Int.J.Curr.Microbiol.App.Sci (2017) *6*(7): 632-640

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 7 (2017) pp. 632-640**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.607.077

Isolation, Characterization and Pathogenesis of *Ustilaginoidea virens* Causing False Smut Disease in Rice (*Oryza sativa* L.)

D.G. Manu[1*], S.S. Pramoda[2], A. Ramanathan[2], S. Ramchander[1], S. Manonmani[1], P. Jeyaprakash[1] and S. Robin[1]

[1]Department of Rice, Centre for Plant Breeding and Genetics,
[2]Department of Plant Pathology, Centre for Plant Protection Studies,
Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India -641003
**Corresponding author*

Keywords

False smut, *Ustilaginoidea virens*, Genetic diversity, Pathogenecity.

Article Info

Accepted:
14 June 2017
Available Online:
10 July 2017

A B S T R A C T

The present study was conducted in order to characterize the isolates of the fungus, *Ustilaginoidea virens* causing false smut disease, collected from different geographical regions of south India and cultured on Potato Sucrose Agar (PSA) medium. Morphological characterization exhibited that, the isolates produced well defined distinct colonies on PSA medium with colony growth ranging from 3.47 cm (TNUv5) to 5.57 cm (TNUv2), colony colour appeared as whitish to yellow except for TNUv5 wherein it showed yellowish to green. Higher mycelia dry weight was observed in TNUv2 (511.00 mg) followed by TNUv1 (503.62 mg) and least weight was recorded by TNUv5 (325.50 mg). Genetic diversity was estimated and dendrogram (UPGMA method) was constructed using 10 polymorphic RAPD markers. It produced two major cluster at 0.62 similarity coefficient, each cluster comprising of two (TNUv1 and TNUv6) and four (TNUv2, TNUv3, TNUv4 and TNUv5) isolates respectively. The divergence among the isolates depended on the geographical location. Beside morphological and molecular identification, the identity of the fungal pathogen was confirmed through ITS sequencing which showed 91 to 99 % identity with *U. virens* in NCBI-BLAST analysis. Pathogenecity test using TNUv2 isolates revealed that, injection of conidial suspension to the plants at booting stage develops symptoms within 12 days of injection and it will be useful for identification of resistance source and breeding resistant varieties against false smut. This study also emphasize that, different isolates of *U. virens* exhibit variation in the ITS (internal transcribed spacer) sequence which may be helpful to study the phylogenetic relationships among the isolates/races and seek the specific resistance genes.

Introduction

False smut (Green smut or pseudo smut) is a common grain disease of rice around the world, caused by *Ustilaginoidea virens* (Cooke) (Takahashi). Recently *Villosiclova virens* has been proposed as the new name for teleomorph of the false smut fungus (White *et al.*, 2000; Tanaka *et al.*, 2008; Ashizawa *et al.*, 2010). The fungus overwinters in soil by means of sclerotia and chlamydospores. *Sclerotia* produce ascospores, which are the primary source of infection to rice plants, whereas secondary infection comes from air-borne chlamydospores (Ashizawa *et al.*, 2010). Infection by the pathogen transforms

individual grains of infected panicles into initially orange, becoming yellowish green or greenish black at maturity (Ou, 1972; Lee and Gunnell, 1992). The disease was first reported from Tirunelveli district of Tamil Nadu State of India (Cooke, 1878). Historically being an uncommon and minor disease by occurring sporadically in certain regions (Ou, 1972; Dodan *et al.,* 1996), now epidemics of the disease is being reported frequently (Rush *et al.,* 2000; Singh and Pophaly, 2010; Ladhalakshmi *et al.,* 2012). This disease is considered as farmer's friendly and locally known as "*Lakshmi*" (meaning wealth) disease because it is found associated with bumper yields. However, the disease has been observed in severe form since 2001 due to widespread cultivation of high fertilizer-responsive cultivars and hybrids, heavy application of nitrogenous fertilizer and change in climate (Yaegashi *et al.,* 1989; Sugha *et al.,* 1993; Lu *et al.,* 2009). In India the disease has been observed in major rice-growing states *viz*., Haryana, Punjab, Uttar Pradesh, Uttaranchal, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Jharkhand, Gujarat, Maharastra, Jammu and Kashmir and Puducherry (Dodan, 1996; Mandhare *et al.,* 2008; Ladhalakshmi *et al.,* 2012).

