False Smut Disease: Importance, Distribution, Ecology and Epidemiology

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Rice crop is known to be attacked by many pests and diseases which cause huge losses annually because of more intensive cultivation and high usage of nitrogenous fertilizers and less availability of resistant varieties. Among diseases of rice, fungal disease cause huge economical yield loss and among fungal diseases rice, false smut caused by *Ustilaginoidea virens* (Cooke) Tak is of significant economic importance in recent years.

Rice false smut, also known as green smut, pseudo smut has been recorded in all rice growing countries worldwide. Earlier it was regarded as a minor disease, occurring sporadically in certain regions, but now epidemics of the disease is being reported in different rice growing regions of the world like Japan, Malaysia and some parts of India like Tamil Nadu. It is an important devastating disease causing yield losses from 1.01 to 10.91% (Atia, 2004). Disease incidence of 10-20% and 5-85% respectively has been reported from Punjab and Tamil Nadu on different rice cultivars (Ladhalakshmi *et al*., 2012). The disease has been reported to cause yield loss up to 17.12 in Karnataka (Muniraju *et al*., 2017a; sharanabasav *et al*., 2021; Huded *et al*., 2022). In recent years, its outbreak might be due to high input cultivation, increased use of hybrid varieties, and climate change.

Economic importance

Rice false smut, also known as green smut, pseudo smut has been recorded in all rice growing countries worldwide. False smut causes chalkiness of grains which leads to reduction in grain weight. It also reduces seed germination. The damages by false smut disease include the reduction in economic yield, contamination of grains and straws with ustiloxins, production of mycotoxins and the formation of antimitotic cyclic peptides in chlamydospores (Interfere and affect on cell division) and these chlamydospores, sclerotia and its mycotoxins which are very poisonous to both human and animals and cause different ill effects on human health on consumption of false smut infected grains, so false smut is having its unique importance.

Extent of damage of the disease

Number of smut balls reached as many as 135 (recorded in one panicle in 2015), In the natural observation 34.4, 53.9 and 11.7% of the smut balls were located at the base, mid and apex part of the infected panicles, respectively. Rice false smut (rfsm) is recognized worldwide as an emerging fungal disease and predominant during Aman (late monsoonal rice) season. Hindering adoption of promising rice varieties, such as BRRI dhan 49. Singh and Dube (1978) reported 44 per cent loss in Ratna and 17 percent in IR 8. Yield loss from rfsm has been reported in the range of below 1 to over 75. Mean disease severity was ranged from 4.44 to 17.12 % from Karnataka (Muniraju *et al*., 2017a). Important problem in the punjab state and widespread in commercial cultivar PR 116. Yield loss due to false smut were reported in many countries such as, Columbia (20 %) in the year of 1952; Peru (25 %), Bangladesh in (50.3 to 75.4) in 1986, china (633,000 ha ; 1.37 billion tonns of yield) (2005); Sichuan china (2005) (75^; 330,000 ha; 20-40*); Northern japan (1998) (Severe incidence); Nigeria (58.3) in (2002); USA (1-15^); Egypt (1.01 to 10.91) (2004); Fizi (10) (1956). Disease within Indian states is varied significantly such as., Uttar Pradesh (0.2 - 44.4); Madhya Pradesh (.6 – 75.4); Orissa (15-20); Punjab (70); Andaman & Nicobar Islands (0.04 -49); Haryana (28.5); Gujarath (10-18); Kashmir (45.7); Maharastra (72); Tamilnadu (5-85); Chhatisgarh (16.8-40.7) and Udupi, Karnataka (23) (Dangi *et al*., 2020).

Symptoms of the disease

False smut disease in rice, belongs to Ascomycetes producing symptoms on rice panicle, can be seen after the panicle open and pathogen infection transforms individual grain into initially orange, green later velvety, finally black smut balls on

panicles and cause economic damage and at the crop maturity stage.

Figure 1. Symptoms at different stage of the disease

Figure 2. Culture and chlamydospore production on the XBZ solid mediu media

The Pathogen

Pathogen has been identified in both sexual and asexual stages. Pathogen belongs to the phylum ascomycota and different taxonomic position is given below.

