the rice false smut pathogen *Ustilaginoidea virens* in Southwest China. Journal of Fungi. 2022 Nov 15; 8(11):1204.
Available: https://doi.org/10.3390/jof8111204
18. Ashizawa T, Takahashi M, Moriwaki J, Hirayae K. A refined inoculation method to evaluate false smut resistance in rice. Journal of General Plant Pathology. 2011; 77:10-6.
Available: https://doi.org/10.1007/s10327-010-0279-5
19. Yong ML, Fan LL, Li DY, Liu YJ, Cheng FM, Xu Y, Wang ZY, Hu DW. *Villosiclava virens* infects specifically rice and barley stamen filaments due to the unique host cell walls. Microscopy research and technique. 2016; 79(9):838-44.
Available: https://doi.org/10.1002/jemt.22710
20. Ladhalakshmi D, Laha GS, Singh R, Krishnaveni D, Prasad MS, Mangrauthia SK. False smut–A threatening disease of rice. Technical Bulletin. 2012; 63.