Original Research Article

Survey and isolation of rice false smut pathogen *Ustilaginoideavirens*(Cooke) Takahashi

Abstract

Roving survey has been conducted during first quarter of 2023 at selected paddy fields of Cauvery Delta zone (Tanjore, Perambalur) and Western zone (Coimbatore, Erode) of Tamil Nadu to assess the Percent Disease Incidence for rice false smut disease in which the maximum disease incidence was recorded in Erode district (40%).The infected spikelet were observed with the characteristic symptoms where the individual rice grains turned into large, fluffy and velvety green balls, later these balls turns hard during maturity.The pathogen *Ustilaginoideavirens* was isolated from the infected smut balls in potato sucrose agar medium in which the fungus appears as small white colonies after seven days of isolation.

Key words: Rice false smut, survey, isolation, *Ustilaginoideavirens*

1. Introduction

Rice (*Oryza sativa*) is one of the most important food crops worldwide, as it is a staple food for more than half of the world population. In India during 2021-22, the area, production and productivity of rice is 46.379 mha, 130.29 MT and 2,809 kg/ha respectively and in Tamil Nadu, the area, production and productivity of rice is 1.9 million ha, 7.55 MT and 3973 kg/ha respectively. Rice is affected by various fungal diseases such as blast, brown spot, false smut, stem rot, sheath blight and sheath rot causing much yield losses. For future food safety and security, the study of these diseases is absolutely essential. Among these diseases, Rice False Smut (RFS) is one of the most important sink infecting ascomycetes fungal diseases caused by *Ustilaginoideavirens*Cooke (Takahashi, 1896) and its teleomorphic stage is *Villosiclava virens* (Tanaka *et al.*, 2008). It was first reported at Tirunelveli district of Tamil Nadu, India (Cooke, 1878). The symptoms produced by *U. virens* are visible only after flowering when the fungus transforms individual grains of the panicle into a yellowish smut ball which further changes to yellowish orange, green, olive green and finally to greenish black (Biswas, 2001). Each matured false smut ball is composed of matured chlamydospore (outer layer), mycelia and immature chlamydospore (middle layer), white endosperm (inner

part), and glume (Tang *et al*., 2013). Mythologically rice false smut infection was recognized as a symbol of a bumper harvest once, it has been considered as a minor disease but now a days it causes high yield losses due to planting of high fertilizer responsive varieties and hybrids, application of enormous nitrogenous fertilizers and most especially global climatic change (Zhou *et al*., 2008; Ladhalakshmi*et al*., 2012). It is reported that the rice false smut disease (RFSD) significantly reduces yield upto 75 % (Upadhyay and Singh, 2013) and releases varieties of secondary metabolites such as ustiloxins, ustilaginoidins and sorbicillinoids which makes it as one of the most harmful fungal diseases been identified in recent years (Lu *et al*., 2015; Sun *et al*., 2017; Wang *et al*., 2017; Jiehua*et al*., 2019; Meng *et al*., 2019; Sun *et al*., 2020).These are the major type of ustiloxinsproducedhighly during its early maturity stage especially in the middle layer containing mycelia and immature chlamydospores (Wang *et al*., 2016) which are easily released into environment by air currents and rain splashes and creating new environmental challenges (Fan *et al*., 2016; Chen *et al*., 2020).

With these points in view, we have carried out survey for assessing the disease incidence of rice false smut in major rice growing areas of Cauvery delta and western zone of Tamil Nadu. Then, the pathogen was isolated from false smut balls collected during survey and characterized morphologically.

2. Materials and Methods

2.1. Survey and collection

Roving survey has been conducted during first quarter of 2023 at major rice growing areas of Cauvery delta zone (Tanjore, Perambalur) and Western zone (Coimbatore, Perambalur) Tamil Nadu were the false smut disease occurrence was recorded.The Percent Disease Incidence (PDI) for rice false smut disease was assessed (Baite *et al*., 2017) and the false smut balls were collected for pathogen isolation. The collected false smut balls were packed in 225 gauge transparent zip seal bags and labeled with date of collection, place of collection, variety of rice, stage of the crop and the collector name. After that the bags containing false smut balls were immediately kept in ice box and brought to the laboratory. The remaining false smut balls other than used for isolation were dried under shade up to getting constant weight and stored at -20 °C. Percent Disease Incidence (PDI) and Disease Severity were calculated by the following formulas.

