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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice false smut, caused by *Ustilaginoidea virens* (*U. virens*), is a highly destructive disease that results in significant grain yield losses. This study focuses on three isolates of *U. virens* (Uv3, Uv4, and Uv15), examining their isolation, cultural morphology, conidiation media, and pathogenicity. During isolation from chlamydospores and mycelial discs of smut balls, Uv3, Uv4, and Uv15 displayed small mycelial growth and varied density 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 count increased significantly with higher concentrations: Uv3 reaches $31.38x10^6$ conidia/ml, Uv4 $27.71x10^6$ conidia/ml, and U15 $20.11x10^6$ 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 media; 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, global rice production stands at 503 million metric tons [1]. 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 [2]. 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% [3].

False smut, caused by the fungus *U. 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, *U. virens* 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 [6,7].

U. virens exhibits diverse colony and chlamydospore characteristics in culture media, varying across geographical rice-growing regions and cultivars [8,9]. Chlamydospores display spherical to elliptical shapes with a thick, double-walled structure [10]. Colonies of *U. virens* in culture media initially appear white, then transition to yellow, and ultimately develop a velvety green appearance [11,12]. Yongli and Qi [13] found that XBZ agar with chloromycetin was an effective medium for isolating *U. virens* and recommended using the chlamydospore suspension for quick isolation of the false smut pathogen. Wang et al. [14] compared different methods for isolating *U. virens* and concluded that isolation from sclerotia (compact mass of mycelium) was the most effective method, though isolation from the central thick mycelium of smut balls was challenging. He et al. [15] reported that PSA (Potato Sucrose Agar) media was the most effective for isolating single spores from yellow chlamydospores of *U. virens*, providing valuable insights into optimal isolation methods. Similarly, Baite et al. [16], Lin et al. [17], Fu et al. [18], and Savitha et al. [8] also successfully isolated the false smut pathogen from chlamydospores of smut balls on PSA media.

Pilla et al. [19] investigated the variability of different *U. virens* isolates, observing colony

diameters ranging from 41.50 to 74.25 mm after 30 days of inoculation. Rani and Sharma [20] collected 35 *U. virens* isolates from various locations in Punjab and nearby states to assess variability. These isolates, cultured on PSA medium, formed well-defined colonies with diameters ranging from 26.0 to 90.0 mm after two weeks of incubation.

Isolation of the false smut pathogen is challenging due to the association of false smut balls with many saprophytes. This experiment details methods for isolating the false smut pathogen, producing conidia using rice leaf extracts, and conducting pathogenicity tests for *U. 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 (OR512524) from Morthad Village, Nizamabad District; Uv4 (OR483808) from RS & RRS, Rudrur, Nizamabad; and Uv15 (OR461676) 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 were isolated using chlamydospores of smut balls (Fig. 1A). Initially, the collected smut balls were washed with tap water and then surface-sterilized with a 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 and streptomycin sulphate (100 ppm) was added to prevent bacterial contamination. The spore suspension was carefully streaked onto Petri dishes containing PSA, and these plates were placed in BOD incubator at a controlled temperature of 26 ± 1°C for seven days. In the second method, pathogen were isolated from the central mycelial portion of the smut ball (Fig. 1B). The inner part of smut ball was cut into small pieces and subjected to surface sterilization with 4% NaOCl solution for 30 seconds, followed by thorough rinsing with sterilized distilled water 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 the first method. After 7 days of incubation, mycelial growth was measured using automatic colony meter (Scan® 300, Interscience, India). The pure culture was prepared using the hyphal tip method for subsequent analysis [16,21]. Mycelium, chlamydospores, and conidia were observed under the compound microscope at 10 and 40x. SEM (Apreo LoVac, FEI, United States) 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 (2, 4, 6, 8 and 10%), along with a control medium of potato sucrose broth (2% sucrose in potato extract). For media preparation, the respective quantity of fresh leaves (2, 4, 6, 8 and 10) 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 121 °C for 20 minutes at 15 psi. Mycelial discs (10 to 15 in number) 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. The formula for spore counting is given below;

> Concentration of spores (spores/ml)=(Total number of spores counted×dilution factor/Number of large squares counted×Volume of each large square (in ml)) x 100

Where:

Total number of spores counted: Sum of spores counted in all large squares.
Dilution factor: Factor by which the original spore suspension was diluted.
Number of large squares counted: Typically 5 (four corners and one center).
Volume of each large square: Each large square has a volume of 0.1 µl (0.0001 ml).

