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Variability studies of *Ustilaginoidea virens* isolates causing false smut and its severity in different rice growing ecosystems of Karnataka

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Abstract
A study was undertaken to know the severity of false smut in rice growing ecosystems of Karnataka and it was ranged from 1.19 to 4.38 per cent with the mean per cent infected tillers of 2.93 on the varieties like Narmage, MO-21, BPT-5204 and Irga with maximum severity in Udupi and Karkala taluk. During survey, samples were collected and isolations were made to study the variability of false smut pathogen. Among the collected isolates, five isolates representing different rice growing ecosystems were studied for their growth on five different media and found that potato sucrose agar media was best for culturing of fungus. The primer pair ITS-1 and ITS-4 amplified the DNA of *U. virens* at 600-700 bp and sequencing of PCR amplified product showed 91 to 99 per cent similarity with NCBI-BLAST analysis and confirms the isolates as *U. virens.* Further, the RAPD analysis indicated the diversity among the *U. virens* isolates collected from different rice growing ecosystem of Karnataka.

Keywords: Ecosystem, false smut, pathogenicity, rice, *Ustilaginoidea virens*

Introduction
Rice suffers from several diseases caused by the fungi, bacteria, viruses, nematodes and non-parasitic disorders, *etc.* Of the diseases, false smut a fungal disease caused by *Ustilaginoidea virens* (Cooke) Takahashi (telomorph: *Villosiclava virens*) attacks the early flowering stage of the rice crop and replaces whole grain into globose, yellowish green and velvety smut sori. In addition, the grains adjacent to the smut balls may remain sterile and makes panicle chaffy [1, 2] and thereby affects the yield potential of the crop. Due to the sporadic occurrence of the disease, emphasis was not given in past for understanding host pathogen interaction, the severity of disease and also about artificial inoculation technique. Recently, false smut of rice attained the status of major importance and gained the attention far and wide due to the introduction of high yielding semi-dwarf varieties, with an associated change in the rice growing technology such as higher plant population, higher dose of fertilizers and increase in irrigation contributed to a gradual shift in the disease pattern of a locality.
Historically being an uncommon and minor disease by occurring sporadically in certain regions [3], now epidemics of the disease are being reported frequently [4, 5]. The disease incidence of 10 to 20, 5 to 85 and 4.44 to 17.12 per cent was reported from Punjab, Tamil Nadu and Karnataka respectively, on different rice cultivars [5]. The estimated yield loss by *U. virens* on different rice varieties in different rice growing areas of the world ranged from 0.2 to 49.0 per cent [6]. Yield loss to the extent of 7 to 75 per cent in India [7], whereas yield loss up to 44 per cent was noticed in Punjab [8].
A cursory perusal of the literature revealed that research on false smut has been negligible in India partly because of its minor importance and partly due to a problem in the artificial culturing and inoculation of the pathogen. Hence, the present study was undertaken to map the extent of disease severity in high-input rice production ecosystems of Karnataka such as irrigated and direct seeded rice (DSR) of Tunga-Bhadra project (TBP) and Upper-Krishna project command area (UKP), hilly upland, irrigated Bhadra, irrigated Kaveri and coastal ecosystems and also reported the diversity of isolates of *U. virens* causes false smut of rice.

Material and Methods
Survey
A random survey was conducted in the taluks of different rice growing ecosystems of Karnataka *viz*., Tunga-Bhadra command (Raichur, Koppal and Ballari districts), Upper

Krishna command (Yadgir district), Bhadra command (Shivamogga, Chickkamagaluru and Davanage districts) and Kaveri command (Mandya district), rainfed hilly (Uttara Kannada, Dharwad, and Kodagu districts) and in coastal ecosystem (Dakshina Kannada and Udupi districts). These areas were selected as they represent the different soil type, climatic condition, diverse rice cultivar pattern with varied input requirement and rice productivity. In each ecosystem, major rice growing taluks were selected and in each taluk, three to four villages were covered and in each village three farmer's fields were selected randomly. In each field, three random plots of 1 m^2 were selected on different rice varieties and observations on the number of infected tillers per m^2 and number of smut balls per infected panicle was recorded, the data was presented as per cent disease severity.

