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Molecular Diagnostics of Pathogen, Status and Management of Newly Emerging False Smut Disease of Rice in different Geographic Areas of North Karnataka

Gururaj Sunkad*, Shivamurthy P., Pramesh Devanna and Kasi Rao Mediga
Department of Plant Pathology,
University of Agricultural Sciences, Raichur (Karnataka), India.

(Corresponding author: Gururaj Sunkad)*
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ABSTRACT: Rice false smut is an important emerging biotic stress caused by the fungus *Ustilaginoidea virens*. Occurrence and distribution of rice false smut disease had been documented from diverse agro-climatic zones of Karnataka are still scanty. In this study, comprehensive surveys, molecular diagnostics and management of disease were carried out to know the disease status, identity of pathogen and best fungicide for the management of the disease. The survey for the disease was carried out during *Kharif* 2019 and 2020 in different parts of North Karnataka. The cultural and morphological characters of the pathogen were studied by using different media. The molecular characterization of the pathogen was carried out by using universal ITS primers. The field evaluation of fungicides during *Kharif* 2019 and 2020, followed by large-scale demonstrations and farm trials in farmer's fields was conducted during *Kharif* 2021 for identification of best fungicide molecule. The results indicated the disease severity varied among different parts of north Karnataka. Potato sucrose agar (PSA) medium found to be superior for ideal growth of pathogen among different media tested. The fungus grows as acute angle branching, smooth and good mycelial growth with dark brown coloured and globular shaped chlamydospores. The DNA of *U. virens* was successfully amplified with universal ITS1 and ITS4 primers as well as specific *uvr-F* and *uvr-R* primers and the band size obtained was 700 bp and 350 bp, respectively. New combi fungicide molecule Trifloxystrobin 25% + Tebuconazole 50% WG @ 0.4 g/lit was very effective for the management of disease by recording lesser disease incidence and higher yields along with BC ratio in farm and large-scale demonstration trials.

Keywords: Characterization, False smut, Management, Rice, Severity.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the leading food crops with regard to human nutrition and caloric intake. Worldwide, India is the largest producer of rice, yielding 177.64 mt. of grains over an area about 43.78 mha (FAOSTAT, 2019). In Karnataka, rice is a major crop in command areas grown over an area of 1.15 mha, with an annual production of 3.63 mt. (Project Coordinators Report, 2019). The productivity of rice is very low due to various biotic and abiotic constraints. Among biotic stresses, rice false smut is one of the emerging and nationally important disease in recent time (Sharanabasav *et al*., 2020). RFS is caused by *Ustilaginoidea virens* (Cooke) Takahashi (teleomorph: *Villosiclava virens*), a pathogenic ascomycete fungus, causes a devastating grain disease in rice. It was first reported from Tirunelveli district of Tamil Nadu State of India by Cooke (1878). For a long time in rice production, RFS disease was categorized as a minor disease with sporadic occurrence in some rice-growing areas such as south and east Asia (Sun *et al.,* 2017). This disease transforms individual grain into initially yellow, green and later into velvety coloured pseudomorphs or smut balls (Sharanabasav *et al.*, 2020; Sekhar *et al.*, 2022) and causes reduction in the quality and quantity of rice grains and also affects the germination vigour of the infected seedlings (Tanaka *et al.*, 2008; Savitha *et al.*, 2019).

In India, disease incidence of 10-20% (Punjab), 5-85% (Tamil Nadu) and 4.44-17.12% (Karnataka) resulting in yield losses of 1–49% depending on rice cultivars and intensity of disease. This loss in yield caused by rice false smut is attributed to both smut balls as well as chaffiness, reduction in grain weight and infertility of the spikelet near the smut balls (Savitha *et al.,* 2019) and even more important, generating toxins poisoning to humans and domestic animals. Meanwhile, the status of disease is assuming epidemic in all rice cultivating ecosystems, it draws the attention towards the holistic approach for the management of disease (Rush *et al.*, 2000). Especially in recent years, its outbreak is anticipated due to high input cultivation, increased use of hybrid varieties, change in climate (Sekhar *et al.*, 2022) and farming systems (Lu *et al.,* 2009). Very few rice cultivars have tolerant to moderate level of resistance and majority of the commercially cultivated

varieties do not show any resistance to false smut (Huang *et al.,* 2016).