Since *U. virens* is very slow growing fungus, it is very difficult to isolate in its pure form. It often gets contaminated by the fast growing saprophytes during isolation, incubation and also difficult to be created artificially under screen house or field conditions. The development of suitable medium for growth and sporulation would facilitate further studies. Practical stand-point, a medium capable of stimulating sporulation of the fungus would aid in the preparation of spore suspension required for inoculation of large populations in disease resistance breeding programs. The disease not only threatens rice production in yield and quality but the chlamydospores also contaminate the rice grains and straw with their antimitotic cyclic peptides (ustiloxin) which are dangerous to the health of human and livestocks (Nakamura *et al.,* 1992; Luduena *et al.,* 1994; Ashizawa *et al.,* 2011). In India the research on false smut has been negligible because of its minor importance and partly due to a problem in the artificial culturing and inoculation of the pathogen. With all these points in mind, the present study was carried out to study/assess the variability of the pathogen collected from various geographical locations and standardization of artificial inoculation technique for large scale screening of rice genotypes in order to identify resistant source against false smut.

Materials and Methods

Sample collection

The samples of typical false smut on rice (Fig. 1) were collected from different geographical regions *viz*., Hyderabad (IIRR), Paddy Breeding Station (Tamil Nadu Agricultural University, Coimbatore), Department of Agronomy (Wetland, TNAU Coimbatore), Hybrid Rice Evaluation Centre (HREC, Gudalur), farmers fields of Thanjavur, and Gobichettipalayam during 2016. Latitude, longitudes and annual mean temperature of site of collection were depicted on map (Fig. 2).

Isolation, purification and maintenance of the pathogen

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

@ 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. A single, well isolated colony of the fungus was picked up using sterilized needle and transferred to the fresh PSA slants and maintained as a pure culture. Six isolates were purified and maintained on PSA medium at 4°C. Thus, the purified cultures were maintained by periodical transfers on PSA slants, they were serially numbered (TNUv1 to TNUv6) and used for further studies (Table 1).

Cultural and morphological characterization

In order to study the detailed cultural characteristics of the pathogen, 5 mm mycelial discs were collected from ten days old culture of *U. virens* and inoculated at the center of PSA plates in three replicates under aseptic cultural condition (27 ± 2°C temp). After 30 days of inoculation (DAI) the observations were recorded on growth of the pathogen on the solid media *viz.,* colony colour, colony diameter, growth type and mycelia dry weight. Radial mycelium growth was measured at 10 days interval (10^{th}, 20^{th}, 30^{th} DAI). Mycelial dry weight of each isolate was observed by culturing the fungus on potato sucrose broth. Two mycelial discs of 5 mm of each isolates were inoculated in the broth and the flasks were incubated at 27 ± 2 °C temperature for 30 days. Culture filtrate along with mycelial mats were filtered on Whatman No. l filter paper and dried in the oven at 60 °C for 3 hours, the weight of the mycelial mats were recorded.

Diversity analysis

DNA extraction

All the six isolates were cultured in 50 ml liquid potato sucrose broth in a 100 ml conical flask at 26°C and shaken at 200 rpm. After 7 days, the culture was transferred to 50 ml liquid potato sucrose broth in a 250 ml conical flask and incubated under the same conditions for 3 days. The mycelia were harvested and immediately ground into a fine powder by liquid nitrogen for DNA extraction following the protocol described by Nakada *et al.,* (1994). Concentrations of DNA were estimated by measuring absorbance at 260 nm with a Nanodrop (GENOVA NANO, UK). DNA samples were diluted to working solutions of 30 ng/ µl and stored at 4 °C until use.

RAPD amplification and data analysis

Fourteen random decamer RAPD primers (Table 2) were used to survey the isolates. Amplifications were performed in a total volume of 25 µl containing 0.5 µl dNTPs mix (10mM), 1 µl primer (12.5 pmol), 2 ml of DNA (30 ng/ µl), 1.2 µl MgCl2, 1.5 µl reaction buffer (10X), 0.3 µl of *Taq* polymerase (1.5 U) and 18.5 µl deionized water. The DNA amplification was done in a thermal cycler (BioRad, USA) using the following PCR cycles. The first denaturation step of 2 minutes at 94 °C, followed by 35 cycles of denaturation for 30 sec at 94 °C, annealing for 1 min at 40°C, extension at 72°C for 2 min and final extension at 72 °C for 5 min with holding temperature at 4°C for 10 min. Reaction products (8 µl) were resolved by electrophoresis in 1.2% agarose gels stained with ethidium bromide in 1X TBE buffer at 100 V for 90 min. Differences in banding patterns among isolates were assessed visually using gel documentation system (BioRad, USA). Polymorphisms including faint bands that could be scored were included in the analyses. Polymorphic RAPD markers were manually scored as binary data with presence as (1) and absence as (0). The similarity matrix was then subjected to the unweighted pair group method with arithmetical mean (UPGMA)

cluster analysis by using NTSYSpc version 2.02i.

