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Prevalence and Diversity of *Ustilaginoidea virens* Inciting Newly Emerging False Smut of Rice in North East Karnataka

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Authors Contribution

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Abstract

False smut of rice (FSR) is an emerging disease posing a major threat to rice cultivation throughout the world. A comprehensive and systematic survey was conducted in the major rice growing regions of Karnataka. There was a large variation in disease severity over the rice growing seasons which ranged from 2.05 to 18.10 per cent. Fifteen isolates of *Ustilaginoidea virens* collected during the survey showed varied morphological characteristics and the isolate Uv-2 was highly virulent. Further, fifteen isolates were confirmed as *Ustilaginoidea virens* upon successful amplification with universal ITS1 and ITS4 primers as well as specific uvr-F and uvr-R primers. Approximately 500-620 bp amplicon was obtained with universal primers, whereas 280-320 bp amplicon was obtained for specific primers. Phylogenetic analysis based on ITS region among the isolates exhibited significant genetic variation especially isolate Uv-2 separated out from rest of cluster.

Keywords : Morphology, Survey, Rice, *Ustilaginoidea virens*, Variability

Rice (*Oryza sativa* L.) is the most important staple food for more than half of the global population. It is a major cereal crop that is widely grown as a food crop around the world. False smut of paddy is a catastrophic grain-infecting disease caused by *Ustilaginoidea virens* (Cook) Takahashi (Telomorph: *Villosiclava virens*), a pathogenic ascomycete fungus. Affected grains turn yellow to black coloured smut balls. The pathogen causes economic losses in rice grain productivity and grain quality (due to toxin contamination of grains) (Dodan and Singh, 1996; Muniraju *et al.*, 2017 and Wang *et al*., 2019). Cooke (1878) was the first to report false smut in India, in the Tirunelveli district of Tamil Nadu state and the disease has since spread to over forty countries, primarily in Asia rice-producing countries, but also in the United States. However, it has recently emerged as one of the most serious diseases in the majority of rice-growing regions around the world (Tanaka *et al*., 2008) and is widespread in India's states of Andhra Pradesh, Assam, Bihar, Haryana, Maharashtra, Karnataka, Orissa, Tamil Nadu and Uttar Pradesh (Singh, 1974).

In India, false smut disease of rice ranged from 5-85 per cent, with yield losses from 1-49 per cent depending on rice cultivars and disease severity (Dodan & Singh 1996; Biswas, 2001; Ladhalakshmi *et al*., 2012; Kumari & Kumar, 2015 and Muniraju *et al*., 2017). It is an ascomycete fungus, not a real smut (fungus), that develops multinuclear, intracellular/ intercellular, homothallic/ heterothallic mycelia that infects panicles, (Sharanabasav *et al*., 2021). False smut does not replace the entire kernel with a mass of black spores; instead, sori form exploding through the palea and lemma, forming a ball of mycelia, with the topmost layers producing spores. False smut balls not only obstruct grain filling, but they also cause the sterility in adjacent kernels, resulting in a large loss in grain output (Dhua, 2015).

A thorough study of the pathogen morphological and molecular diversity can provide important information for developing more effective disease management measures. The *U. virens* isolates collected from various agro-ecosystems represent both molecular heterogeneity and morphometric variability (Sun *et al.*, 2013). The majority of the preceding studies in India were reported and documented on disease status and pathogen management, whereas studies on variability at the cultural, morphological, and molecular levels of *U. virens* isolates are limited to a small number of isolates (Ladhalakshmi *et al.*, 2012 and Baite *et al.*, 2014). However, Rice being an important crop in command areas of Karnataka, data on disease status, morphological and molecular characterization of the pathogen is scanty. Hence, there is a need to understand the status of disease, to develop and to know the molecular diagnostics and diversity of pathogen under changing climatic scenario for the management of the disease for the benefit of farming community. In this direction, the present study was carried out to know the newly emerging disease status, to characterize the fifteen geo-distinct isolates of *U. virens* from Karnataka for their cultural, morphological and molecular diversity.