Rice is a major crop in Tungabhadra and Krishna command areas of north Karnataka and cultivated in two different ecosystems *viz.,* transplanted and dry seeded rice (DSR). Hence, a thorough understanding of morphological and molecular detection of the pathogen will provide suitable information which is useful to design more appropriate disease management strategies (Sharanabasav *et al.,* 2021). The bio-efficacy of many solo fungicides against false smut has been reported by various workers from different parts of the world (Sharanabasav *et al.,* 2020). Though, these chemicals have been reported to be effective in reducing the false smut severity in different locations such as, Kerala, Punjab and Uttar Pradesh, no systemic studies are found for its efficacy from irrigated ecosystems of Karnataka such as Tunga Bhadra and Krishna command areas. Moreover, there was no recommendation for the management of disease so far in the university package of practices (PoP). Considering the above facts and research gaps, the present investigations were planned and carried out with the main objectives, to know the disease severity, to diagnose the pathogen at morphological and molecular level and to manage the disease using fungicides.

MATERIALS AND METHODS

Survey for the disease severity: Major Rice growing districts of north Karnataka *viz*., Raichur, Bellary, Yadgir and Koppal mainly covering command areas were surveyed for the incidence of false smut during *Kharif* 2019 and 2020, when the crop was between maturity to harvesting stage. Per cent disease severity was assessed as per Singh and Dube (1978).

Disease severity (%) = Per cent infected tillers × Per cent infected grains

Effect of different solid media on *U. Virens:* During the survey, pathogen isolate from Koppal taluk was collected to diagnose the pathogen at cultural, morphological and molecular level at Rice Pathology Laboratory, ACRIP, Gangavathi and the isolate was named as Koppal-isolate. An experiment was conducted to find out the suitable medium for the growth and sporulation of the Koppal-isolate with the following media *viz.,* Potato Dextrose Agar (PDA), Potato Sucrose Agar (PSA), Yeast Glucose Agar (YGA), XBZ agar (XBZA) and Yeast Peptone Potato dextrose Agar (YPPDA). Three replications were maintained for each treatment. The petri plates were incubated at 28±1°C. Observations on colony growth were taken when the maximum growth was attained in any one of the media tested.

Morphological and molecular characterization: The Koppal-isolate was characterized with respect to morphological characters *viz.,* mycelial characters (color, width and branching of mycelium), colony characters (growth and morphology), chlamydospore characters (color, size and shape) on PSA media. The cultures were identified according to cultural descriptions given by Sharma and Joshi (1975). Molecular identity of Koppal-isolate of *U. virens* was also studied by PCR amplification of ITS rDNA conserved region. Isolate was grown in potato dextrose broth (PDB) for mycelium production to be used for DNA extraction. Cetyl Trimethyl Ammonium Bromide (CTAB) method (Zhou *et al.,* 2003) was adopted to extract the total DNA from the mycelium of *U. virens*. Two sets of primers *viz.,* ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3'), ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.,* 1990), and *uvr-F*(5′-CTTGTGTTTTCCAATGCATGT-3′), *uvr-R* (5′-ATTCAGTTATCCTCGCACTT G-3′) (Chen *et al.,* 2014) were used in the present study. The PCR was done by 35 cycles of denaturation at 94°C for 60 sec., annealing at 55°C for 60 sec. and extension at 72°C for 1.5 min. with an initial denaturation of 5 min at 94°C before cycling and final extension of 5 min. at 72°C after cycling. Amplified DNA product was resolved by gel electrophoresis on agarose gel (1%) in TAE (1X) buffer and photographs were taken using a gel documentation system.

Management of false smut under field conditions: A field experiment was conducted at Agricultural and Research Station, Gangavathi, Karnataka (15.4319° N, 76.5315° E), during two consecutive seasons, *Kharif* 2019 and 2020 to identify the effective fungicide in managing false smut of rice. Nine fungicides which were found most effective under *in vitro* were evaluated for their field efficacy in randomized block design with three replications. The popular and highly susceptible rice cultivar BPT-5204 was sown at a spacing of 30×10 cm and bio-efficacy was evaluated under natural epiphytic conditions. All the recommended package of practices for tillage, manuring and irrigation *etc*. were followed. Nine treatments *viz.,* five solo fungicide molecules and three combination fungicide molecules along with untreated control were evaluated. Two sprays of each fungicide was given for each treatment at booting stage (50 days after transplanting (DAT) and post-flowering (70 DAT) stage. Disease severity was calculated by using the formulae described by Singh and Dube (1978).