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.

The false smut susceptible rice cultivars TN 1 and BPT 5204 were used for the experiment. The seeds were surface-sterilized by soaking them in 4% NaOCl for 30 seconds and then washed thoroughly with sterilized distilled water three times to remove 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 a 1:3 ratio. The nursery was established and transplantation was performed after 30 days in pots under polyhouse conditions. Inoculation was performed with a surgical syringe loaded with 2 ml of conidial suspension of *U. virens* (2×10^5 conidia ml-1) by injecting into leaf sheaths covering the developing panicle of BPT 5204 and TN 1 plants 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 a fogging system. Subsequently, they were maintained at normal room temperature until the appearance of false smut balls from the emerging spikelets [10]. Data on the number of smut balls per panicle (NSBPP) and percent infected grains (PIG) were calculated as given below [22,23].

Number of smut balls per panicle (NSBPP)

$$= \frac{\text{Total number of observed smut balls/hill}}{\text{Total number of infected panicles/hill}}$$

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 on PSA media (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 incubation, Isolate Uv3 displays raised elevation with irregular growth forms and entire mycelial margins. It shows zonation and has a white-yellow surface color with yellow-green reverse pigmentation. Chlamydospores were produced, and it had the highest radial mycelial growth among the three isolates, measuring 77.00 mm. Isolate Uv4 was characterized by a flat elevation, circular growth form, and filiform mycelial margins. It lacked zonation and sectoring and exhibited a white-green surface color with yellow-green reverse pigmentation. It produced chlamydospores and shows a mycelial growth of 64.00 mm. Isolate Uv15 had a flat elevation, circular growth form, and filiform mycelial margins. It did not exhibit zonation or sectoring. The surface color was white-yellow-green, and the reverse pigmentation was yellow-green. Chlamydospores were produced, and the radial mycelial growth was 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^{c} ±1.03*	7.20^{c} ±1.52	77.00^{c} ±3.33
Uv4	3.94^{b} ±0.52	5.31^{b} ±0.57	64.00^{b} ±2.65
Uv15	3.31^{a} ±0.51	4.43^{a} ±0.37	55.66^{a} ±2.46
C.D. (p<0.05)	0.374	0.407	3.265
SEm±	0.106	0.115	0.925

**Mean standard error, means followed by different letters are significantly different from each other according to Duncan's Multiple Range Test at the 0.05 significance level*

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

Media	Conidiation (x10^{6} Conidia/ml) at 7 days after incubation		
	Uv3	Uv4	U15
2% BRLE	5.32^{bc}±0.05	4.11^{b}±0.04	1.^{64ab}±0.02
4% BRLE	9.91^{d}±0.13	8.63^{c}±0.04	4.37^{c}±0.05
6% BRLE	15.67^{f}±1.21	13.87^{d}±0.26	8.72^{d}±0.16
8% BRLE	23.28^{h}±1.16	20.16^{e}±0.83	14.49^{f}±1.13
10% BRLE	31.38^{i}±1.91	27.71^{f}±1.24	20.11^{g}±0.71
2% TRLE	3.67^{b}±0.07	1.45^{a}±0.02	0.57^{a}±0.01
4% TRLE	7.14^{c}±0.06	3.16^{b}±0.04	2.14^{b}±0.04
6% TRLE	12.94^{e}±0.62	7.22^{c}±0.01	5.31^{c}±0.03
8% TRLE	19.48^{g}±0.01	13.93^{d}±0.86	11.75^{e}±0.61
10% TRLE	25.56^{h}±1.17	21.48^{e}±0.66	22.16^{h}±0.77
PSB	0.84^{a}±0.02	0.57^{a}±0.01	0.15^{a}±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, means followed by different letters are significantly different from each other according to Duncan's Multiple Range Test at the 0.05 significance level*

