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Cultural, Morphological and Molecular Characterization of *Ustilaginoidea virens* Causing Rice False Smut in Tamil Nadu, India

K. Devadharshini [a], T. Anand [a*], C. Gopalakrishnan [a], R. Umarani [b] and V. Babu Rajendra Prasad [c]

[a] Department of Plant Pathology, TNAU, Coimbatore - 641 003, Tamil Nadu, India.
[b] Directorate of Seed Centre, TNAU, Coimbatore - 641 003, Tamil Nadu, India.
[c] Department of Crop Physiology, TNAU, Coimbatore - 641 003, Tamil Nadu, India.

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 the fungus *Ustilaginoidea virens* is one of the most destructive and re-emerging disease in many rice growing areas of Tamil Nadu, India. The current study was carried out to characterize different isolates of *Ustilaginoidea virens* collected from major rice growing areas in Tamil Nadu, India. The pathogen was isolated by different isolation methods, out of which inner spore dust was effective. The colony was milky white to yellowish white, fluffy, leathery and compact. The colony diameter ranged between 35.33 mm and 65.33mm. The mycelium was hyaline, septate and width of the hyphae ranged between 1.84 and 3.10 µm. The size of the conidia was also varied among the isolates and the maximum size (6.35 µm) was observed in FS 6 isolate and minimum in FS 2 (4.10 µm). The growth of fungus in different media was examined. The

**Corresponding author: E-mail: anandpath10@yahoo.com;*

maximum colony diameter was observed in Potato Sucrose Agar (PSA) medium and minimum growth in Czapek Agar (CA) medium. The molecular confirmation was done through *U. virens*-specific internal transcribed spacer (ITS) region amplification using the primers, US 1-5/US3-3. The primers amplified a product of 380 bp which are specific for the fungus *U. virens*. The identity of the fungus was further confirmed through ITS sequencing which showed 98-99% sequence homology. The dendrogram showed two main clusters; cluster I comprised of five isolates (FS1, FS2, FS9, FS10 and FS3), while Cluster II consisted of five isolates (FS4, FS8, FS6, FS5 and FS7).

Keywords: Characterization; false smut; media; rice; Ustilaginoidea virens.

1. INTRODUCTION

Rice is one of the most important and widely grown food crops in the world. The production and productivity of rice is affected by various biotic and abiotic factors. Rice false smut, which is also known as pseudo-smut, or green smut is one of the most severe re-emerging fungal diseases in rice. False smut is caused by an ascomycetous fungus *Ustilaginoidea virens* (Cooke) (Takahashi) (Teleomorph: *Villosiclava virens*) which cause significant quantitative and qualitative yield losses in grain [1]. Excessive usage of nitrogen fertilizer, large scale cultivation of hybrids and high yielding variety with low disease resistant made the false smut as destructive disease of rice worldwide [2]. Initially the white fungal mass emerges from the inside of a spikelet during the initial infection stage, which then changes into a light-yellow smut ball that eventually turns greenish-black. The false smut ball fills with powdery chlamydospores as it matures, and the colour changes to yellowish, yellowish orange, green, olive green, and at last greenish black when smut balls rupture, powdery dark green spores are released. The pathogen produces sclerotia (sexual stage) and chlamydospore (asexual stage). The sclerotia are the main source of primary inoculums which usually develop in the autumn [3]. The sclerotia germinate to produce millions of ascospores. These ascospores produce conidia which infects the rice plant during flowering stage [4]. During sexual cycle, chlamydospores are produced which cause secondary infection via wind and rain [5].

The duration and severity of a disease epidemic are highly influenced by the weather. The disease rate will be high if the temperature ranges between 22°C and 30°C, and rainy and humid condition during the rice booting stage [6,7]. Although the pathogens begin to affect rice during the growth of the panicle, symptoms don't appear until flowering. Initially affects a small number of grains and it spreads to the entire panicle, yet in cases of severe disease incidence, the number may rise to 100% [8]. The causative organism was also identified to produce an enormous amount of mycotoxin, such as ustiloxins and ustilaginoidins which is toxic to human and animal health [9,10]. Due to the pathogen's difficulties with artificial culturing and inoculation, research on false smut was very less in Tamil Nadu. With this in consideration, the present study was undertaken to know about cultural, morphological and molecular diversity of false smut pathogen collected from various rice growing areas of Tamil Nadu.