Farm Trials (FT) and Large-scale Demonstrations (LSD): Based on the experimental results over two seasons, farm and large-scale trials were conducted to test the efficacy of the superior fungicide molecule in comparison with an untreated control during *Kharif*, 2021 in farmers fields to confirm the findings through KVKs, AEECs and line departments in farmers fields through farm and large-scale demonstration trials. Seventeen farm and large-scale demonstration trials were laid out in farmer's fields of Raichur, Yadgir, Koppal, Gangavathi, B-Gudi, Dharwad and Sirsi districts with a spacing of 30×10 cm during *Kharif* 2021. All the recommended package of practices for tillage, manuring and irrigation etc. were followed. Two sprays of test superior fungicide were given for each treatment at booting stage (50 DAT) and post-flowering (70 DAT) stage. Yield (q/ha) in fungicide sprayed plot and untreated control plot was recorded.

Statistical analysis. Analysis of the experimental data was done by using suitable software by subjecting data of experiments.

RESULTS AND DISCUSSION

Symptomology of *U. Virens*: The symptoms of false smut started on the panicle, the spore balls were small at first and visible in between the glumes growing gradually to reach one cm or more in diameter, and enclosing the floral parts. They were slightly flattened, smooth and yellow and covered by a membrane. The membrane bursts as a result of further growth and color of the ball became orange and later yellowish green or greenish black. At this stage, surface of the ball cracks. When cut opened, smutted grains were white in the centre and consist of tightly woven mycelium together with the glumes and other tissues of the host (Fig. 1).

Disease severity of false smut: The results on two season data indicated that the mean disease severity of Tungabhadra command area ranged from 9.77-16.72% (Table 1). Among three districts, Koppal district recorded highest mean disease severity of 16.72% followed by Bellary district (15.92%) and least mean disease severity of 9.77% was recorded in Raichur. In Upper Krishna command area, Yadgir district recorded mean disease severity of 4.62% during *Kharif* 2019. Similarly, during *Kharif* 2020, mean disease severity of Tungabhadra command area ranged from 7.60-13.37% (Table 2). Among three districts, Koppal district recorded highest mean disease severity of 13.37% followed by Bellary district (10.52%) and least mean disease severity of 7.60% was recorded in Raichur. In Upper Krishna command area, Yadgir district recorded mean disease severity of 2.89%. Overall, Koppal district recorded highest false smut disease severity. Mandhare *et al.* (2008) reported that incidence of false smut disease was more in commercially cultivated varieties under irrigated condition.

Effect of different solid media on *U. Virens*: Koppal isolate exhibited varied diversity in its radial growth, colony color and texture on different solid media (Table 3 and Fig. 2). Radial growth was the maximum on PSA media (88.33 mm) which was significantly superior over all other tested media followed by XBZA (71.17 mm), PDA (68.50 mm) and YGA (61.00 mm). However, YPPDA media recorded the least radial growth (59.08 mm) as compared to all other media. PSA, XBZA and PDA supported good growth of fungus colony, whereas moderate colony growth was observed on rest of the media tested. Mycelium was whitish in all of the media, except in case of PDA and PSA wherein it was greyish to white and dirty brown at later stage of the growth of the pathogen. Raised mycelial growth with circular margin was observed in the all the tested media except PSA and XBZA which produced flat mycelium growth with regular margin on XBZA, whereas on PSA margin was wavy.

Several experiments were documented by earlier workers for identification of specific media for the growth of RFS pathogen. Li Yong *et al.* (2008); Wang *et al.* (2008) tested different solid and liquid media for radial growth and sporulation of *U. virens* and reported that among the solid media tested, PSA supported the fastest mycelial growth of pathogen.

Morphological and molecular characterization of pathogen: The mycelium of the fungus was white, acute angle branching, smooth and good growth with dark brown coloured and globular shaped chlamydospores. Mycelial width was 6.35µm and size of chlamydospore was 55.05 µm. Further, the isolate was subjected DNA extraction and PCR amplification with general ITS-1 and ITS-4 primers and specific *uvr-F* and *uvr-R* primers. Approximately 700 bp amplicon was obtained for universal ITS primers, wherein 350 bp amplicon was obtained for specific primer (Fig. 3). Fu *et al.* (2012) studied cultural characters on synthetic XBZA medium, the colony resembled white bread. To understand extent of diversity of the pathogen, PCR based technique with ITS and specific primers were attempted. In the present study, ITS1 and ITS-4 amplified 700 bp in the Koppal-isolate of *U. virens* genomes ITS region, which was within the range for ascomycetes, wherein amplicon size of 350 bp was observed with the pathogen specific primers (*uvr-F* and *uvr-R*).