Table 4. Scientific classification of pathogen

Kingdom	Telemorph	Anamorphic
Phylum:	Ascomycota	Ascomycota
Class:	Ascomycetes	Ascomycetes
Sub class:	Sordariomycetes	Incertaesedis
Order:	Hypocreales	Incertaesedis
Family:	Claviceptaceae	Incertaesedis
Genus:	*Villosiliclova*	*Ustiloginoidea*
Species:	*virens*	*virens*

False smut spore balls or sclerotia (sclerotia = pseudomorph) (Figures 1.1 and 1.2) are found growing in association with *Oryza sativa*, on *Zea mays* L. and a few other tropical Graminae hosts that have no significant economic importance. Spore balls are comprised of a proliferation of branched, radial and compacted hyphae that give rise to spherical to elliptical (3-5 X 4-6 µm), warty olivaceous chlamydospores at the terminus. The chlamydospores are smooth, round to elliptical when immature but warty, spiny and yellow to orange pigmented when mature. The chlamydospores become olivaceous, globose to irregularly round, as they mature and the surface is ornamented with prominent spines. In culture, the chlamydospores germinate and produce fine germ tubes that give rise to 1- 3 small ovoid secondary conidia. *U virens* can be cultured on potato dextrose agar (PDA) or potato dextrose sucrose agar (PDSA), but the fungus is slow growing and has two different colony types. The two colony types are either green, hardened sclerotia-like after incubation for two weeks or white, spreading mycelia after incubation for three weeks (Figure 1.3). Further, the fungus can also be grown in liquid broth. Descriptions of the teleomorphic stage, *Villosiclava virens*, include flat, botuliform, reniform, horseshoe-shaped or differently shaped, 1 to several, usually 2, protruding from sclerotia (or pseudosclerotia) overwintering in the field and producing a stalked stromata (=4 ascomata) containing perithecia with about 300 asci in the summer or fall of the following season. The asci are described as cylindrical, hyaline, filiform, unicellular, 120-180 X 4 µm (130-300 X 4-7 µm) and contain eight ascospores that are hyaline, filiform, septate disarticulating at septa to form four part-spores 120-180 X 0.5-1 µm (140-230 X 1.3-1.8 µm) (Tanaka *et al*., 2008). The four part-spores are aseptate and are 30-60 µm.

Sharanabasav *et al*., 2021 characterized the sixty one geo-distinct isolates of *Ustilaginoidea virens* for morpho-molecular and mating-type locus diversity. *MAT1* loci analysis indicated the distribution of heterothallic mating types in south Indian paddy fields. This is the first report describing the sexuality of Indian strains of the *U. virens*, which would help better understand the genetic diversity of the *U. virens* prevailing in Southern India.

Isolation of *U. virens*

The collected smut balls were surface sterilized by dipping them in 70% ethanol followed by 0.1% mercuric chloride and subsequently washed with sterile distilled water. Using a sterilized inoculation loop, the mass of chlamydospores were streaked onto Petri dishes containing potato sucrose agar medium (PSA) under aseptic condition. To avoid bacterial

contamination, Streptomycin at 100 ppm per litre was added in the medium at lukewarm stage before pouring into petri plates. The Petri dishes were incubated at 27 ± 2°C until the appearance of mycelium.

Morphological characters of disease signs

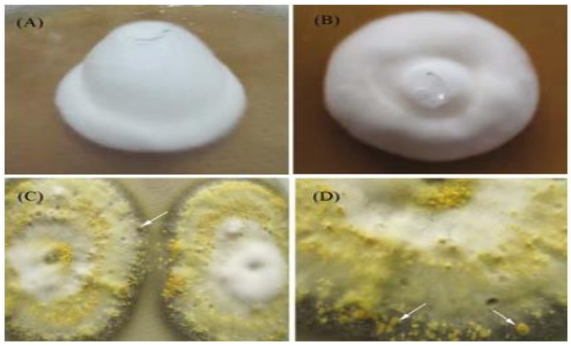


Figure 3. Colonies of *V. virens* on the XBZ solid medium. A) An isolate of *V. virens*, 10 days after inoculation. (B) The morphology of colony resembled a straw hat, days after inoculation. (C D) There were many mounds of chlamydospores (arrows) formed on the colony margin and a great number of chlamydospores dispersed on the whole colony, 40 days after inoculation.