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of tillers infected}}{\text{Total no. of tillers}} \times 100$$

Disease severity = Percent tillers infected × Percent smut balls

In order to compare the percent disease incidence and disease severity, the number of infected tillers/paniclesand the number of smut balls per panicle was recorded. All the experiment datas were represented in mean.

2.2. Isolation of rice false smut pathogen

The pathogen which has been causal of rice false smut disease was isolated from false smut balls collected from different districts of Tamil Nadu as per the procedure mentioned by Ladhalakshmi*et al*., (2012). Initially, surface sterilization of infected spikelets was carried out using 1% sodium hypochlorite for a minute and washed repeatedly thrice with sterile distilled water. Using a sterilized scalpel, the smut ball was cut into small pieces and placed at the center of the plate containing Potato Sucrose Agar (PSA) medium (potato = 200 g, sucrose = 20 g, agar agar = 20 g, distilled water = 1000 ml). To avoid bacterial contamination chloramphenicol (0.3 g / 1000 ml) was added to the PSA medium. The plates were incubated for 7-20 days at 27 ℃ for pathogen growth. After growth of the pathogen, the mycelial disc was taken by using sterilized cork borer and placed on the center of the petri plate containing PSA medium to get pure culture of the pathogen.

3. Results and discussion

3.1. Survey for disease assessment of rice false smut

Survey results revealed that the variations in the disease incidence of false smut of rice was observed in Erode, Tanjore, Perambalur and Coimbatore districts of Tamil Nadu from January-2023 to March-2023 **(Table.1)**. In Erode, the disease incidence was noticed between 5 to 40% among different cultivars. In Tanjore, the disease incidence was comparatively less among the varieties with the percentage range between 7 to 21%. In Perambalur, the false smut incidence was reported as 5.26%. In Coimbatore, the disease incidence varies from 12 to 32%, which causes reduction in grain yield. Among the areas surveyed, higher disease incidence was recorded in the rice variety Bhavani cultivated at Bhavanisagar, Erode district with the incidence of 40% **(Figure.1, Table.2).**The minimum disease incidence was recorded in the variety ADT 54 collected from Perambalur district of Tamil Nadu having the disease incidence of 5.26%. The infected spikelets from the regions of Erode, Tanjore, Perambalur

and Coimbatore districts of Tamil Nadu revealed that the disease incidence varied widely from one place to another through which disease severity was assessed. The maximum disease severity (342.8) was recorded in the variety Bhavani collected from Bhavanisagar, Erode followed by the variety TN 1 collected from Coimbatore **(Table.2)**. The incidence of infected tillers was found to range between 5% and 40%, causing a substantial reduction in grain yield.

Figure.1 Map depicting the surveyed areas of Tamil Nadu

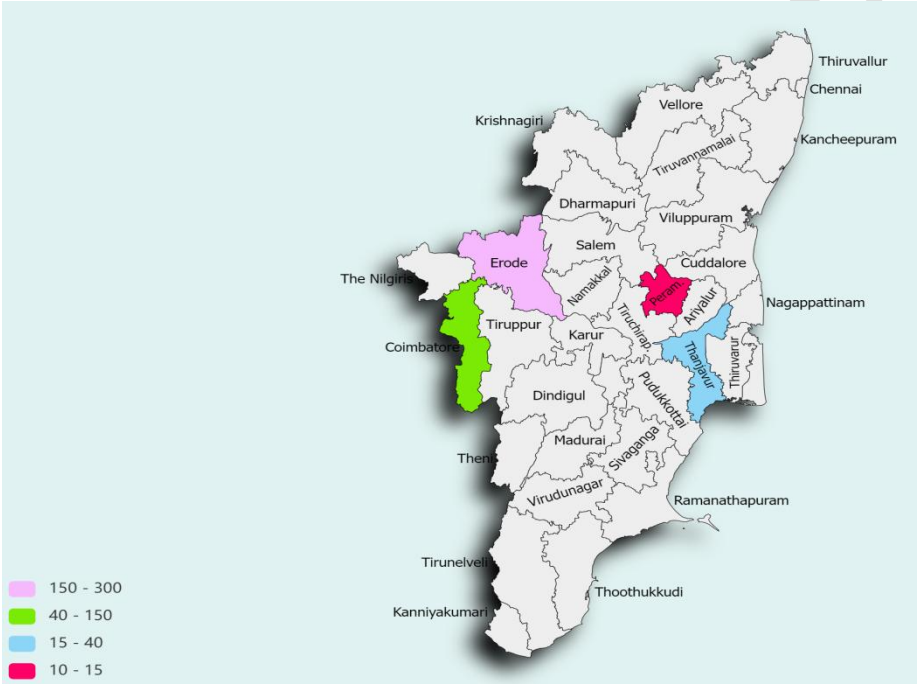

Table.1 Collection of rice false smut isolates from major rice growing areas of Tamil Nadu