Material and Methods

Survey and Disease Severity

Major Rice growing districts of Karnataka mainly covering command areas were surveyed for the incidence of false smut during *kharif* and *rabi* 2020 when the crop was between maturity to harvesting stage. The disease incidence in each field was determined by counting the number of infected tillers from total number of tillers by selecting three random spots of 1×1m and per cent disease severity was assessed by using the method described previously (Singh & Dube, 1978). For recording per cent diseased grains, five spikelets were selected randomly, and observations on diseased grains/ spikelet from total number of grains per spikelet were recorded.

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

Collection, Isolation and Maintenance of *Ustilaginoidea virens* Isolates

A total of fifteen false smut infected rice samples showing typical yellow smut ball symptoms were collected from different districts of north east Karnataka *viz.,* Raichur, Bellary, Yadgir, and Koppal to study the cultural, morphological and molecular diversity (Table 1). The fungus was isolated and purified from yellow smut balls using a modified single spore isolation approach (Ladhalakshmi *et al.*, 2012). The pure culture of all 15 isolates were mass multiplied on PSA (Potato Sucrose Agar) media and stored separately at 28 ± 1ºC on PSA slants for maintenance using the periodical transfer method (15days interval).

Table 1

Source and designation of isolates of *Ustilaginoideavirens* infecting rice

District name	Taluk name	Village name	Name of the isolate(it is better mentioned as code of the isolate instead of the name of the isolate)
Raichur	Raichur	Kalmala	Uv1
		Nelhal	Uv2
	Manvi	Sirawara	Uv3
		Neermanvi	Uv4
	Sindhunur	Javalagera	Uv5
		RH Camp 2	Uv6
Ballari	Siraguppa	Tekkalakote	Uv7
	Ballari	Somasamudra	Uv8
		Vijayanagara camp	Uv9
	Hospet	Kampli	Uv10
		Ramasagar	Uv11
Yadagiri	Shorapur	Devapur	Uv12
Koppal	Gangavati	Devi Camp	Uv13
		ARS, Gangavathi	Uv14
	Koppal	Hitnal	Uv15

Evaluation of Different Solid Media for Growth of *U. virens* (Uv-2 isolate)

As the fungus is very shy for growth, an experiment was carried out to determine the best medium for its growth and sporulation. In this study, the media such as potato dextrose agar (PDA), potato sucrose agar (PSA), yeast glucose agar (YGA), XBZ agar (XBZA), and yeast peptone potato dextrose agar (YPPDA) were used for the study. In sterilized petri dishes, 15 to 20 ml medium was poured and a sterilized needle was used to extract a 4 mm culture disc from the periphery of a 20-days-old pathogen culture (Uv-2) and three replications were maintained for each medium. To prevent bacterial contamination, the media was infused with streptomycin (100 ppm) before being dispensed to the petri plates. Petri plates were incubated at 28 ± 1ºC, when the maximum growth was achieved in any of the media tested; observations on colony growth were made.

Cultural and Morphological Diversity of *U. virens* Isolates

On PSA medium, all 15 isolates were evaluated for cultural and morphological diversity with respect to mycelium (color, breadth and branching of mycelium), colony (growth and morphology) and chlamydospore (color, size and form). Isolates were identified by following standard cultural discretions prescribed by Sharma & Joshi (1975) and Verma & Singh (1988).

Molecular Diversity of *U. virens* Isolates

Total DNA Extraction, PCR Amplification, Sequencing and Phylogenetic Analysis

Molecular diversity of 15 isolates of *U. virens* isolated from rice were studied by sequencing ITS rDNA conserved region. Fifteen isolates of *U. virens* were cultured on potato dextrose broth (PDB) for mycelium production to be used for DNA extraction. Cetyl Trimethyl Ammonium Bromide (CTAB) method with partial modification was adopted to extract the total DNA from the mycelium of *U. virens*. The DNA purity and quality were assessed using a spectrophotometer (Nano Drop 8000, Thermo-Fisher Scientific) and stored at -20°C. Two sets of primers *viz.,* ITS-1(5'-TCCGTAGGTGAACCTGCGG-3'), ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990) and uvr-F (52 -CTTGTGTTTTCCA ATGCATGT-32), uvr-R (52 -ATTCAGTTATCCTC GCACTT G-32) (Yu Chen *et al.,* 2014) were used. PCR was performed in a total volume of 25µl containing 2.5µl of 10X 3 PCR buffer, 3.0µl of $MgCl_2$, 0.5µl of Taq DNA polymerase (GeNei), 0.50µl of dNTP mixture, 0.5µl of each ITS1 and ITS4 primers and 1µl of genomic DNA with17µl of sterile distilled water. PCR reactions (25µl) were performed by 35 cycles of denaturation at 94°C for 60s, annealing at 55°C for 60s 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. All the amplified DNA products were resolved in a 1.2 per cent TAE agarose gel, visualized and documented by a Gel-doc system (Major Science-image analyzer).Sequencing was carried out by Sanger'sdideoxy chain-termination method. Phylogenetic analysis of 15 *U. virens* was constructed using UPGMA-NJ online software.