PCR amplification and sequencing of ITS region

Based on the RAPD diversity study, four diverse isolates were selected out of six and examined for the amplification of ITS region, which is having gene cluster of ITS1, 5.8S and ITS2. ITS region specific primers ITS1 F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 R (5′-TCCTCCGCTTATTGATATGC-3′) designed by White *et al.,* (1990) were used for PCR amplification. Reaction products (8µl) were resolved by electrophoresis in 1.2% agarose gels stained with ethidium bromide in 1X TBE buffer at 100 V for 90 min. After electrophoresis the gel slice along with PCR fragments were excised with a clean, sharp scalpel. Then, they were purified using QIAquick Gel Purification Kit (QIAGEN, California) according to the manufacturer's protocol. The purified DNA from ITS-PCR was given for sequencing to Bioserve solutions, Hyderabad. The partial sequence of rDNA was obtained and it was submitted to the National Centre for Biotechnology Information (NCBI), Gene Bank, New York, USA and accession numbers were obtained (KX421100, KX421101, KX421102 and KX421103) (Table 3).

Pathogenicity test

Paddy variety BPT 5204 (Samba Mashuri) plants were brought to the screen house from main field at booting stage. The plants were inoculated with conidial suspension as described by Fujita *et al.,* (1989). Pure culture of fast growing (TNUv2) isolate was inoculated into 100 ml of potato sucrose broth (PSB) and kept in an incubator shaker (125 rpm) at 28°C for 2 weeks for incubation. The conidia were harvested and suspended in sterile distilled water. Plants at booting stage were selected for inoculation and injected with 2 ml of conidial suspension (1×10^5 conidia ml^{-1}). The inoculated plants were kept in a humidity chamber with an RH of 95% and 24-26°C temperature in screen house. The rice panicles injected with sterile distilled water served as control.

Results and Discussion

Morphological characterization of *U. virens*

The isolates produced well defined colonies on PSA medium. Maximum colony growth of 5.57 cm was observed in TNUv2 followed by TNUv3 (5.07 cm), whereas minimum colony growth of 3.47cm in TNUv5. The colony colors in most of the isolates were initially white and turned to yellow. However, TNUv5 isolate alone showed greenish yellow even after incubation period of 30 days. Most of the isolates produced raised hat like structures except TNUv1and TNUv2, which showed flat growth in culture media. The highest mycelial dry weight was observed in TNUv2 (511.00 mg) followed by TNUv1 (503.62 mg) and the lowest was in TNUv5 (325.50 mg) (Table 4).

Molecular characterization of *U. virens* using RAPD markers

Genomic DNA was isolated from six different isolates and subjected to RAPD-PCR amplification using 14 random decamer primers. Out of 14 primers, 10 RAPD (Table 2) primers produced reproducible and scorable polymorphic bands. The size of RAPD fragments on 1.2% agarose gel ranged from 100 to 2000 bp (Fig. 3). The Jaccard similarity coefficient values among *U. virens* isolates ranged from 0.51 to 0.96. The dendrogram constructed through UPGMA analysis clearly grouped the isolates into two major clusters at 0.62 similarity coefficient (Fig. 4). Dendrogram consist of two major

clusters A and B. The isolates TNUv1 (Hyderabad) and TNUv6 (Gobichettipalayam) coming under cluster A and TNUv2 (PBS, Coimbatore), TNUv3 (Wetland, Coimbatore), TNUv4 (Gudalur) and TNUv5 (Thanjavur) belongs to cluster B. There were two sub clusters (BI and BII) were observed under major cluster B, BI consist of three isolates TNUv2 (PBS, Coimbatore), TNUv3 (Wetland, Coimbatore), TNUv4 (Gudalur), where isolates TNUv3 and TNUv4 showed a relative genetic similarity value of 0.96. The isolate TNUv5 (Thanjavur) belongs to BII sub cluster. Although, major clustering supported the geographical origin of isolates in general, critical analysis revealed that fine clustering does not support exact geographical origin of isolates and their genetic properties. The information obtained from sequencing of isolates *viz.,* TNUv1, TNUv2, TNUv4 and TNUv5 had 678 (95%), 612 (99%), 674 (97%) and 649 (91%) base pairs respectively. Inside the parenthesis says per cent coverage identity in BLAST search http://www.ncbi.nih.gov/index.html (Table 3).