2. MATERIALS AND METHODS

2.1 Collection and Isolation of False Smut Pathogen

Paddy seeds infected with false smut pathogen were collected from major rice growing areas of Tamil Nadu by conducting roving surveys. The incidence (%) of false smut per panicle was also recorded during the survey. The infected panicle having smut ball symptoms were collected in a polythene bag and bought to the laboratory. The fungus strains were isolated on Potato Sucrose Agar (PSA) plates by inner spore dust method as described by Bashyal et al. [11]. The smut balls were surface sterilized by dipping them in 1% sodium hypochlorite solution for 1 min followed by 70% ethanol wash for 1 min and subsequently washed three times with sterile distilled water. The smut balls were cut into two halves using a sterilized scalpel and spores were dusted from the inner surface of the ball with sterilized needle onto the Petri plates containing PSA medium amended with streptomycin sulphate @ 100 ppm. The plates were incubated at 27±1 °C for 7 days. Pure culture was obtained by hyphal tip method using sterilized needle and transferred to PSA plate. The cultures were maintained in slants at 4°C for further use and the culture was periodically transferred to fresh media. To compare the isolation efficiency of inner spore dust method, different isolation techniques such

as entire smut ball tease and outer spore dust method was also done.

2.2 Cultural and Morphological Characterization

2.2.1 Cultural characterization

Ten different isolates of *U. virens* were isolated and used for further studies. The 8-mm mycelial discs were cut from the 3 weeks old culture and placed in the Petri plate containing PSA media. The plates were incubated at 27°C and three replications were maintained. The colony characters such as colony shape, colour and growth type were observed.

2.2.2 Growth on different media

In order to study the variations in mycelial growth, two *U. virens* isolates (FS 1 and FS 8) were grown in seven different media. The composition for these culture media were as follows: Paddy Straw Extract Agar (PSEA): 100 g paddy straw, 15 g agar and 1000 ml distilled water; Rice Extract Agar (REA): 200 g rice, 15 g agar and 1000 ml distilled water; Potato Dextrose agar (PDA): 200 g potato, 20 g dextrose, 15 g Agar and 1000 ml distilled water; Potato Sucrose Agar (PSA): 200 g potato, 20 g sucrose,15 g agar and 1000 ml distilled water; Corn Meal Agar (CMA): 200 g corn meal, 15 g agar and 1000 ml distilled water; Oat Meal Agar (OMA): 30 g oatmeal, 1000 ml distilled water; Czapek Agar (CA): 30 g sucrose, 3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$, 15 g agar, and 1000 ml distilled water. The 8-mm mycelial discs were cut from three-week-old culture and placed in the Petri plate containing different media and incubated at 27ºC. Three replications were maintained. The colony diameter on different media was measured at different time interval *viz*. 7^{th}, 14^{th} and 21^{st} days of incubation.

2.2.3 Morphological characterization

Yellow coloured false smut spores were spread over the cleaned glass-slide having a drop of distilled water using a needle and covered with a cover slip and observed under the compound microscope under 40X. The mycelial character and septation were observed and photographed. The colour and shapes of spores were also observed. The morphological characters like width of mycelium and size of conidia of isolates were measured under phase contrast microscope-image analyzer.

2.2.4 Molecular Characterization

2.2.4.1 DNA extraction

The total DNA of *U. virens* was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) technique [12] with partial modification. A pure culture of *U. virens* mycelia was inoculated in potato sucrose broth and cultured at 28°C in a rotary incubator shaker at 125 rpm for 2 weeks [8]. The mycelial mat was collected by filtering the broth through Whatman filter paper No.1 and dried under sterile condition. About 100 mg of the dried mycelium was powdered using liquid nitrogen, to which 750µl of CTAB buffer (100mM TrisHCl, pH 8.0, 1.4 M NaCl, 50mM EDTA, pH 8.0 and 2% CTAB) was added. The finely ground mycelium was added into a sterile 1.5 ml centrifuge tube, mixed thoroughly, and incubated for an hour in a water bath at 65°C with intermittent vortex. After incubation, equal volume of phenol: chloroform: isoamyl alcohol (24:1:1 v/v) was added and mixed gently and was centrifuged at 13000 rpm for 15 min at 4°C. The aqueous phase was transferred to a new eppendorf tube and equal volume of ice-cold iso-propanol was added and kept for overnight incubation at -20°C. After incubation, DNA was precipitated by centrifugation at 13,000 rpm for 15 min. Ethanol wash was given twice for the DNA pellet using 70% ethanol and air dried at 37°C. The pellet was suspended in 50 µl of the sterile distilled water and stored at -20°C.