Results and Discussion

Symptomatology of *U. virens*

False smut symptoms first appeared on the panicle, where little spore balls were seen in between glumes, eventually developing to be one centimeter in diameter and surrounding the floral components. They were somewhat flattened, smooth and yellow in colour coved under membrane. As the ball grew larger, the membrane burst and the hue changed to orange, later turn to yellowish green and further, greenish black in colour. The surface of the spore ball ruptures at this point. However, smutted grains were white in the center when sliced open and they were made up of tightly woven mycelium, glumes and other host tissues. The mature head of the diseased plant is replaced with velvety smut balls that are globose and yellowish green in color. Powdery dark green spores were expelled when the smut balls burst open (Fig. 1).

Fig. 1 : Typical symptoms of false smut of rice under field conditions
A- Initial stage; B-Advanced stage; C- Individual infected grain

Severity of False Smut

Results of two consecutive seasons indicated that the varied range of disease severity was observed among the districts (2.13-19.09 per cent) (Table 2). The taluka wise severity data of false smut revealed that, highest disease severity was recorded in Gangavathi (19.09%) followed by Manvi (15.05%) and Siruguppa (14.74%) and the least severity was in Yadgiri taluk (2.23%). With reference to the districts, maximum disease severity was recorded in Koppal followed by Ballari and Raichur whereas the least was recorded in Yadagiri. The mean disease severity of 15.04 per cent was recorded in Koppal, followed by Ballari (13.21%)

TABLE 2
Severity of rice false smut during *Kharif* and *rabi*, 2020

District	Taluka	Disease severity (%)		Mean disease severity (%)
		kharif 2020	*rabi* 2020	
Raichur	Raichur	10.85	8.76	9.80
	Lingasugur	2.93	1.84	2.38
	Devadurga	2.05	2.21	2.13
	Sindhanur	17.19	10.90	14.04
	Manvi	15.85	14.26	15.05
	9.77	7.59	8.68	
Koppal	Koppal	15.33	6.67	11.00
	Gangavathi	18.10	20.08	19.09
		16.71	13.37	15.04
Bellary	Siraguppa	16.87	12.61	14.74
	Bellary	16.66	6.53	11.58
	Hospet	14.23	12.42	13.32
		15.92	10.52	13.21
Yadgir	Yadgir	2.34	2.12	2.23
	Shahapur	5.41	2.57	3.99
	Shorapur	6.11	4.00	5.05
		4.62	2.90	3.76

and Raichur (8.68%) when compared to least severity of 3.76 per cent in Yadagiri district.

These results are in accordance with the findings of Baruah *et al.* (1992) they reported that, out breaks of false smut disease was more during wet season in a large number of rice varieties including the most popular variety Mahsuri when compared to summer. Among the two seasons, the mean disease severity was ranged from 2.13-19.09 per cent. Wherein Gangavathi taluk recorded significantly highest mean (19.09%) disease severity, while least was recorded in Devadurga (2.13%). In some areas, the severity was more severe due to conginial conditions for growth of pathogen. Similarly, our present findings are in agreement with findings of Ikegami (1960). He reported Ballari district, Ballari and Hospete taluks recoded relatively moderate diseases severity on rice variety BPT-5204. Ladhalakshmi *et al.* (2012) reported that maximum infection was recorded in southern state of Tamil Nadu, the disease incidence varied from 5-85 per cent and a heavy incidence was noticed on variety BPT-5204.