The rice variety BPT 5204 was used to test the pathogenicity of the isolated fungus by artificially injecting the conidial suspension of TNUv2 to the tillers at the booting stage. Out of ten inoculated plants, three plants produced typical false smut balls on the panicles with 30 percentage of diseased hill (PDH) after 12 days of inoculation. *U. virens* infected the young ovary of the individual spikelets and transformed them into large, velvety green balls. Young smut balls were fleshy inside and became hard after some time. Initially the smut balls were small, visible between glumes and were covered with a membrane that burst at a later stage (Fig. 5).

False smut disease is commonly occurring in the wet season all over the world. Flowering stage coinciding with rains and high humidity, many of the high yielding rice varieties succumb to varied level of infection. Limited information is available on the pathogen diversity and hence this study becomes relevant. The pathogen was characterized based on its colony and spore morphology, which produced pure white coloured, raised, smooth colony margin and on continuous incubation, the colour converted to light yellow and in some cases green. Similar morphological characteristics of the pathogen were described by Hegde *et al.,* (2000) and Ladhalakshmi *et al.,* (2012) in rice. The isolates produced well defined colonies on potato sucrose agar medium with colony growth ranging from 3.47 to 5.57 cm after 30 days of incubation. Initially the colony growth was creamy white colour resembling straw hat shape with undulations, which turned yellowish after 20-25 days of incubation. In the culture medium the chlamydospore burst and appears after 30- 40 days after incubation at 27 ^{o}C. The colour of the mycelium changed white to yellow and later greenish yellow resembling symptoms in the field. These results agreed with Ladhalakshmi *et al.,* (2012) and Baite *et al.,* (2015). In culture medium *U. virens* proliferated and produced creamy-white colony, flat or rose with slight undulations, mycelium fluffy, compact and leathery. Chlamydospores were observed at the centre or margin of the colony at later stage of growth.

RAPD markers were used to estimate genetic variation in *U. virens* and to determine if any of population structure occurred with regard to geographical origin. The UPGMA algorithm constructed a neighbor joining tree which grouped the isolates into two major clusters at 0.62 Jaccard similarity coefficients (Fig. 4). Cluster A comprised of two isolates TNUv1 (Hyderabad) and TNUv6 (Gobichettipalayam). Cluster B comprised of four isolates which were again subdivided into two subgroups. The first subgroup (BI)

comprise of three isolates, out of which two isolates TNUv3 (Wetlands, Coimbatore) and TNUv4 (Gudalur) with 0.96 similarity coefficient and TNUv2 (PBS, Coimbatore) different from other isolates of same subgroup, the only isolate TNUv5 (Thanjavur) belongs to second subgroup (BII). In earlier studies RAPD marker system has been used successfully to characterize molecular variation in *U. virens* (Li *et al.*, 2004; Xiao-ping *et al.*, 2008; Mathew *et al.*, 2014). Our results suggest that RAPD markers can be used to evaluate genetic diversity within the species *U. virens* through estimates of variation at multiple loci across the genome.

The advent of molecular biology has caused a significant shift in the types of approaches used to identify and characterize plant pathogens and to device management strategies. The polymerase chain reaction (PCR) method has been developed for the *in vitro* amplification of nucleic acid sequence and has been used to detect a number of plant pathogens based on the specific nucleotide sequences. This method is highly sensitive and capable of detecting even a single copy of DNA molecule (Henson and French, 1993). PCR based technique was used for detection and characterization of *U. virens* based on the Internal Transcribed Spacer (ITS) regions of ribosomal DNA (rDNA).