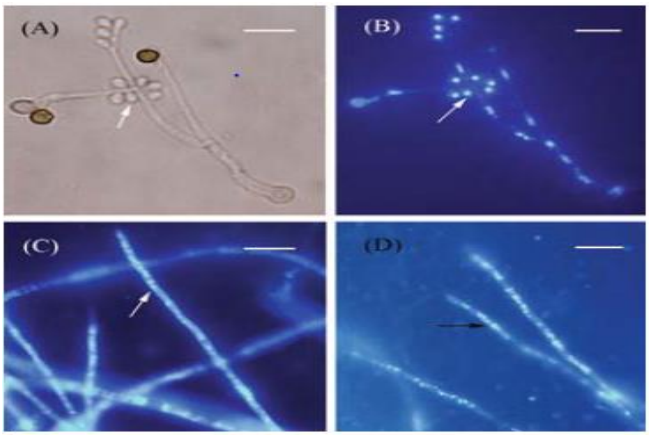


Figure 4. Chlamydospores of germinated *V. virens* and karyological observation. (A) The conidia are holoblastically and sympodially produced at the apex of each conidiophore cells. (B) Conidia were mononuclear (arrows). (C–D) Somatic hyphae were multinuclear (arrows).Bars: A–D=10 µm.

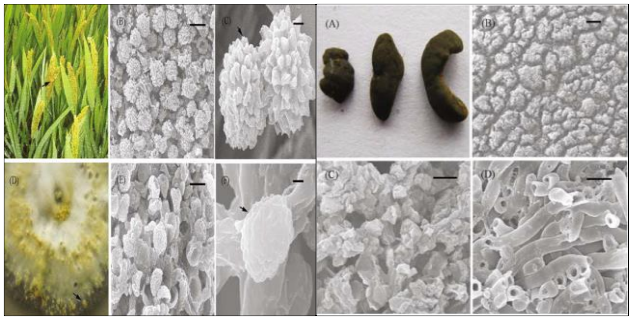


Figure 5. Symptom of rice false smut and scanning electron micrographs of *V. virens* chlamydospores came from natural false smut balls and laboratorial culture A) A naturally infected rice kernel (arrows). (B) Chlamydospores masses. (C) Higher magnification of the chlamydospores. (D) A large number of chlamydospores balls piled on the XBZ solid medium. (E) Chlamydospores masses. (F) Higher magnification of a chlamydospore,with wavy spines (arrows) were prominent on the chlamydospores surface. Bars: B, E=10 µm.C, F=1 µm. Figure 6. Scanning electron micrographs of *V. virens sclerotia.* (A) Sclerotia of *V. virens*, which appeared black horseshoe-shaped and irregular oblongor flat and sizes ranged from 2 to 20 mm. (B–C) Surface shape of asclerotium. (D) Internal structure of a sclerotium, comprising dense mycelium. Bars: B=5 µm. C–D=10 µm.

5. Mode of infection

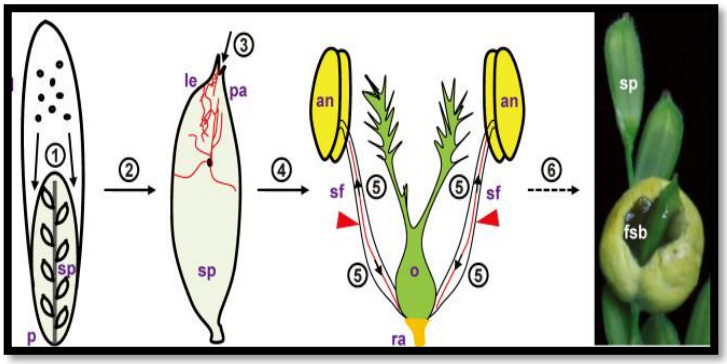


Figure 7. Modes of infection

Villosiclava virense (Vv) spores landing on the top of the second leaf (l) can enter the developing panicle (p) along with rainwater (Fig.9) 1. Subsequently, *Vv*spores are attached to the developing spikelet (sp) and germinate to produce hyphae 2. The hyphae extend into the inner space of the spikelet via the gap between the palea (pa) and lemma (le) 3. Primary infection sites are found in the

stamen filaments (sf) 4. where the hyphae extend intercellularly in both directions 5. Hyphae can extend into anthers (an) and reach the rachilla (ra), and intertwine with the ovary (o) and other floral organs. *Vv* hyphae acquire abundant nutrients to form false smut balls (fsb), hypothetically through hijacking of the rice nutrient reservoir 6. This step (arrow with dotted line) is of particular interest and requires further exploration. Red curved lines represent *V. virens* hyphae. Red arrowheads point to the primary infection sites at the stamen filaments.

Epidemiology and Disease cycle

Predisposing factors

High relative humidity and rainfall accompanied by cloudy days during flowering favoured the disease incidence and the high humidity and lower maximum temperature favours the disease. False smut incidence is favoured by relative low (around 20^0C) temperature and high relative humidity more than 90 per cent coupled with well distributed moderate rainfall during flowering also by late sowing and high soil fertility with N,P. and K at 100, 50, and 50 kg/ha.