Evaluation of Different Solid Media for Growth of *U. virens* (Uv-2 isolate)

The results indicated that Uv2 isolate exhibited varied diversity in their radial growth, colony color and texture on different solid media and results are presented in Table 3 and Fig. 2. The maximum radial growth of Uv2 isolate was recorded on PSA medium (88.33 mm) which was significantly superior over all other tested media and it was followed by XBZ agar (71.17 mm), PDA (68.50 mm) and YGA (61.00 mm), while YPPDA medium recorded least radial growth (59.08 mm). The results also indicated that PSA, XBZ agar and PDA media 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 grayish at initial growth stage and further turn to dirty brown in case of later stage of the growth of the culture. Raised mycelium growth with circular margin was observed in the all-tested media except PSA and XBZ agar media. On XBZ agar mycelium growth was flat with regular margin, whereas on PSA margins were wavy.

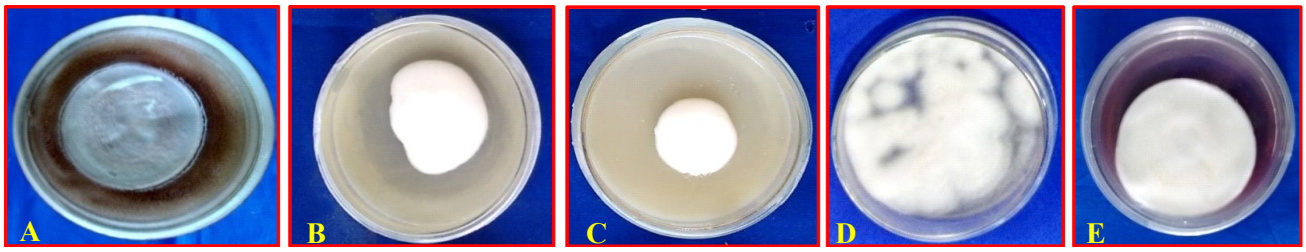

Fig. 2 : Growth of *U. virens*on different soild media

Arranger the photos as per the list mentioned in the above table for better understanding

A-PDA; B-YPPDA; C- YGA; D-PSA; E-XBZ

TABLE 3

Evaluation of different solid media for cultural characteristics of *U. virens* *(Uv-2)*

Medium	Radial growth (mm)	Growth characters		
		Colour of colony	Type of growth	Shape of colony
Potato sucrose agar	88.33	Grayish to white	Good	Flat and wavy margin
Yeast peptone potato dextrose agar	59.08	Cream white	Moderate	Raised and circular margin
Yeast glucose agar	61.00	Whitish	Moderate	Raised and circular margin
XBZ agar	71.17	Bright Whitish	Moderate	Flat and regular margin
Potato dextrose agar	68.50	Cream white	Moderate	Raised and circular margin

Li Yong *et al.* (2008) tested different solid and liquid media for radial growth and sporulation of *U. virens* and they found that, among the solid media tested, fastest mycelial growth was observed on PSA media. As per the study conducted by Wang *et al.*, 2008 they observed that Potato sucrose broth was found to be optimal medium to promote conidial production of *U. virens.* Similarly LU *et al.* (2009) reported that PDA was suitable for growth of pathogen. Successful isolation of single spore of false smut balls from yellow rice reached 90 per cent when cultured on PSA medium and they reported that PSA was the most effective medium to isolate *U. virens* from single spores method (Haiyong *et al.,* 2012). Therefore, PSA supported the fastest mycelial growth of the fungus with less or no contamination by other pathogens.

Cultural and Morphological Diversityof *U. virens.*

Cultural and morphological diversity was studied in all the fifteen isolates after 15 dpi (expand) on PSA media and the results are summarized (Table 4 and Fig. 3). The isolates exhibited five different types of mycelial color patterns such as white (9 isolates), white to light brown (Uv1), light white (Uv12) and white to light pink (4 isolates). Growth of the colony was good in 6 isolates, moderate in 8 isolates and poor in isolate Uv10. However, morphology was smooth in most of isolates (9 isolates), whereas Uv6 and Uv8 were exhibited partially smooth and Uv4, Uv7, Uv9, and Uv10 were produced fluffy growth. Further, all fifteen isolates produced chlamydospores on PSA medium and color of chlamydospores also varies wherein 10 isolates isolates produces brown coloured chlamydospores; similarly light brown (Uv2 and Uv10) and dark brown (Uv3, Uv13 and Uv15). The shape of the chlamydospore was globular in 7 isolates, ovoid in two isolates (Uv2 and Uv5) and round irregular in 6 isolates (Uv3, Uv9, Uv11, Uv12, Uv13 and Uv14). Which indicated that, there is a greater level of variation in terms of cultural and morphological characters among the collected isolates of *U. virens*