S.No	Variety	Isolate	Place	Latitude	Longitude
1.	CR 1009	UvE-1	Erode	11°28’46”N	77°7’6”E
2.	ADT 45	UvE-2	Erode	11°28’59”N	77°7’8”E
3.	Bhavani	UvE-3	Erode	11°29’0”N	77°7’52”E
4.	VGD 1	UvT-1	Tanjore	10°59’56”N	79°28’47”E
5.	ADT 38	UvT-2	Tanjore	10°59’56”N	79°28’50”E
6.	CO 38	UvT-3	Tanjore	10°28’59”N	79°7’8”E
7.	ADT 54	UvP-1	Perambalur	11°22’12”N	78°47’46”E

8.	CO 51	UvC-1	Coimbatore	10°59’43.5”N	76°54’56.5”E
9.	TN 1	UvC-2	Coimbatore	11°00’10.3”N	76°55’29.0”E
10.	CO 52	UvC-3	Coimbatore	11°03’58”N	76°50’8”E

Similarly during2012, Ladhalakshmi*et al.,* conducted survey in northern states of India and revealed the variations of incidence observed between 2 to 75%. Singh *et al*., (2014) conducted survey in different regions of Uttar Pradesh and Tamil Nadu in which the incidence of the infected tillers ranged between 5 - 80%. During Kharif (2017) and (2018), Savitha *et al*., (2019) conducted a survey among different regions of Karnataka in which the maximum disease severity was recorded in Udupi taluk of coastal areas having the severity of 22.99%. Baite*et al*., (2017) conducted survey at Odisha among different varieties during kharif, in which the maximum incidence of 33.33% was observed at Pooja variety of rice. Also, Navarasu*et al*., (2022) conducted survey in different regions of Tamil Nadu with the false smut incidence ranged from 1.36 to 36.36% and the disease severity ranged between 1.08 to 379.23%.

Table.2 Occurrence of rice false smut disease incidence and Disease severity in Tamil Nadu

S. No	Isolate	Total no. of tillers	Infected tillers	Percent disease incidence (%)	Total no. of grains*	Smutted grains*	Percent smutted grains (%)	Disease severity (%)
1.	UvE-1	17	2	11.76^{f}	195	4.3	2.20	25.87^{f}
2.	UvE-2	19	1	5.26^{i}	123	2.7	2.19	11.51^{j}
3.	UvE-3	25	10	40^{a}	112	9.6	8.57	342.8^{a}
4.	UvT-1	28	6	21.42^{d}	197	2.2	1.11	23.77^{g}
5.	UvT-2	26	2	7.69^{h}	129	7	5.42	41.67^{e}
6.	UvT-3	32	3	9.37^{g}	167	3.1	1.85	17.33^{h}
7.	UvP-1	19	1	5.26^{i}	154	4.6	2.98	15.67^{i}
8.	UvC-1	16	2	12.5^{e}	136	5.1	3.75	46.87^{d}
9.	UvC-2	28	9	32.14^{b}	131	7.7	5.87	188.66^{b}
10.	UvC-3	23	6	26.08^{c}	102	2.3	2.25	58.68^{c}
							Mean =	77.283

*denotes mean of the replications

CD (p=0.05) = 3.9095 for percent disease incidence

CD (p=0.05) = 0.6537 for Disease severity

The values denoted by same letter are not significant at 5% level of DMRT

3.2. Symptomatology

The infected spikelets were observed with the characteristic symptoms such as the individual rice grains turned into large, fluffy and velvety green balls, later these balls became hard after maturity. Initially, these balls were observed as yellow in colour and at later stages it was turned into olive green to greenish black. The visible symptom of RFS includes grain replacement with fungal mycelium known as false smut balls. On maturity, the smut balls was covered with powdery chlamydospores from which the colour changes from yellowish orange to olive green and finally leads to greenish black **(Figure.2)**.