2.2.4.2 Specific internal transcribed spacer (ITS) region analysis

The ten different isolates of *U. virens* were confirmed using the specific internal transcribed spacer (ITS) primers US1-5, Forward (CCGGAGGATACAACCAAAAAA ACTCT) and US3-3, Reverse (GCTCCAAGTGCGAGGATA ACTGAAT) primer [13]. The 20 µl of Polymerase Chain Reaction (PCR) mixture consisted of 0.5 µl of 2.5 mM of dNTPs, one unit of Taq polymerase, 10 pmol of each primer (US1-5/US3-3), and DNA template (20 ng). The PCR reaction was performed with following condition: an initial denaturation at 96°C for 2 min, and 30 cycles of amplification (denaturation at 96°C for 20 s, primer annealing at 53°C for 30 s and extension at 70°C for 30 s) and single cycle of final extension was done at 72°C for 7 min. PCR amplification was performed on a Thermocycler. The amplified products were then resolved in 1.2% agarose gel electrophoresis and viewed under a gel documentation unit (Alpha Imager

2000; Alpha Innotech Corporation, San Leandro, CA, USA). The amplified PCR products were sequenced using by adopting the Sanger dideoxy sequencing method.

2.3 Statistical Analysis

The *in vitro* experiments were performed by using a Completely Randomized Design (CRD). The data generated from different experiments were analyzed statistically using statistical package of INDOSTAT and the ANOVA determined the probability for significant variation among the treatments. Phylogenetic analysis was carried out using the MEGA 11 software and dendrogram was constructed by UPGMA (Unweighted Paired-Group Method with Arithmetic Average) method.

3. RESULTS AND DISCUSSION

3.1 Severity of Rice False Smut and *Ustilaginoidea virens* Isolates

The false smut infected panicles were collected from different regions of Tamil Nadu and totally 10 isolates of *U. virens* were collected. Among 10 different samples used for the study, maximum false smut disease incidence per panicle was observed in variety ADT 37 collected from Nallathur, Cuddalore district (7.8%) followed by var. CO 43 (6.2%) and Amman Ponni (5.0%) collected from Pollachi, Coimbatore district and Omalur, Salem district, respectively and minimum disease incidence was observed in the rice variety Super Aman collected from Chinnampalli, Dharmapuri district (1.6%) (Table 1). Similarly, numerous surveys carried out in various locations revealed a significant variance in the incidence of false smut disease [14-16].

For isolation of false smut pathogen, different isolation techniques were followed, such as the outer spore dust method, the entire smut ball tease, streaking and the inner spore dust method. Out of these four methods, the inner spore dust was found to be more effective for *U. virens* isolation. The pathogen was not able to isolate from the outer spore dust and the entire smut ball tease method. The isolation efficiency was lesser in streaking method compared to the inner spore dust method (Fig. 1a & 1b). Hence, inner spores were used for isolation of *U. virens* and dusted on the PSA medium as reported by Bashyal et al. [11], Savitha et al. [15] and Nithila et al. [17].

Table 1. Isolates and severity of false smut in different rice growing areas of Tamil Nadu

S. No.	Name of the isolates	Place of sample collection		GPS coordinates	Variety	False smut incidence (%)
		District	Village			
1.	FS 1	Coimbatore	Wetland, TNAU	11.0019ºN 76.9267ºE	ADT 45	4.6
2.	FS 2	Coimbatore	Pollachi	10.6583ºN 77.0085ºE	CO 43	6.2
3.	FS 3	Erode	Bhavanisagar	11.29 ºN 77.80ºE	ASD 16	2.5
4.	FS 4	Cuddalore	Nallathur	12.2998ºN 79.7506ºE	ADT 37	7.8
5.	FS 5	Madurai	Narasingam	9.9686ºN 78.2029ºE	ADT 36	1.8
6.	FS 6	Thanjavur	Koilvenni	10.7824ºN 79.3617ºE	ASD 16	2.6
7.	FS 7	Tiruppur	Thittuparai	11.1105ºN 77.5902ºE	BPT 5204	4.2
8.	FS 8	Erode	Bhavani	11.4506 ºN 77.6260 ºE	CO 43	5.0
9.	FS 9	Dharmapuri	Chinnampalli	12.0106ºN 77.9644ºE	Super Aman	1.6
10.	FS 10	Salem	Omalur	11.7291ºN 78.0200ºE	Amman Ponni	5.2