TABLE 4

Cultural and morphological diversity of *U.virens* isolates on PSA medium

Isolate	Cultural diversity		Morphological diversity			
			Mycelial characters		Chlamydospore characters	
	Growth	Morphology	Colour	Branching type	Colour	Shape
Uv1	Moderate	Smooth	White to light brown	Acute	Brown	Globular
Uv2	Moderate	Smooth	White to light pink	Acute	Light brown	Ovoid
Uv3	Good	Smooth	White	Acute	Dark brown	Round irregular
Uv4	Moderate	Fluffy	White	Acute	Brown	Globular
Uv5	Moderate	Smooth	White	Acute	Brown	Ovoid
Uv6	Moderate	Partially smooth	White to light pink	Acute	Brown	Globular
Uv7	Good	Fluffy	White	Acute	Brown	Globular
Uv8	Good	Partially smooth	White	Acute	Brown	Globular
Uv9	Moderate	Fluffy	White	Acute	Brown	Round irregular
Uv10	Poor	Fluffy	White to light pink	Acute	Light brown	Globular
Uv11	Good	Smooth	White	Acute	Brown	Round irregular
Uv12	Good	Smooth	Light White	Acute	Brown	Round irregular
Uv13	Good	Smooth	White to light pink	Acute	Dark brown	Round irregular
Uv14	Moderate	Smooth	White	Acute	Brown	Round irregular
Uv15	Moderate	Smooth	White	Acute	Dark brown	Globular