Isolation and proving pathogenicity of *Ustilaginoidea virens*

Initially, the rice panicles showing typical false smut spikelets of yellow colour or pseudosclerotia with green or greenish black colour measuring 8 x 6 mm were surface sterilized in 70 per cent ethanol for one min followed by surface sterilization with sodium hypochlorite (0.1%) for one min. Such smut balls were subsequently washed three times in sterile distilled water and then the chlamydospores from both yellow and greenish black coloured smut balls were scraped using sterilized needle and streaked separately with the help of sterilized inoculating loop on the sterilized potato sucrose agar (PSA) plates supplemented with 100 ppm streptomycin sulphate antibiotic. The plates were incubated at 25 ± 2 ℃ for seven days for growth of the fungus. Later, the single white pure colony was transferred to PSA slants.

The pathogenicity studies were carried out by adopting injection [9] and spray method [10]. Paddy variety BPT 5204 grown under glasshouse conditions was used for pathogenicity studies. Pure culture of the *U. virens* was inoculated by using injection method at booting stage with 2 ml of conidial suspension (2× 10^6 conidia ml^{-1}) and in spray method, spore suspension was sprayed by using an atomiser on the leaf surface and on the panicles which had just emerged from the boot leaf (50% of flower opening). The inoculated plants were kept at 95 per cent relative humidity in a humid chamber for one week, followed by normal room temperature (27 ℃) on glasshouse benches. The plants were observed for symptom expression after 15 days of inoculation of the pathogen. The rice panicles inoculated with sterile distilled water served as control.

Cultural conditions

The growth characters of five representative isolates of *U. virens* collected from different rice ecosystems of Karnataka were studied on five different solid media *viz*., Potato dextrose agar (PDA), Potato sucrose agar (PSA), Malt extract agar (MEA), Corn meal agar (CMA) and Czapek Dox agar (CDA). All the media used in the study were sterilized at 121 ℃ temperature and 1.1 kg cm^{-2} pressure for 15 min and streptomycin antibiotic at 100 ppm was added to each medium before dispensing to plates to avoid bacterial contamination. About 15- 20 ml of each of the medium was poured into sterilized Petri dishes. Such plates were inoculated with 6 mm disc taken from the periphery of actively growing culture to the centre of Petri dish and incubated at 25 ± 2 ℃. Each treatment was replicated twice. Observations on colony diameter, colony colour, type of growth, elevation and shape of the colony were taken at three weeks after incubation.

DNA extration

Monosporic culture of *U. virens* isolates were inoculated in 100 ml potato dextrose broth (PDB) in the 250 ml conical flasks and incubated at 25 ± 2 ℃ in BOD incubator for 20-30 days. Mycelial mat was harvested by filtering through Whatman filter paper no.1, washed repeatedly with distilled sterilized water, blot dried and stored in Al-foils at -80 ℃ for genomic DNA extraction. Genomic DNA was extracted from the mycelium harvested from broth media based on cetyl trimethyl ammonium bromide (CTAB) method. The DNA pellet was rehydrated in 100 µl TE buffer and allowed to re suspend at 4 ℃ overnight. The quality and quantity of DNA was estimated using nano drop spectrophotometer (Denovix, USA).