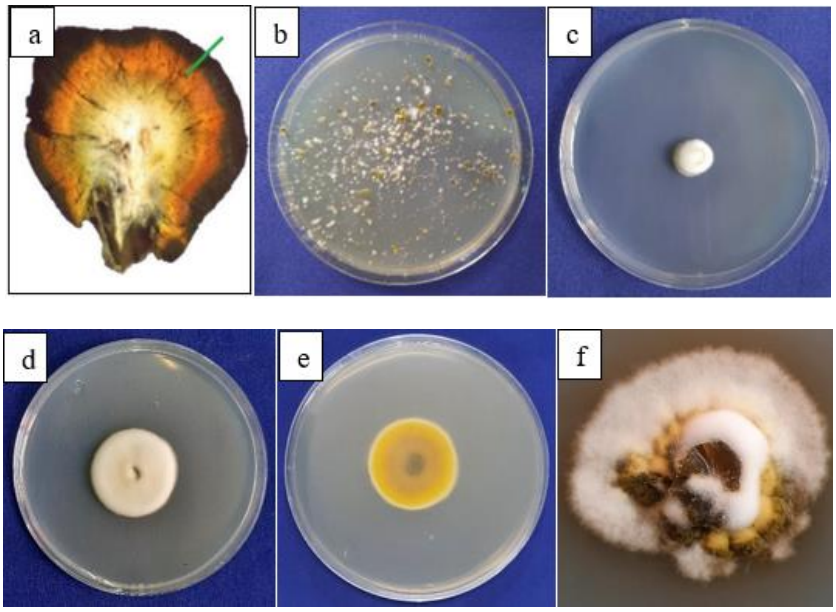

Fig. 1. Isolation of *U. virens* by inner spore dust method; (a). Sampling location of spore dust from false smut ball; (b). Growth of *U. virens* after 3 days; (c). 5-days old culture of *U. virens*; (d) & (e) 14-days old culture of *U. virens* (Front side and obverse side); (f). Spores formed by isolate FS 8

3.2 Cultural and Morphological Characterization of *Ustilaginoidea virens*

3.2.1 Cultural characteristics

The growth of *U. virens* was very slow which took 3-5 days for formation of tiny white colony. After 14 days, large colony developed on the culture plate. The colony was creamy white to milky white in colour with fluffy mycelium, flat and leathery. On obverse side, mycelium produced yellow with olive green tinch. Some isolates formed chlamydospores at the colony margin or the centre of the colony, which was yellowish orange and later became greenish (Fig. 1c - f). The maximum colony growth was observed in the isolate FS 6 (65.33 mm), followed by FS 5 (61 mm) and FS 1 (59.00 mm). The minimum colony diameter was observed in FS 8 (35.33 mm) (Table 2). The colony colour varied from creamy white to milky white on front side and yellowish green or olive green on obverse side. Almost all isolates showed fluffy and raised growth except FS 5 and FS 6 which formed flat colony on PSA media. Two isolates FS 2 and FS 7 formed leathery mycelial growth with undulations (Fig. 2a & 2b). The results were in accordance with Ladhalakshmi et al. [8], Baite et al. [18], Lin et al. [19] and Nithila et al. [17]. Goswami et al. [20] reported that white-reddish yellow, fluffy, compact, undulated colony to without undulation, wavy colony edge and white greenish grey to black colony.