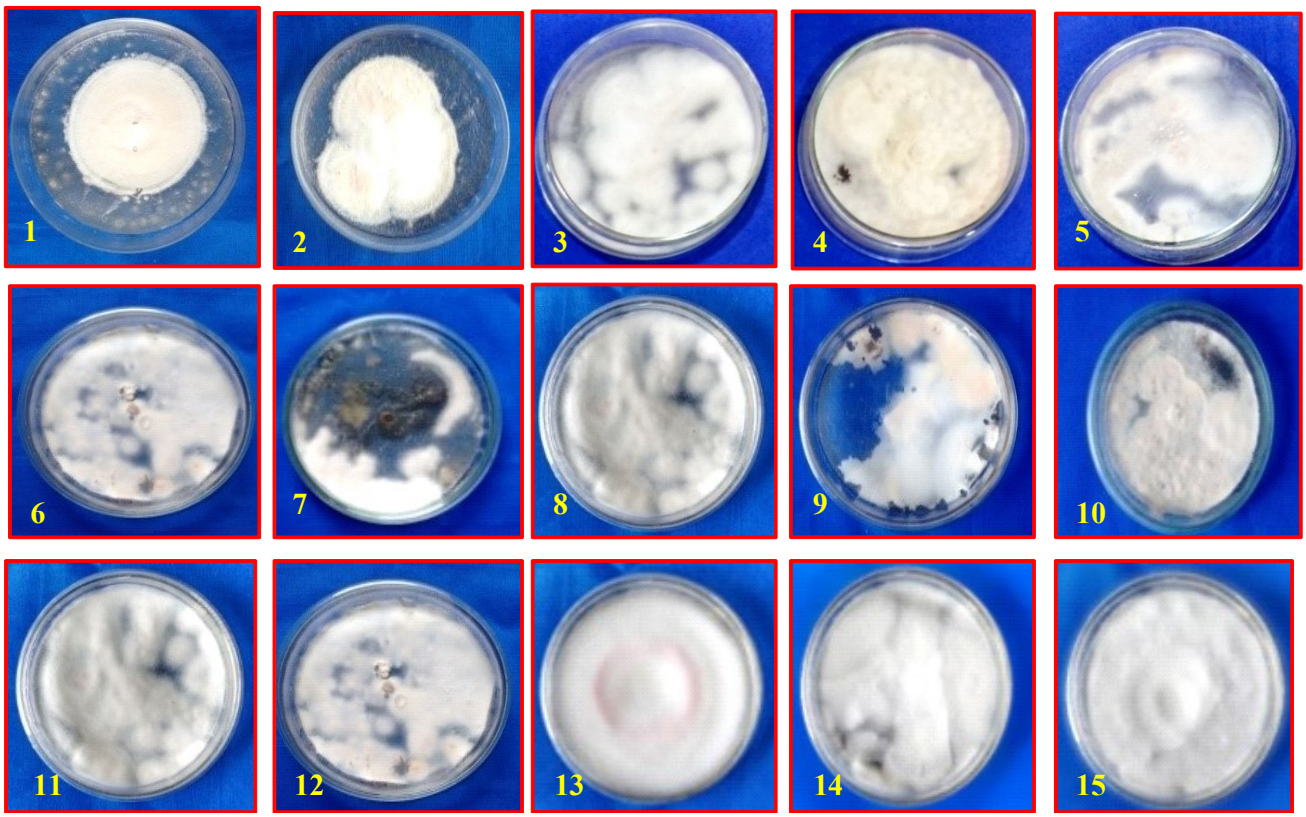

Fig. 3 : Cultural and morphological diversity of *U.virens* isolates (Uv-1 to Uv-15)

The morphological characters of the fungus such as mycelial width and chlamydospore size were also recorded by using fluorescent microscope with 400X magnification. The considerable variation in mycelial width and conidial morphology were observed in all the 15 isolates of pathogen and the results are given in Table 5. The mycelial mean width ranged from 5.65 to 7.10 µm, wherein, Uv6 formed a wider mycelial width measuring 7.10 µm and Uv13 isolate formed narrow mycelial width measuring 5.65 µm. Similarly, chlamydospore morpho-metrics also revealed considerable variation, the mean size of the chlamydospore ranged from 47.09 to 67.45 µm. However, Uv9 recorded the highest chlamydospore size (67.45 µm) and Uv2 least size (47.09 µm). Further, grouping of all the isolates with respect to chlamydospore size and mycelial width is presented in Table 6.

The diversity of *U. virens* at morphological and molecular level has been reported by researchers (Ladhalakshmi *et al.,* 2012 and Baite *et al.,* 2014). However, few isolates collected from limited geographical regions were studied in the earlier reports. In the present investigation, the detailed morphological and molecular diversity of geo-distinct isolates from north east Karnataka was studied .The mycelium was whitish and raised in all of the media, except in case of PDA and PSA wherein, it was grayish to white. Fu *et al.* (2012) studied cultural characters on synthetic XBZ agar medium, the colony resembled white bread.

Variations in different isolates of *U. virens* with respect to size, color and shape and width of the mycelium chlamydospore have been documented by Ladhalakshmi *et al.* (2012) and Baite *et al.* (2014). In the present study, majority of isolates showed white color mycelium and growth of the colony was good, moderate and poor. Further, morphology of isolates was categorized into smooth, partially smooth and fluffy. Chlamydospores formation was observed in all the isolates and color varied from brown, light brown and dark brown. The shape of the chlamydospore was

TABLE 5

Diversity in mycelial width and chlamydospores size of isolates of *U. virens*

Isolate	Mycelial width		Chlamydospore size	
	Range (µm)	Mean (µm)	Range (µm)	Mean (µm)
Uv1	5.3-7.8	6.55	43.58-66.67	55.12
Uv2	5.5-7.7	6.60	34.54-59.65	47.09
Uv3	4.6-7.5	6.05	56.89-52.11	54.5
Uv4	5.2-7.3	6.25	55.11-59.26	57.18
Uv5	5.3-6.9	6.10	65.33-48.60	56.96
Uv6	5.9-8.3	7.10	53.24-4297	48.10
Uv7	6.1-7.8	6.95	58.11-63.16	60.63
Uv8	6.2-7.4	6.80	65.79-42.11	53.95
Uv9	4.3-6.9	5.75	73.68-61.23	67.45
Uv10	5.4-7.0	6.20	57.22-52.34	54.78
Uv11	5.4-7.4	6.40	67.79-51.30	59.54
Uv12	4.5-6.8	5.65	65.76-54.27	60.01
Uv13	4.4-6.5	5.45	68.92-56.10	62.51
Uv14	5.1-7.7	6.4	55.23-48.29	51.76
Uv15	4.9 -7.8	6.35	60.38-49.73	55.05