Figure.2 Collection of false smut balls from infected rice panicles

3.3. Isolation of the pathogen

The pathogen *Ustilaginoideavirens* was isolated from the infected smut balls in Potato Sucrose Agar (PSA) medium in which the fungus appears as small white colonies after seven days of inoculation. On observing the culture plate at 15th day after inoculation, the white mycelium turned yellow on maturity that was found similar to the smut balls found in the field condition **(Figure.3)**.

Figure.3 Isolation of *U. virens* in PSA medium

1 2 3 4 5

6 7 8 9 10

Different isolates of false smutgrown in PSA medium.1-CR 1009, 2- ADT 45, 3- Bhavani, 4-VGD 1, 5- ADT 38, 6- CO 38, 7-ADT 54, 8-CO51, 9- TN 1, 10-CO 52

On observing the plate at 15th day after inoculation, the white mycelium turned into yellow on maturity that was found similar to the smut balls found in the field condition. According to Ladhalakshmi*et al*., (2012) the pathogen of RFS is slow growing in PDA plates in which the fungi appears as tiny white colonies after 7 days of inoculation. They isolated the pathogen by placing the mycelial discs in the plates containing PDA medium. Manu *et al*., (2017) isolated the RFS pathogen by streaking the mass of chlamydospores in petri dishes containing PSA medium. Similarly, Khedkar *et al*., (2017) reported that during maturity, chlamydospores becomes dark brown, echinulated with rough surface and the diameter is found to be 5.0 to 6.0 µm. Large colonies are appeared after 7-14 days. The morphological characters were descrided as milky white or olive green colonies with flat or convex surface, fluffy mycelium, compact and leathery (Baite*et al*., 2014 and Baite& Sharma, 2015).

4. Conclusion

In recent years the rice false smut incidence has increased due to planting of high fertilizer responsive varieties and hybrids, high application of nitrogenous fertilizers and most inevitably global climatic change. So, the high incidence of RFS not only reduces the yield but also produces high amount of toxins. Therefore screening of virulent pathogen from the infected field has to be carried out to evaluate of RFS resistant rice germplasm and to generate eco-friendly management strategies for the control of pathogen and toxins produced by them.

References

1. Baite, M.S., S. Raghu, S. Lenka, A.K. Mukherjee, S.R. Prabhukarthikeyan, and M. Jena. 2017. "Survey of rice false smut caused by *Ustilaginoidea virens* in Odisha." *The Bioscan* 12 (4):2081-2085.
2. Baite, M.S., and R.K. Sharma. 2015. "Isolation technique and culture conditions of false smut pathogen (*Ustilaginoidea virens*) on rice." *Indian Phytopathology* 68 (1):50-55.
3. Baite, M.S., R.K. Sharma, T.P. Devi, P. Sharma, and D. Kamil. 2014. "Morphological and molecular characterization of *Ustilaginoidea virens* isolates causing false smut of rice in India." *Indian Phytopathol* 67 (3):222-227.
4. Biswas, A. 2001. "False smut disease of rice: a review." *Environment and Ecology* 19 (1):67-83.
5. Chen, X., D. Hai, J. Tang, H. Liu, J. Huang, C. Luo, T. Hsiang, and L. Zheng. 2020. "*UvCom1* is an important regulator required for development and infection in the rice false smut fungus *Ustilaginoidea virens*." *Phytopathology* 110 (2):483-493.
6. Cooke, M.C. 1878. "The fungi of Texas." *Botanical Journal of the Linnean Society* 17 (99):141-144.
7. Fan, J., J. Yang, Y.Q. Wang, G.B. Li, Y. Li, F. Huang, and W.M. Wang. 2016. "Current understanding on *Villosiclava virens*, a unique flower- infecting fungus causing rice false smut disease." *Molecular Plant Pathology* 17 (9):1321-1330.
8. Jiehua, Q., M. Shuai, D. Yizhen, H. Shiwen, and K. Yanjun. 2019. "Ustilaginoidea virens: A fungus infects rice flower and threats world rice production." *Rice Science* 26 (4):199-206.
9. Khedkar, D.T., M.S. Joshi, V.M. Karade, S.V. Pawar, and R.A. Karande. 2017. "Morphological characteristics of *Ustilaginoidea virens*." *Annals of Plant Protection Sciences* 25 (1):226-227.
10. Ladhalakshmi, D., G.S. Laha, R. Singh, A. Karthikeyan, S.K. Mangrauthia, R.M. Sundaram, P. Thukkaiyannan, and B.C. Viraktamath. 2012. "Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India." *Phytoparasitica* 40:171-176.
11. Lu, S., W. Sun, J. Meng, A. Wang, X. Wang, J. Tian, X. Fu, J. Dai, Y. Liu, and D. Lai. 2015. "Bioactive bis-naphtho-γ-pyrones from rice false smut pathogen

Ustilaginoidea virens." *Journal of agricultural and food chemistry* 63 (13):3501-3508.