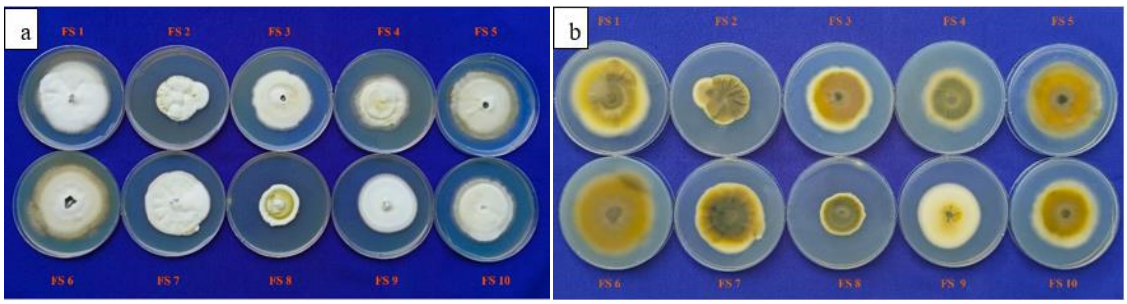

Fig. 2. Different isolates of *Ustilaginoidea virens* (FS 1 to FS 10); a. Front side b. Obverse side

Table 2. Cultural characteristics of different isolates of *Ustilaginoidea virens*

Isolates	Radial growth (mm)			Colony colour	Growth type
	10th day	20th day	30th day		
FS 1	24.33^{b} (29.556)	46.00^{b} (42.705)	59.00^{c} (50.186)	Milky white	Fluffy and circular
FS 2	14.00^{f} (21.965)	25.67^{g} (30.438)	41.33^{h} (40.009)	Creamy white	Leathery and irregular with undulation
FS 3	20.67^{cd} (27.030)	31.67^{e} (34.241)	50.67^{e} (45.382)	Creamy white	Fluffy and circular
FS 4	11.33^{f} (19.646)	22.00^{h} (27.957)	45.00^{g} (42.130)	Creamy white to yellow	Fluffy and circular with undulation
FS 5	25.33^{ab} (30.219)	41.67^{c} (40.202)	61.00^{b} (51.355)	Creamy white to yellow	Flat and circular
FS 6	26.67^{a} (31.090)	50.33^{a} (45.191)	65.33^{a} (53.930)	Creamy white	Flat and circular
FS 7	22.00^{c} (27.968)	36.33^{d} (37.067)	51.00^{e} (45.573)	Milky white	Leathery, circular with undulation
FS 8	11.67^{f} (19.969)	26.67^{g} (31.090)	35.33^{i} (36.471)	Milky white with yellowish green	Fluffy and circular
FS 9	16.33^{e} (23.835)	29.00^{f} (32.581)	48.67^{f} (44.236)	Milky white	Fluffy and circular
FS 10	20.00^{d} (26.561)	33.00^{e} (35.055)	54.00^{d} (47.295)	Milky white	Fluffy and circular
CD @ 5%	**1.278**	**1.391**	**1.016**	-	-
SE(d)	**0.613**	**0.666**	**0.487**	-	-

Values are the means of three replications.
Figures in parentheses represent arcsine transformation.
Means in a column followed by same superscript letters are not significantly different according to DMRT

3.2.2 Growth on different media

The colony diameter and density of *U. virens* were highly influenced by the media. The maximum colony diameter was observed on PSA media (FS 1- 43.67 mm & FS 8- 39 mm) and minimum on CA media (FS 1- 10 mm & FS 8- 19.67 mm) (Table 4). Similar results were reported by Fu et al. and Baite et al. [14]. Fu et al. reported that maximum growth of about 36 mm diameter was observed in PSA media and minimum growth was observed in CA media after 14days of inoculation. Baite et al. [18] reported that on PSA media, the mycelial growth rate of the fungus was 2.54 mm/ day, whereas PDA showed comparatively slow growth. This may be due to the preference of *U. virens* for sucrose over dextrose. The mycelial density was thick on PSA, PDA, OMA and CMA media, whereas on REA, PSEA and CA media, the density was very less (Fig. 3a & 3b).