globular, ovoid and round irregular. Similar reports were documented by Baite *et al.* (2014), they reported that variation in isolates with respect to mycelial width as well as chlamydospore diameter. Sharanabasav *et al.* (2021) also reported wider mycelial width in UV26 and narrow in Uv29 and they also observed that diameter of chlamydospores were bigger on PSA medium than the field borne chlamydospores.

Molecular Characterization of *U. virens* Isolates and Phylogenetic Analysis

To understand variation of the 15 isolates collected from different districts through molecular tools, DNA was extracted and amplified separately with universal ITS1 and ITS4 primers and specific uvr-F and uvr-Rprimers. The results indicated that, approximate size of amplicon was 500-620 bp with universal primers, whereas 280-320 bp ampliconsize was obtained with specific primers (Fig. 4 and 5). The NCBI BLAST analysis conformed that all the fifteen isolates belonged to the *U. virens*. The nucleotide sequences of fifteen isolates of *U. virens* were subjected to the phylogenetic analysis (Fig. 6). Two

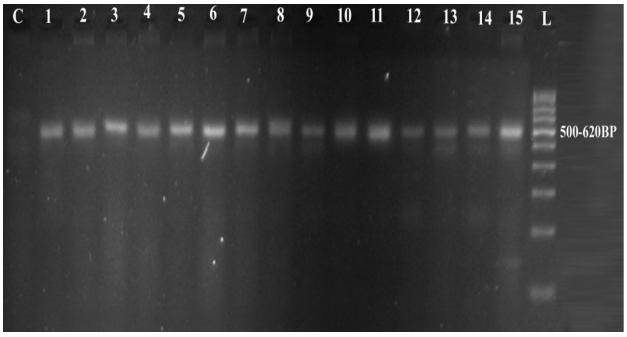

Fig. 4 : Molecular characterization of *U. virens*isolates using universal primers (ITS-1 and ITS-4)

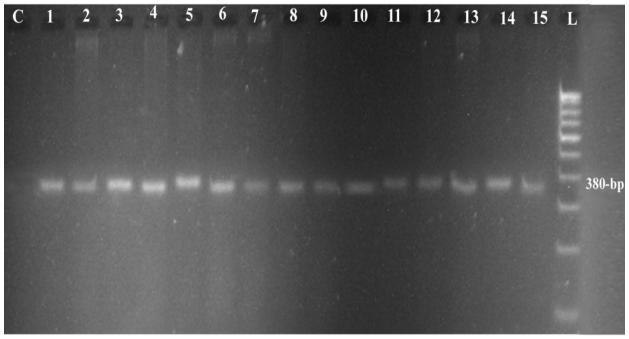

Fig. 5 : Molecular characterization of *U. virens*isolates using specific primer (uvr-F and uvr-R)

TABLE 6
Grouping of isolates based on cultural and morphological variability

Growth character	Nature	Isolate /s
Mycelial colour	White (n = 9)	Uv3, Uv4, Uv5, Uv7, Uv8, Uv9, Uv11 Uv14, Uv15
	White to light brown (n = 1)	Uv1
	White to light pink (n = 4)	Uv2, Uv6, Uv10, Uv13
	Light white (n = 1)	Uv12
Colony morphology	Smooth (n = 9)	Uv1, Uv2, Uv3, Uv5, Uv11, Uv12, Uv13, Uv14, Uv15
	Partially smooth (n = 2)	Uv6, Uv8
	Fluffy (n = 4)	Uv4, Uv7, Uv9, Uv10
Chlamydospore Shape	Globular (n = 7)	Uv1, Uv4, Uv6, Uv7, Uv8, Uv10, Uv15
	Round irregular (n = 6)	Uv3, Uv9, Uv11, Uv12, Uv13, Uv14
	Ovoid (n = 2)	Uv2, Uv5
Chlamydospore colour	Light Brown (n = 2)	Uv2, Uv10
	Brown (n = 10)	Uv1, Uv4, Uv5, Uv6, Uv7, Uv8, Uv9, Uv11, Uv12, Uv14
	Dark Brown (n = 3)	Uv3, Uv13, Uv15