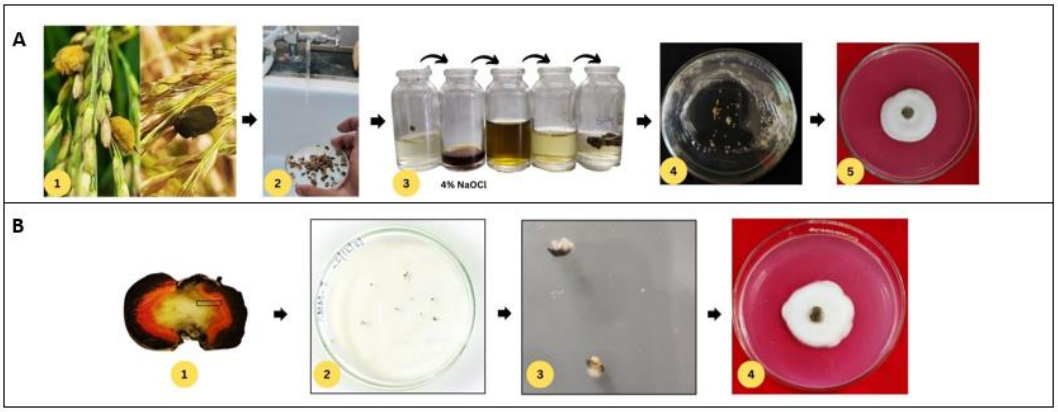

Fig. 1. Isolation of rice false smut pathogen (*U. virens*) from smutted grain

A- pathogen isolation from chlamydospores of smut balls, 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. B- pathogen isolation from mycelial part of smut balls, 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

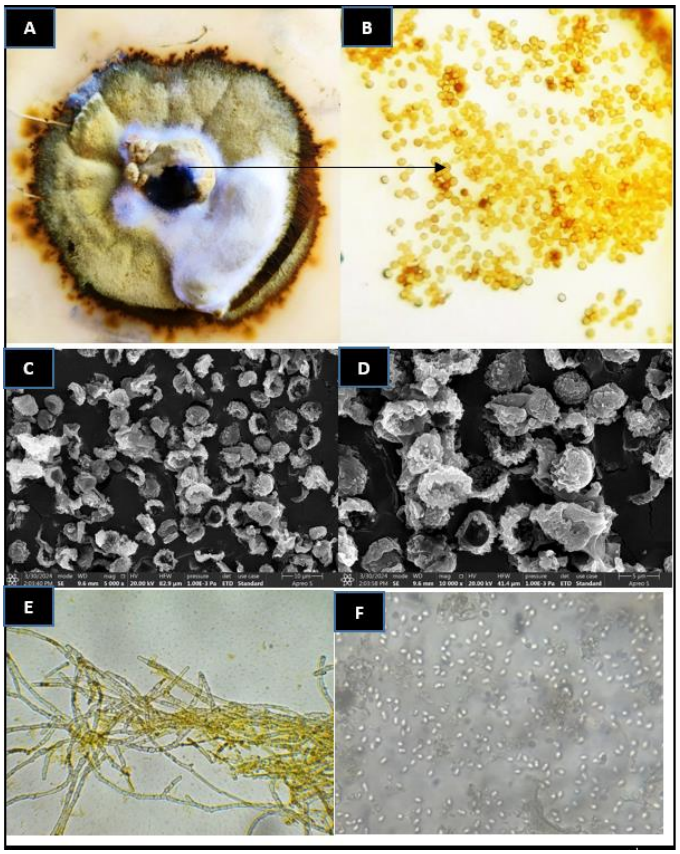

Fig. 2. Microscopic images of rice false smut pathogen (*U. virens*)
A) Chlamydospores (Uv3) formed on PSA media, B) Microscopic observation of chlamydospores (40x), (C, D) SEM images of Chlamydopsores (5000x and 10000x), (E) Geniculate mycelium of U. virens (40x), (F) Oval shaped conidia formation in rice leaf extract media (40x)

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 includes BPT 5204 rice leaf extract (BRLE) and TN1 rice leaf extract (TRLE) at various concentrations (2, 4, 6, 8, and 10%), along with a control medium (PSB) have been provided in Table 2. In the BRLE medium, the number of conidia for Uv3 starts at $5.32x10^6$ conidia/ml at a concentration of 2% and increases significantly with higher concentrations, reaching $31.38x10^6$ conidia/ml at 10%. 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 2% and rising to 20.11 $x10^6$ conidia/ml at 10%. These results indicates 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 were generally lower compared to the BRLE medium. For Uv3, the conidia count is $3.67x10^6$ at 2% and increases to $25.56x10^6$ at 10%. 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 2% and increasing to $22.16x10^6$ at 10%. Despite the lower overall conidia counts in TRLE, a noticeable increase in conidia was still observed at higher concentrations.