Table.1 Isolates collected from different locations

Isolates	Place of collection
TNUv1	Indian Institute of Rice Research, Hyderabad
TNUv2	Paddy Breeding Station, Coimbatore
TNUv3	Wetland Farm, Coimbatore
TNUv4	Hybrid Rice Evaluation Centre, Gudalur, The Nilgiris
TNUv5	Farmer field, Thanjavur
TNUv6	Farmer field, Gobichettipalayam

Table.2 Primers used for RAPD-PCR amplification of *U. virens* isolates

Primer name	Primer sequence 5'-3'	Nature of primer
OPK07	AGCGAGCAAG	**Polymorphic**
OPF03	CCTGATCACC	**Polymorphic**
OPG02	GGCACTGAGG	**Polymorphic**
OPD20	ACCCGGTCAC	**Polymorphic**
OPA-08	GTGACGTAGG	Monomorphic
OPB-09	TGGGGGACTC	Monomorphic
OPG17	ACGACCGACA	**Polymorphic**
OPE03	CCAGATCGAC	**Polymorphic**
OPL17	AGCCTGAGCC	**Polymorphic**
OPC10	TGTCTGGGTG	**Polymorphic**
OPG06	GTGCCTAACC	**Polymorphic**
OPC-11	AAAGCTGCGC	Monomorphic
OPE-14	TGCGGCTGAG	Monomorphic
OPM16	GTAACCAGCC	**Polymorphic**

Table.3 Fungal identification based on NCBI-BLAST search (for ITS1-5.8s-ITS4 region)

Isolates	Source	Accession number	Base pair length	Species identified as	Coverage identity (%)
TNUv1	Hyderabad	KX421100	678	*U. virens*	95
TNUv2	Coimbatore	KX421101	612	*U. virens*	99
TNUv4	Gudalur	KX421102	674	*U. virens*	97
TNUv5	Thanjavur	KX421103	649	*U. virens*	91

Table.4 Cultural and morphological characteristics of different isolates of *U. virens* on PSA medium

Isolates	Radial mycelium growth (cm)			Dry weight (mg) 30 (DAI)	Colony colour	Growth type
	10th day	20th day	30th day			
TNUv1	1.3 (1.14)^{c}	3.07 (1.75)^{c}	5.00 (2.24)^{b}	503.62 (22.44)^{a}	Creamy white	Flat, Fluffy and Circular
TNUv2	1.88 (1.37)^{a}	3.93 (1.98)^{a}	5.57 (2.36)a	511.00 (22.61)^{a}	White to yellow	Flat and Circular
TNUv3	1.61 (1.27)^{b}	3.46 (1.86)^{b}	5.07 (2.25)^{b}	421.75 (20.54)^{b}	Yellowish white	Raised, Fluffy and Circular
TNUv4	1.17 (1.08)^{c}	2.8 (1.67)^{d}	4.5 (2.12)^{c}	479.75 (21.90)^{a}	White to yellow	Flat, Fluffy and irregular
TNUv5	0.88 (0.94)^{d}	2.35 (1.53)^{e}	3.47 (1.86)^{e}	325.50 (18.04)^{c}	Yellow to green	Raised, irregular
TNUv6	1.00 (1.00)^{d}	2.3 (1.52)^{e}	3.92 (1.98)^{d}	339.25 (18.42)^{c}	White to yellow	Raised, fluffy irregular
SE (d)	**0.03**	**0.03**	**0.03**	**0.42**	-	-
CD(P=0.05)	**0.07**	**0.07**	**0.06**	**0.90**	-	-

Figures in parentheses represent square root transformation. Means in a column followed by same superscript letters are not significantly different according to DMRT

Results revealed that all the four isolates amplified a fragment of 625 bp for universal ITS primer (ITS1/ ITS 4). The results agreed with Baite *et al.*, (2015) who observed sequence length of Uv2 (Bulandshahr, Uttar Pradesh) and Uv3 (Haridwar, Uttarakhand) with 645 and 634 respectively and the identity was 98-99%. It is thus indicated that identity of the fungus could be confirmed by sequencing PCR products of ITS regions using primers. It is necessary to develop an efficient inoculation method for investigation of the rice-*U. virens* interaction. Up to date, quite a few reports consistently conclude that inoculation by injection of spores into the sheath during booting stage is sufficient and efficient to induce disease symptoms. Wang *et al.*, (2008) obtained a white smut strain that is virulent to rice plants, with this strain, it was found that disease severity is higher by injection than by spraying. Low temperature exposure after inoculation has a strong stimulatory effect on disease development (Kulkarni and Moniz, 1975; Fujita *et al.*, 1989;