major clusters *viz.*, cluster 1 and cluster 2 were formed, the cluster 1 again divided into two sub-clusters (cluster 1A and cluster 1B). Cluster 1A comprised of eightr isolates *viz.,* Uv6 (RH camp 2), Uv10 (Kampli), Uv7 (Tekkalakote), Uv1 (Kalmala), Uv3 (sirawara), Uv11 (Ramasagar), Uv5 (Javalagera) and Uv8 (Somasamudra), while Cluster 1B comprised 6 isolates *viz.,* Uv4 (Neermanvi), Uv12 (Devapur), Uv13 (Devi camp), Uv14 (ARS, Gangavathi), Uv15 (Hitnal) and Uv9 (Vijayanagara camp). Similarly, cluster 2 comprised of only one isolate, Uv2 (Nelhal).

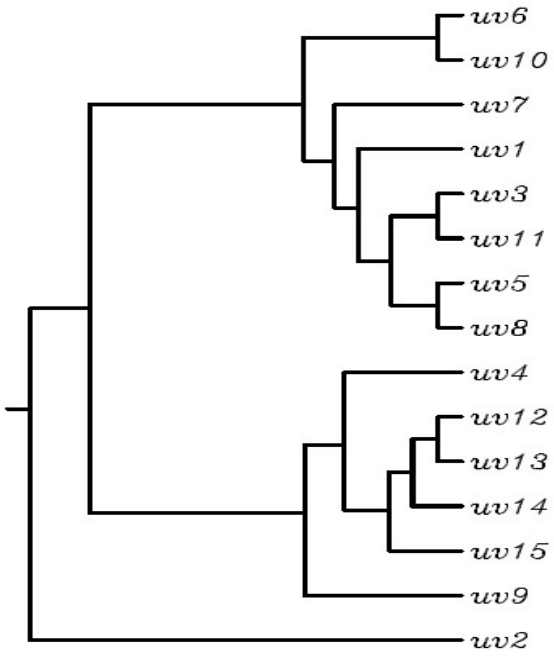

Fig. 6 : Phylogenetic tree analysis of *U. virens* isolates based on ITS1 and ITS4

Porter and Golding (2011) determined the degree of diversity among the *U. virens* isolates by PCR-based method called ITS. ITS1 and ITS-4 primers amplified 500-620 bp in the ITS region of genome, which was within the range for ascomycetes, but uvr-F and uvr-R amplified 280-320 bp. Based on BLAST results, all of the isolates were belonging to the *U. virens*. Furthermore, the present study also reports that phylogenetic analysis of ITS sequences divided the fifteen 15 isolates into two groups: cluster 1 and cluster 2. However, previous about genetic diversity studies of *U. virens* isolates revealed that they were not closely grouped (Li *et al.,* 2004; Xiao-ping *et al.,* 2008; Min *et al.,* 2009; Yu *et al.,* 2013 and Baite *et al.,* 2014). As a result, isolates that were geographically close to each other in the current investigation belonged to the same group and other isolates displayed some diversity depending on the geographical locations to which they belonged.

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The false smut disease was prevalent with less to moderate in all the districts whereever survey is carried out and its incidence varied from season to season and location to location. Potato sucrose medium supported maximum growth of pathogen and can be used for other studies by researchers. All fifteen isolates showed cultural, morphological and molecular diversity. Diversity in mycelial characters (color, branching type and breadth), colony characters (growth & morphology) and chlamydospore characters (color, shape and size) are explored in depth among the isolates. The universal primers, ITS1 and ITS4 successfully amplified the genome of pathogen and phylogenetic tree indicated two large clusters indicating diversity at the genetic level. The specific primers uvr-F and uvr-R also confirmed the identity of all pathogen isolates.

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