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 *U. virens*.

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 exhibited the symptoms of smut balls (Fig. 3e). Again, the *U. virens* was successfully isolated from those 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 chlamydospores 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 which 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 *U. virens* isolates Uv3, Uv4, and Uv15 on two rice varieties, TN1 and BPT 5204, demonstrated significant differences in the 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.

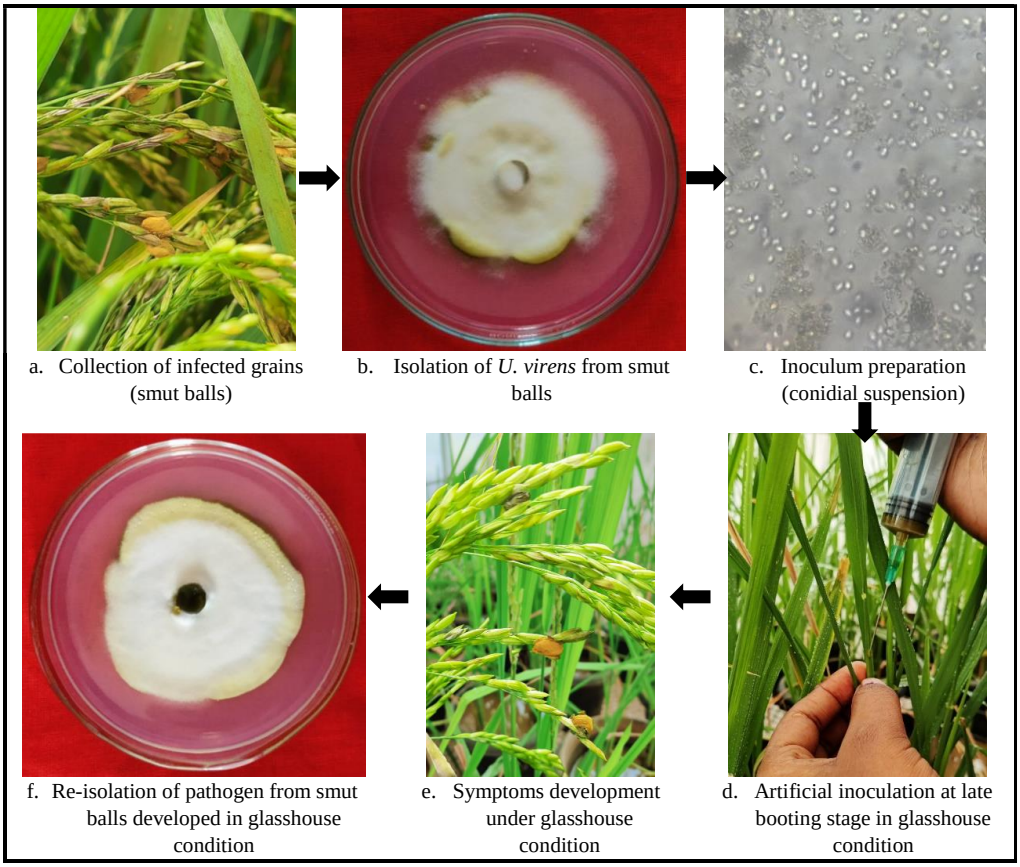

Fig. 3. Pathogenicity test for false smut of rice caused by *Ustilaginoidea virens*