Hegde *et al.,* 1998; and Xiu *et al.,* 2011; Ladhalaxmi *et al.,* 2012). Lu *et al.,* (2009) studied the pathogenic diversity of *U. virens* by injecting the conidial inoculum of the fungus during the booting stage under field conditions. Ashizawa *et al.,* (2010) developed a modified method of artificial inoculation by injecting the conidial inoculum of the fungus. Haiyong *et al.,* (2015), injected a conidial suspension (1 x 10^6 conidia ml^{-1}) of *U. virens* at the booting stage and incubated the plants in a moist cabinet initially at 15°C for 2 days, and then at 26°C for 5 days. Similar results were observed by injection of the conidial inoculum produced by artificially culturing the fungus, led to typical false smut symptoms under glasshouse conditions.

Genetic improvement of rice for false smut resistance is in need of a robust and moderate throughput screening methods for identification of genes and genotypes conferring resistance to false smut. The study has brought out some leads on the pathogenesity and isolates diversity that would carry forward the research in the right context.

Acknowledgement

The authors are grateful to Indian Council of Agricultural Research (ICAR), New Delhi for providing financial assistance through the Crop Research Platform, “Incentivizing Agriculture”.

Conflict of Interest

Authors certify that there is no financial commitment to the sponsor. Authors also state that they have full control of all primary data and that they agree to allow the journal to review their data if requested.

References

Ashizawa, T., Takahashi, M., Moriwaki, J., Hirayae, K. 2010. Quantification of the rice false smut pathogen *Ustilaginoidea virens* from soil in Japan using real-time PCR. *Eur. J. Plant Pathol.,* 128(2): 221-232.

Ashizawa, T., Takahashi, M., Moriwaki, J., Hirayae, K. 2011. A refined inoculation method to evaluate false smut resistance in rice. *J. Genet. Pl. Pathol*., 77(1): 10-16.

Baite, M.S., Sharma, R.K. 2015. Isolation technique and culture conditions of false smut pathogen. *Ustilaginoidea virens*. of rice. *Indian Phytopathol.,* 68(1): 50-55.

Cooke, M.C. 1878. Some extra European fungi. *Grevillea*, 7: 13-15.

Dodan, D.S., Ram, S., Sunder, S. 1996. False smut of rice: Present status. *Agril. Rev.,* 17: 227-240.

Fujita, Y., Sonoda, R., Yaegashi, H. 1989. Inoculation with conidiospores of false smut fungus to rice panicles at booting stage. *Ann. Phytopathol. Soc. Japan*, 55: 629-634.

Haiyong, H., Wongkaew, S., Jie, Y., Xuehui, Y., Xiaojun, C., Shiping, W., Qigqun, T., Lishuang, W., Sompong, M., Buensanteai, N. 2015. Biology and artificial inoculation of *Ustilaginoidea virens*. Cooke). Takahashi in rice. *Afr. J. Microbiol. Res.,* 9(11): 821-830.

Hegde, Y.R. 1998. Biology and Management of False Smut of Rice. *Oryza sativa* L. caused by *Claviceps oryzae sativa* Hashioka in Karnataka. *Karnataka J. Agric. Sci.,* 13(3): 623-627.

Hegde, Y.R., Anahosur, K.H., Kulkarni, S. 2000. Morphological and cultural studies on *Claviceps Oryzae sativae* a causal agent of false smut of rice. *Karnataka J. Agric. Sci.,* 13(4): 1005-1008.

Henson, M.J., French, R. 1993. The polymerase chain reaction and plant disease diagnosis. *Annu Rev. Phytopathol.*, 31: 81-109.

Kulkarni, C.S., Moniz, L. 1975. False smut of Rice -An airborne disease. *Curr. Sci.,* 44: 483-484.

Ladhalakshmi, D., Laha, G.S., Ram, S., Karthikeyan, A., Mangrauthia, S.K., Sundaram, R.M., Thukkaiyannan, P., Viraktamath, B.C. 2012. Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India. *Phytoparasitica*, 40: 171-176.

Lee, F.N., Gunnell, P.S. 1992. False smut Compendium of rice diseases St Paul: *American Phytopathol. Soc.,* 28.