Management of false smut under field conditions: The results revealed that all tested fungicides differed significantly with respect to disease incidence and yield compared to untreated control (Table 4). Among them, two sprays of Trifloxystrobin 25% + Tebuconazole 50% WG @ .04 g/lit was highly effective in managing the disease with least mean disease incidence (15.01%) as well as highest yield (54.66 q/ha) followed by Penconazole 25% EC @ 0.1 ml/l was also effective with mean disease severity of 19.67 and yield of 51.68 followed by Propiconazole 25% EC (21.43% and 51.31 q/ha). The highest mean disease severity (43.88%) and lowest yield (42.33 q/ha) was obtained in untreated control. In addition to this, similar trend was observed with respect to B:C ratio, highest BC ratio of (1:2.10) was recorded in Trifloxystrobin (25%) + Tebuconazole (50%) 75% WG @ 0.4 g/lit, while penconazole 25% EC @ 1 ml/lit and propiconazole 25% EC @ 1 ml/lit recorded (1:2.08) and 1: 208, respectively.

Farm trials (FT) and large-scale demonstration trials (LSD): The results revealed that the combi fungicide molecule that is Trifloxystrobin (25%) + Tebuconazole (50%) 75% WG@ 0.4 g/lit was highly effective in recording the least average disease severity (3.97) as well as higher yield (37.34 q/ha) of rice crop when compared to higher disease severity (14.15) and lesser yield (33.93 q/ha) confirming the results of field trials.

Table 1: Taluk and district wise severity of false smut of rice in different command areas of north Karnataka during *Kharif* 2019.

Command area	District	Taluk	Disease severity (%)	Mean (%)
Tungabhadra	Raichur	Raichur	10.85	9.77
		Sindhanur	17.19	
		Manvi	15.83	
		Devadurga	2.05	
		Lingasuguru	2.93	
	Bellary	Siruguppa	16.87	15.92
		Bellary	16.66	
		Hospet	14.23	
	Koppal	Gangavathi	18.10	16.72
		Koppal	15.33	
Upper Krishna	Yadgir	Shahapur	5.41	4.62
		Shorapur	6.11	
		Yadgir	2.34	

Table 2: Taluk and district wise severity of false smut of rice in different command areas of north Karnataka during *Kharif* 2020.

Command area	District	Taluk	Disease severity (%)	Mean (%)
Tungabhadra	Raichur	Raichur	8.76	7.60
		Sindhanur	10.90	
		Manvi	14.26	
		Devadurga	2.21	
		Lingasuguru	1.84	
	Bellary	Siruguppa	12.61	10.52
		Bellary	6.53	
		Hospet	12.42	
	Koppal	Gangavathi	20.08	13.37
		Koppal	6.67	
Upper Krishna	Yadgir	Shahapur	2.57	2.89
		Shorapur	4.00	
		Yadgir	2.12	

Table 3: Effect of different solid media on growth characters of *U. virens.*

Medium	Radial growth (mm)	Growth characters		
		Colour of colony	Type of growth	Shape of colony
PSA	88.33	Greyish to white	Good	Flat and wavy margin
YPPDA	59.08	Cream white	Moderate	Raised and circular margin
YGA	61.00	Whitish	Moderate	Raised and circular margin
XBZA	71.17	Bright Whitish	Moderate	Flat and regular margin
PDA	68.50	Cream white	Moderate	Raised and circular margin

Table 4: Management of false smut of rice under field conditions during *Kharif* 2019-20.