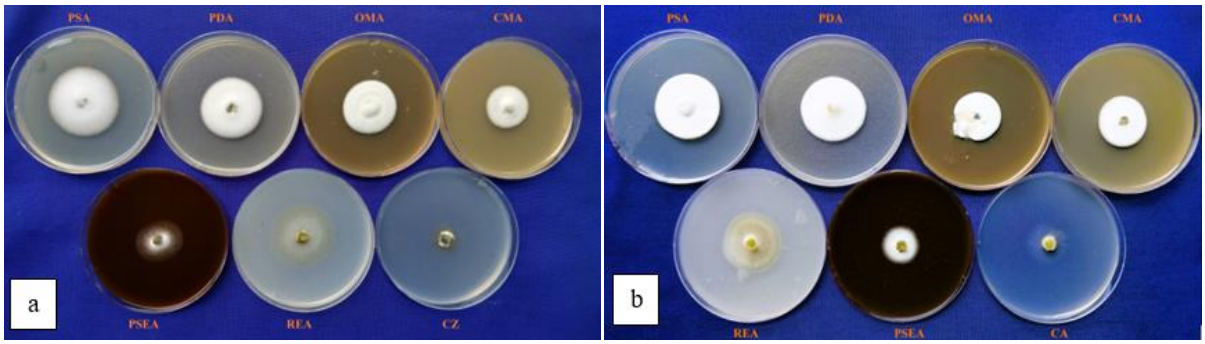

Fig. 3. Growth of isolate FS 1(a) and FS 8(b) different media PSA: Potato Sucrose Agar; PDA: Potato Dextrose Agar; OMA: Oat Meal Agar; CMA: Corn Meal Agar; PSEA: Paddy Straw Extract Agar; REA: Rice Extract Agar; CA: Czapek Agar

Table 3. Mycelial growth of *Ustilaginoidea virens* on different culture media

Media	Radial growth (mm)					
	Isolate FS 1			Isolate FS 8		
	7th day	14th day	21st day	7th day	14th day	21st day
PSA	21.00[a] (27.236)	31.67[a] (34.227)	43.67[a] (41.344)	20.00[a] (26.547)	27.33[a] (31.510)	39.00[a] (38.645)
PDA	16.00[ab] (23.542)	28.67[b] (32.354)	37.67[b] (37.842)	17.67[a] (24.853)	25.00[ab] (29.997)	37.33[a] (37.662)
OMA	14.33[bc] (22.235)	24.00[c] (29.319)	35.33[b] (36.456)	12.67[b] (20.826)	20.00[d] (26.552)	29.33[b] (32.790)
CMA	12.33[c] (20.529)	21.33[d] (27.488)	26.67[c] (31.072)	11.33[bc] (19.646)	22.67[bc] (28.422)	30.67[b] (33.626)
REA	8.67[d] (17.091)	12.33[e] (20.548)	23.67[d] (29.090)	10.00[cd] (18.421)	21.33[cd] (27.503)	30.00[b] (33.203)
PSEA	6.33[de] (14.561)	10.67[e] (19.024)	16.00[e] (23.562)	9.67[cd] (18.095)	15.33[e] (23.043)	22.33[c] (28.194)
CZ	4.00[e] (11.473)	7.00[f] (15.311)	10.00[f] (18.387)	8.33[d] (16.736)	13.67[e] (21.693)	19.67[d] (26.315)
CD @ 5%	**2.293**	**1.766**	**1.769**	**2.144**	**1.703**	**1.482**
SE(d)	**1.059**	**0.816**	**0.578**	**0.999**	**0.776**	**0.690**

Values are the means of three replications.
Figures in parentheses represent arcsine transformation.
Means in a column followed by same superscript letters are not significantly different according to DMRT

3.2.3 Morphological characterization

The symptoms on paddy grains differed greatly among the false smut infected samples. During the initial stages of infection, the smut balls were orange or yellowish in colour then they gradually turned to dark olive-green colour at later stage of infection (Fig. 4a, 4b & 4c). The smut balls were divided into two halves and inner region containing different layers were examined. The innermost layer of the orangish yellow balls was white, whereas the outer two layers were yellow. The inner region of the dark olive-green balls was white, the second layer was yellow, and the outermost layer was dark green in olive. Nessa [21] reported similar results with slight variations. The hyphae under compound microscope appeared hyaline and septate. The yellow smut spores appeared spherical to elliptical in shape, and no prominent spines were noticed under compound microscope (Fig. 4d &4e). The width of the hyphae was varied among the isolates and ranged between 1.84 and 3.10 µm (Table 4). The maximum width of the hyphae (3.10 µm) was observed in isolate FS 2 and minimum in FS 7 (1.84 µm). The size of the conidia also differed between isolates and ranged from 4.10 to 6.35 µm. The isolate FS 2 contained the smallest conidia, while isolate FS 6 contained the largest. The morphological characteristics of *U. virens* isolates have been agreed with the descriptions given by Baite et al. [22]. Recently, Nithila et al. [17] reported that the conidia were hyaline, spherical to globose in shape and warty with a width of 5.24 µm approximately and the chlamydospores were echinulated and had prominently decorated spines.