Li, Z.Y., Juan, F.J., Zhen, Z.C., Zhou, L.X., Shu, W., Jun, K.K. 2004. Preliminary analysis of genetic diversity and population structure of *Ustilaginoidea virens*. *Acta Phytopathol.*, 34(5): 442-448.

Lu, D.H., Yang, X.Q., Mao, J.H., Ye, H.L., Wang, P., Chen, Y.P., He, Z.Q., Chen, F. 2009. Characterizing the pathogenicity diversity of *U virens* in hybrid rice in China. *J. Plant Pathol.*, 91: 443-451.

Ludueńa, R.F., Roach, M.C., Prasad, V., Banerjee, M., Koiso, Y. 1994. Interaction of ustiloxin with bovine brain tubulin. *Biochem. Pharmacol.*, 47:1593-1599.

Mandhare, V.K., Gawade, S.B., Game, B.C., Padule, D.N. 2008. Prevalence and incidence of bunt and false smut in paddy. *Oryza sativa* L. seeds, *Maharashtra Agric. Sci. Digest*, 28(4): 292-294.

Mathew, S.B., Sharma, R.K., Prameela, D.T., Pratibha, S., Deeba. 2014. Morphological and molecular characterization of *U virens* isolates causing false smut of rice in India. *Indian Phytopathol*, 67(3): 222-227.

Nakada, M., Tanaka, C., Tsunewaki, K., Tsuda, M. 1994. RFLP analysis for species separation in genera *Bipolaris* and *Curvularia*. *Mycosci.*, 35: 271-278.

Nakamura, K., Izumiyama, N., Ohtsubo, K., Koiso, Y., Iwasaki, S., Sonoda, R., Fujita, Y., Yaegashi, H., Sato, Z. 1992. Lupinosis in mice caused by ustiloxin and a crude extract of fungal culture of *U virens*. *Proc. Jpn. Assoc. Mycotoxicol.*, 35: 41-43.

Ou, S.H. 1972. Rice diseases Commonwealth Mycological Institute Kew.

Rush, M.C., Shahjahan, A.K.M., Jones, J.P. 2000. Outbreak of false smut of rice in Louisiana. *Pl Dis.*, 84(1): 100.

Singh, A.K., Pophaly, D.J. 2010. An unusual rice false smut epidemic reported in Raigarh District Chhattisgarh. *Int. Rice Res. Notes*, 35: 1-3.

Sugha, S.K., Sharma, O.P., Kaushik, R.P. 1993. Performance of rice genotypes against false smut pathogen under rainfed conditions. *Pl. Dis. Res.*, 8: 76-77.

Tanaka, E., Tanaka, C. 2008. Phylogenetic study of Clavicipitaceous fungi using acetaldehyde dehydrogenase gene sequences. *Mycosci.*, 49:115-125.

Wang, S., Li, M., Dong, H., Liu, X.Z., Bai, Y.J. 2008. Sporulation inoculation methods and pathogenicity of *Ustilaginoidea albicans* the cause of white rice false smut in China. *J. Phytopathol.*, 156: 755-757.

White, J.F., Sullivan, R., Moy, M., Patel, R., Duncan, R. 2000. An overview of problems in the classification of plant parasitic Clavicipitaceae. *Studies in Mycol.*, 45: 95-105.

White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to Methods and Applications*, 315-322.

Xiao-ping, T., Jian-wei, S., Er-ming, L., Nian-xi, L., Jin-hui, W., Qi-ming, X. 2008. Analysis of genetic diversity of *U. virens* in Hunan. *Natural Sci.*, 34(6): 694-697.

Xiu, Y., Shu, W., Lin, L., Xin, Y.D., Raun, C. 2011. Study on characteristics of the mycelial growth and conidia germination of U virens. *J. Fujian Agric. Forestry Univ.*, 40(6): 576-581.

Yaegashi, H., Fujita, Y., Sonoda, R. 1989. Severe outbreak of false smut of rice in 1988. *Plant Prot. Tokyo*, 43: 311-314.

How to cite this article:

Manu, D.G., S.S. Pramoda, A. Ramanathan, S. Ramchander, S. Manonmani, P. Jeyaprakash and Robin, S. 2017. Isolation, Characterization and Pathogenesis of *Ustilaginoidea virens* Causing False Smut Disease in Rice (*Oryza sativa* L.). *Int.J.Curr.Microbiol.App.Sci.* 6(7): 632-640.
doi: https://doi.org/10.20546/ijcmas.2017.607.077