12. Manu, D.G., S.S. Pramoda, A. Ramanathan, S. Ramchander, S. Manonmani, P. Jeyaprakash, and S. Robin. 2017. "Isolation, characterization and pathogenesis of *Ustilaginoidea virens* causing false smut disease in rice (*Oryza sativa* L.)." *Int J Curr Microbiol App Sci* 6:632-640.
13. Meng, J., W. Sun, Z. Mao, D. Xu, X. Wang, S. Lu, D. Lai, Y. Liu, L. Zhou, and G. Zhang. 2019. "Main ustilaginoidins and their distribution in rice false smut balls." *Toxins* 7 (10):4023-4034.
14. Navarasu, S., E.G. Ebenezar, K.E.A. Aiyanathan, and B. Jeberlin. 2022. "Survey for the incidence of false smut disease of rice in Tamil Nadu." *The Pharma Innovation* SP-11 (7):4461-4465.
15. Savitha, A.S., A. Nagaraja, S.B. Goudar, and N.L. Rajesh. 2019. "Assessing and mapping the severity of false smut of rice in different rice ecosystems of Karnataka." *Journal of Pharmacognosy and Phytochemistry* 8 (5):2020-2025.
16. Singh, S., A.A. Lal, S. Simon, A. Singh, R. Taduman, D.A.A. Kamaluddeen, and A.A. David. 2014. "Survey of false smut (*Ustilaginoidea virens*) of rice (*Oryza sativa* L.) in selected districts of Utter Pradesh, India." *The Bioscan* 9:389-392.
17. Sun, W., A. Wang, D. Xu, W. Wang, J. Meng, J. Dai, Y. Liu, D. Lai, and L. Zhou. 2017. "New ustilaginoidins from rice false smut balls caused by *Villosiclava virens* and their phytotoxic and cytotoxic activities." *Journal of agricultural and food chemistry* 65 (25):5151-5160.
18. Sun, W., J. Fan, A. Fang, Y. Li, M. Tariqjaveed, D. Li, D. Hu, and W.-M. Wang. 2020. "*Ustilaginoidea virens*: Insights into an emerging rice pathogen." *Annual review of phytopathology* 58:363-385.
19. Tang, Y.X., J. Jin, D.W. Hu, M.L. Yong, Y. Xu, and L.P. He. 2013. "Elucidation of the infection process of *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*) in rice spikelets." *Plant Pathology* 62 (1):1-8.
20. Takahashi, Y. 1896. "On *Ustilago virens* Cooke and a new species of *Tilletia* parasitic on rice-plant." *Shokubutsugaku Zasshi* 10 (109):en16-en20.

21. Tanaka, E., T. Ashizawa, R. Sonoda, and C. Tanaka. 2008. "*Villosiclava virens* gen. nov., comb. nov., the teleomorph of *Ustilaginoidea virens*, the causal agent of rice false smut." *Mycotaxon* 106 (1):491-501

22. Upadhyay, A.L., and R.V. Singh. 2013. "Yield Loss Assessment In Rice Due To False Smut." *Annals of Plant and Soil Research* 15 (2):173-174.

23. Wang, X., J. Wang, D. Lai, W. Wang, J. Dai, L. Zhou, and Y. Liu. 2017. "Ustiloxin G, a new cyclopeptide mycotoxin from rice false smut balls." *Toxins* 9 (2):54.

24. Wang, X., X. Fu, F. Lin, W. Sun, J. Meng, A. Wang, D. Lai, L. Zhou, and Y. Liu. 2016. "The contents of ustiloxins A and B along with their distribution in rice false smut balls." *Toxins* 8 (9):262.

25. Zhou, Y.L., Y.J. Pan, X.W. Xie, L.H. Zhu, J.L. Xu, S. Wang, and Z.K. Li. 2008. "Genetic diversity of rice false smut fungus, *Ustilaginoidea virens* and its pronounced differentiation of populations in North China." *Journal of Phytopathology*. 156 (9):559-564.