3.2.4 Molecular characterization

The study on the morphology and growth characteristics of *U. virens* was significant for further understanding the occurrence regularity of RFS and control measures against RFS [6]. The fungus' identity was validated by PCR using *U. virens*-specific primer, US1-5/US3-3 [9]. The desired specific length of 380 bp band was observed in all 10 isolates and no band was formed in control lane (Fig. 5). The amplified DNA samples were purified and sequenced. The sequences were blasted in NCBI database which shows that all 10 isolates belong to *U. virens* with homology as shown in Table 5. The sequences were submitted to GenBank and accession numbers were obtained. The dendrogram constructed through UPGMA analysis clearly grouped the isolates into two major clusters (Fig. 6). The cluster I comprised of five isolates: FS1 (Wetland, TNAU), FS2 (Pollachi), FS9 (Chinnampalli), FS10 (Omalur) and FS3 (Bhavanisagar). The cluster II comprised of five isolates: FS4 (Nallathur), FS8 (Bhavani), FS6 (Koilvenni), FS5 (Narasingam) and FS7 (Thittuparai). This shows that isolates taken from

Table 4. Hyphal and conidial characteristics of *Ustilaginoidea virens*

Isolate	Width of the hyphae (µm)	Conidial size (µm)
FS 1	2.78	4.38-5.36
FS 2	3.10	4.10-6.10
FS 3	1.97	4.50-5.65
FS 4	2.56	4.87-6.27
FS 5	2.90	4.40-5.42
FS 6	2.15	4.78-6.35
FS 7	1.84	4.85-5.77
FS 8	2.42	4.65-5.69
FS 9	2.08	4.54-5.90
FS 10	2.65	4.75-5.81
CD @ 5%	**0.40**	**0.36**
SE(d)	**0.21**	**0.18**

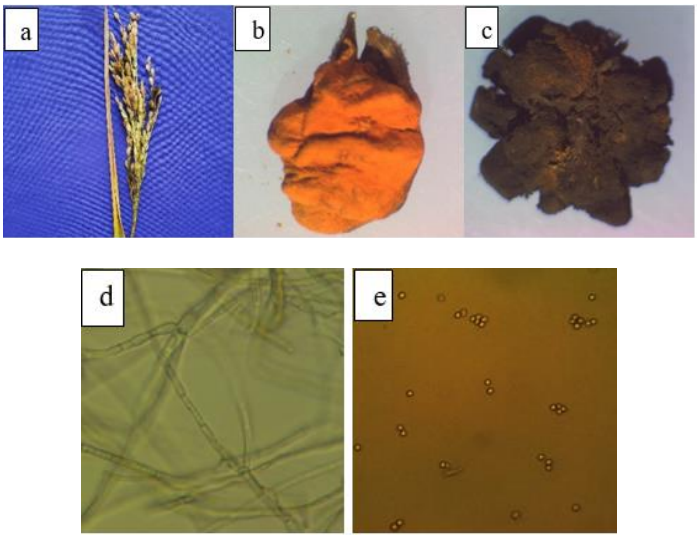

Fig. 4. (a). False smut infected panicle; (b). Orange smut spore ball; (c). Dark olive-green smut ball; (d) & (e). Septate hyphae and smut spores viewed in compound microscope at 40x magnification

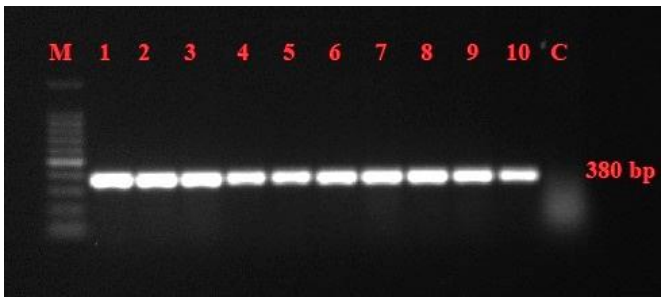

Fig. 5. Polymerase chain reaction (PCR) amplification of internal transcribed spacer (ITS) regions of *U. virens* with specific ITS primers (US1-5/US3-3). Lane M – 100 bp DNA marker; lane 1- FS1: lane 2- FS2; lane 3- FS 3; lane 4- FS 4; lane 5- FS 5; lane 6- FS 6; lane 7- FS 7; lane 8- FS 8; lane 9- FS 9; lane 10- FS 10; lane C- control

nearby location showed less variability and belonged to the same cluster, whereas those collected from locations farther away exhibited more variation and were grouped into separate clusters. Ze Tan et al. [23] also reported variations in the isolates of rice false smut