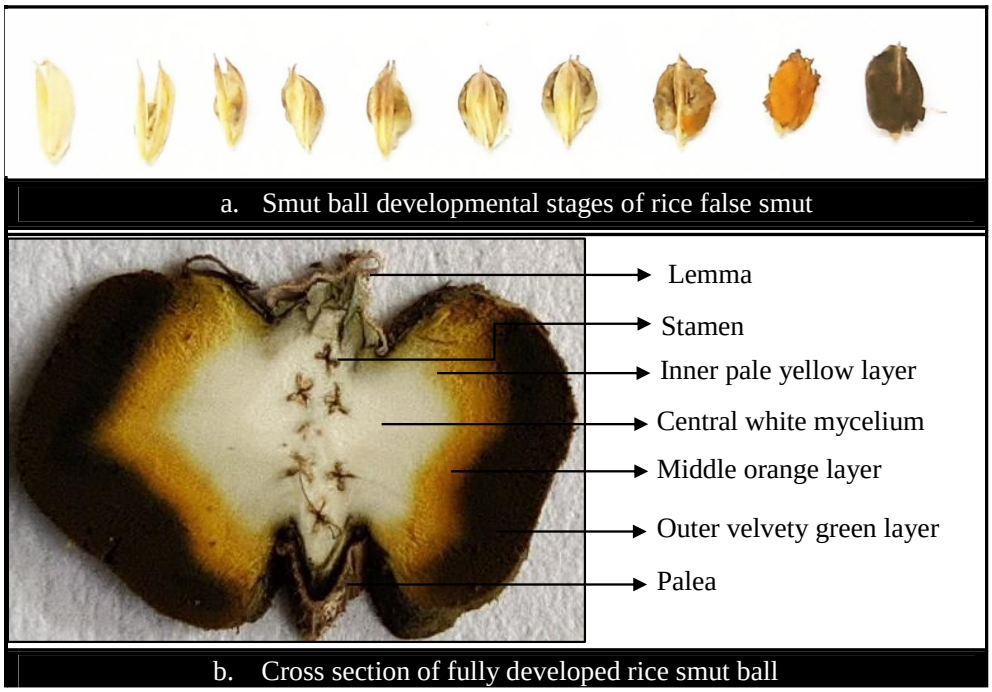

Fig. 4. Developmental stages and cross section of rice false smut ball

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^{b}±1.89*	23.32^{c}±1.28	9.56^{b}±1.01	21.03^{b}±1.32
Uv4	8.41^{a}±1.48	12.62^{b}±1.22	3.71^{a}±0.68	8.16^{a}±0.57
Uv15	5.02^{a}±0.51	7.53^{a}±1.14	2.75^{a}±0.50	6.05^{a}±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, means followed by different letters are significantly different from each other according to Duncan's Multiple Range Test at the 0.05 significance level*

4. DISCUSSION

Rice false smut pathogen, *U. 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 [21], noting its slow growth over one to two weeks to form large colonies [17]. After obtaining pure cultures, the growth characteristics of *U. 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 [24, 20]. Savitha et al. [8] 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. [24] 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. [16] also reported mean colony diameters ranging from 25

mm to 40 mm, further supporting the variability in growth characteristics observed in *U. virens* isolates.

The study demonstrated that *U. virens* produced more conidia in BPT 5204 rice leaf extract (BRLE) compared to TN1 rice leaf extract (TRLE). This differential conidia production can be attributed to the inherent biochemical and nutritional differences between the two rice varieties, which influence the growth and reproductive capabilities of the pathogen. 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 *U. virens*. These findings are consistent with those of Wang et al. [25] who reported that among the tested media, a 10% panicle medium was most efficient for conidiation. Furthermore, certain rice leaf media, except for the 10% 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. [18], Yong et al. [26], Ladhalakshmi et al. [27] and Ashizawa et al. [28], who examined the pathogenicity of false smut fungi in rice cultivars.

5. CONCLUSION

This study examined the growth, conidiation, and pathogenicity of three *U. virens* isolates (Uv3, Uv4, and Uv15) causing false smut disease in rice. The main findings showed that Uv3 had the highest radial mycelial growth on PSA media, followed by Uv4 and Uv15. Conidia production was significantly higher in BPT 5204 rice leaf extract (BRLE) compared to TN1 rice leaf extract (TRLE), with Uv3 producing the most conidia at higher extract concentrations. Uv3 was also the most aggressive isolate, causing the highest number of smut balls per panicle and the greatest percentage of infected grains in both TN1 and BPT 5204 rice varieties. These results highlight the variability among *U. virens* isolates and the effectiveness of BRLE in promoting conidia production, which could influence future management practices. Understanding these patterns can help in developing better disease management strategies and guide rice breeding programs towards resistant varieties. Future research should focus on the genetic diversity of *U. virens* isolates, conduct large-scale field trials to validate findings, and explore integrated disease management approaches.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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