Sr. No.	Treatment	Dosage (g/l or ml/l)	PDI (%)		Pooled Mean	Yield (q/ha)		Pooled Mean	BC ratio
			Kharif 2019	*Kharif* 2020		*Kharif* 2019	*Kharif* 2020		
T_1	Trifloxystrobin 25% + Tebuconazole 50% WG	0.4	15.46 *(23.15)	14.56 *(22.42)	15.01 *(22.79)	53.96	55.36	54.66	2.10
T_2	Mancozeb 75% WP	2.0	23.96 (29.30)	25.55 (30.35)	24.75 (29.83)	48.58	50.48	49.53	2.02
T_3	Propiconazole 25% EC	1.0	19.32 (26.07)	23.55 (29.01)	21.43 (27.58)	51.91	50.71	51.31	2.08
T_4	Penconazole 25% EC	1.0	20.00 (26.56)	19.35 (26.08	19.67 (26.33)	49.83	49.53	49.68	2.03
T_5	Hexaconazole 5% SC	1.0	24.71 (29.80)	27.77 (31.07)	26.24 (30.81)	48.01	47.05	47.53	1.98
T_6	Tebuconazole 25.9% EC	1.0	20.30 (26.77)	22.33 (28.1)	21.31 (27.49)	49.01	50.03	49.52	1.97
T_7	Carbendazim 12% + Mancozeb 63% WP	1.0	32.88 (34.98)	29.55 (32.9)	31.21 (33.96	47.08	45.08	46.08	1.88
T_8	Hexaconazole 4% + Zineb 68% WP	1.0	30.56 (33.56)	33.52 (35.3)	32.04 (34.47)	49.70	46.35	48.03	1.96
T_9	Untreated control	---	42.21 (40.51)	45.55 (42.4)	43.88 (41.48)	43.23	41.43	42.33	1.81
S. Em±			0.96	0.92	-	0.65	0.63	-	-
CD at 5%			3.20	3.46	-	2.34	3.10	-	

*Figures in parenthesis are arc sin transformed values

Table 5: Performance of Trifloxystrobin 25% + Tebuconazole 50% against false smut and yield of rice in farm trial and large-scale demonstrations during *Kharif* 2021.

Location /Station	No. of trials	Mean Disease severity (%)		Mean Yield (q/ha)	
		Trifloxystrobin + Tebuconazole 75% WG@ 0.4 g/lit		Untreated control	Untreated control
JDA, Yadgir	2	2.50	7.25	40.00	37.00
JDA, Raichur	2	5.30	17.25	49.50	38.50
JDA, Koppal	2	3.50	11.00	57.50	38.35
KVK, Raichur	2	3.60	14.05	35.00	33.00
KVK, Gangavathi	2	8.10	26.15	28.00	27.00
AEEC, B'Gudi	2	2.50	16.25	26.50	28.00
KVK, Dharwad	2	2.25	10.60	31.20	22.00
KVK, Sirsi	1	3.50	11.25	45.00	52.50
AC, Raichur LSD	1	4.25	15.20	28.25	25.00
ARS, Gangavathi LSD	1	4.15	12.50	32.52	38.00
Grand Mean		**3.97**	**14.15**	**37.34**	**33.93**

Fig. 1. False smut (*Ustilaginoidea virens*) symptoms on Rice. A) initial stage, B) advanced stage and C) individual grain infected.

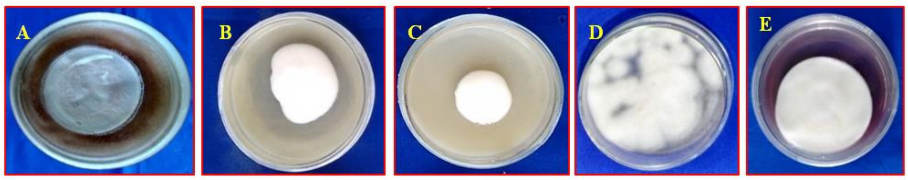

Fig. 2. Effect of different solid media on growth of *U. virens.* A) PDA, B) YPPDA, C) YGA, D) PSA, and E) XBZA.

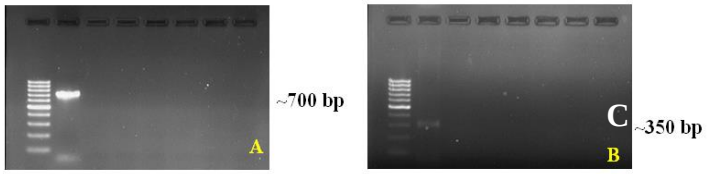

Fig. 3. PCR amplification of *U. virens.* A) ITS primers and B) Specific *uvr-F* and *uvr-R* primers.

CONCLUSIONS

In the present study, the incidence of false smut varied from season to season as well as location to location. Potato sucrose agar was found ideal for growth and development of pathogen under *in vitro*. The pathogen was identified and characterized as *U. virens* based on morphological characters as well as at molecular level by using both universal and specific primers. On the basis of performance in field and farm as well as large scale demonstration trials, Trifloxystrobin (25%) + Tebuconazole (50%) 75% WG @ 0.4 g/lit was found highly effective in the management of false smut and has found a place in the university package of practices for the benefit of farmers of the region. Now, the fungicide molecule has become very popular among farmers for the management of false smut.

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Conflict of Interest. None.

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