Table 5. Fungal identification based on NCBI-BLAST search (for ITS1-5.8s-ITS2 region)

Isolate	Accession number	Species identified as	Coverage identity (%)
FS 1	OR193799	*U. virens*	98
FS 2	OR223235	*U. virens*	99
FS 3	OR225647	*U. virens*	99
FS 4	OR225649	*U. virens*	98
FS 5	OR225650	*U. virens*	99
FS 6	OR225651	*U. virens*	98
FS 7	OR225654	*U. virens*	98
FS 8	OR225656	*U. virens*	98
FS 9	OR232535	*U. virens*	99
FS 10	OR232680	*U. virens*	99

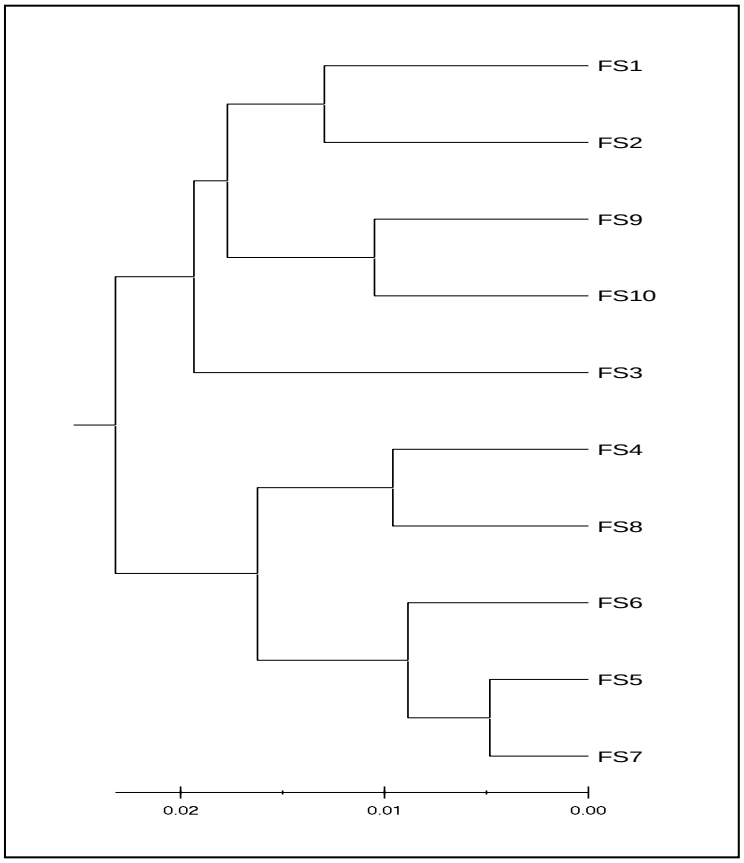

Fig. 6. Dendrogram constructed by UPGMA (Unweighted Paired-Group Method with Arithmetic average) method

pathogens collected from different locations compared to the same locations. In contrast, Fu et al. [24] reported that the most of the strains of false smut pathogen were not clustered according to their geographical origin and showed the rich genetic diversity. Similarly, Yang et al. [25] pointed out that the clustering of isolates had no clear relationship with their geographical location. However, Fang et al. [26] found a high degree of genetic variation of *U. virens* among geographical populations [27,28].

4. CONCLUSION

Ten fungal isolates of false smut infected panicles collected from different rice growing areas of Tamil Nadu were identified as *Ustilaginoidea virens* based on morphological and cultural characters. The fungal pathogen was further confirmed molecularly through PCR analysis using *U. virens* specific ITS primer (US1-5/US3-3). The dendrogram constructed by UPGMA method grouped the isolates into two

clusters which showed the molecular diversity between the ten isolates. The information obtained from this study will help to understand the population structure and evolution of rice false smut fungus in Tamil Nadu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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