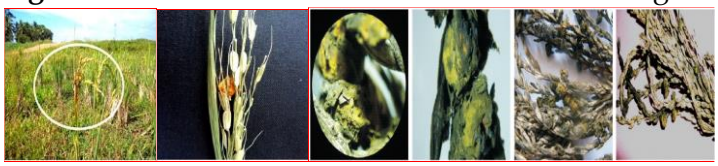
Alase *et al*., 2021 conducted field experiment during two consecutive kharif seasons (2019-20 and 2020-21) to find out the influence of weather parameters on disease development.

Off season survival of *Ustilaginoidea virens*

The pathogen survives as dormant structures such as sporeballs, chlamydospores, sclerotia etc. in soil, stubbles of the crop and on collateral hosts. It is reported that false smut disease survived on *Oryza officinalis* from India. Shetty and Shetty (1985) reported *Digitaria marginata*as the collateral host of *U. virens* from Dakshina Kannada district of Karnataka. False smut was also observed from India on *Panicum trypheron*

Echinochloa crusgalli, and *Imperata cylindrical* are the other reported collateral hosts of *U. virens*. Ratoon rice and weedy rice have some role to play in the off season active survival of the false smut pathogen, *U. virens* in Kerala. False smut disease of maize (*Zea mays* L.) has been observed in the Panchmahals and Dahod districts of Middle Gujarat, India during *kharif* 2013 (Shetty and Shetty, 1987).

Figure 8. False smut disease on ratoon rice emerged



from the stubbles of the previous crop and False smut disease on *Oryza spontanea*. 10. Yellow and black color smut balls produced by the false smut pathogen, *Ustilaginoidea virens* on the tassels of male flowers of maize.

Disease cycle of the fungus

As the fungus has been recorded on many grasses and wild rice, it is presumed that the spores produced on the collateral hosts become air-borne and serve as the main source of inoculum. Primary Infection is believed to be caused mainly by ascospores produced from the Sclerotia. Chlamydospores play an important role the secondary infection which is a major part of the disease cycle. Chlamydospores are air borne and are profusely present at the time heading of rice plant.

Infection of a few individual grains only lends support to the presumption that the infection is floral. If the infection takes place in the early stages of flower opening, the ovary is destroyed, whereas if the infection is later than the grain is set, the mycelium invades the endosperm and produces masses of the spores. The disease is not seed borne. Rainfall accompanied by cloudy days during the period between flowering and maturity of the grain increases the incidence of the disease.

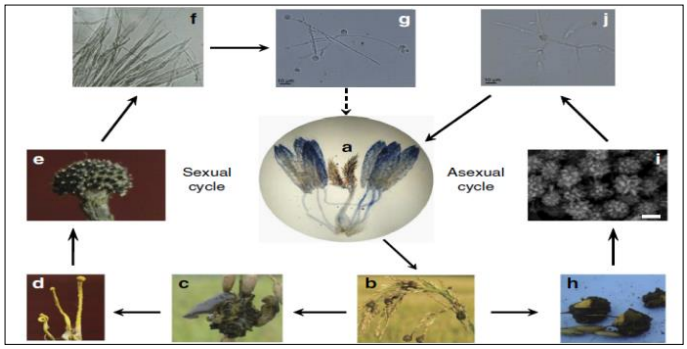


Figure 11. Major stages in the infection cycle of *U. virens*. a) Stamen filaments in the florets infected by *U. virens*. Hyphae were stained with trypan blue to show

primary infection sites. (b) False smut balls formed in the rice spikelets. (c) Sclerotia formed on the surface of spore balls. (d) Stroma produced by a germinating sclerotium. (e) Ascocarp formed on the stroma. (f) Asci. (g) Ascospore germination, Scale bar, 10 mm. (h) Spore balls. (i) Chlamydospores under scanning electron microscopy. Scale bar, 3 mm. (j) Chlamydospore germination.

Conclusion

False smut was recognized as a symbol of a bumper harvest and was categorized as a minor disease due to its sporadic occurrence. However, the disease has been observed in severe form since 2001. Hence to manage this disease future work should pay attention to developing a more rapid and effective system to evaluate rice resistance and susceptibility to the disease, screening of rice germplasm for disease-resistance breeding, studying the resistance inheritance and investigating the molecular mechanism of rice-false smut fungus interaction.

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