PCR amplification of ITS region and sequencing

The universal internal transcribed spacer primers such as ITS1 F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 R (5′-TCCTCCGCTTATTGATATGC-3′) were used for specific amplification of ITS region of 20 isolates of *U. virens* isolated from different rice growing ecosystems of Karnataka and the amplification was performed as per the procedure [11]. Reaction products (8 µl) were resolved by electrophoresis in agarose gels (1.4%) stained with ethidium bromide in 1X TBE buffer at 65 V for 90 min. The gel was observed under UV light and documented using gel documentation unit (Bio-Rad, USA). The amplified PCR product of both the regions (ITS1and ITS4) were sent for purification through gel elution using gel extraction kit (Qiagen gel extraction kit) and sequenced at Eurofins Genomics Pvt. Ltd., Bengaluru from both the ends (ITS1and ITS4). The sequence data was assembled and analysed using the programme Cap3 online software. Homology search was done using BLAST algorithm available at the http://www.ncbi.nlm.nih.gov. The sequences were compared with the previously published database sequences and were deposited in the NCBI GenBank, Maryland, USA, to get accession numbers. Multiple alignments for homology search were performed using the clustral W algorithm software and the phylogenetic tree was constructed by using MEGA 7.0 online software version [12].

Analysis of genetic variability among *U. virens* using PCR-RAPD

The genetic diversity of *U. virens* isolates was assessed with RAPD (random amplified polymorphic DNA) using pair wise combination of 5 primers (OPA-08, OPB-09, OPC-11, OPE-14 and OPG-05) selected from random primers based on reproducible and scorable amplifications made by the previous workers (Rani, 2014). The primers for amplification were custom synthesized at Eurofins Genomics, Pvt. Ltd. Bengaluru and were supplied as lyophilized products of desalted oligos. The molecular diversity of 11 isolates was assessed using RAPD method[13]. Different PCR reactions were carried out to get the best amplification. Composition of PCR reaction was optimized by varying the concentration of template DNA (25 ng, 50 ng, and 75 ng), Taq DNA polymerase (0.5 unit, 1.0 unit and 1.5 units) and $MgCl_2$ concentration (1.5 mM, 2.5 mM and 3.5 mM). RAPD condition for *U. virens* isolates in the present investigation

was standardized and the amplification assay with the following conditions was formulated. The reaction was performed in 25 µl volume containing template DNA 50 ng, Taq DNA polymerase 3U/ µl, $MgCl_2$ 2.5 mM, dNTPs mix 0.6 mM and 10X PCR buffer. Nuclease free water was used to bring the reaction volume to 25 µl. The standardized temperature profiles of 94 ºC for 5 min followed by 40 cycles at 94 ºC for 1 min, 35 ºC for 1 min, 72 ºC for 2 min, with an elongation at 72 ºC for 5 min yielded best results and was used for further experiments. Agarose gel electrophoresis was performed to resolve the amplified product using 1.4 per cent agarose in 1 X TAE (Tris Acetate EDTA) buffer, 0.5 μgml^{-1} of ethidium bromide and loading buffer (0.25% Bromophenol Blue in 40% sucrose). Four µL of the loading dye was added to 20 µl of the PCR product and loaded to the agarose gel. The electrophoresis was carried at 65 V for 1.5 hrs. The gel was observed under UV light and documented using gel documentation unit. RAPD bands were visualised under the UV light. The band size was estimated by comparing with 1 Kb marker (Eurofins Genomics, Pvt. Ltd., Bengaluru).

Scoring and statistical analysis

Clearly visible RAPD products were scored across the isolates as well as primer combinations from the image of ethidium bromide stained gels. The presence or absence of bands were recorded and entered in a binary data matrix, where the presence of band was indicated by "1" and absence of band or its presence was not possible to determine with certainty was considered as "0". The binary matrix was analysed by cluster analysis, the NTSYS.PC (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) computer programme. The generated pair wise similarity matrix was used to group the strains by the unweighted pair-group method with arithmetic average (UPGMA). A dendrogram was derived from the similarity matrix [14].

Results and Discussion

Survey

A survey on the occurrence of false smut disease of rice in different rice growing ecosystems of Karnataka during *Kharif* 2017 and *Kharif* 2018 revealed that the disease severity varied widely from ecosystem to ecosystem (Table 1). Within the ecosystem, the intensity of the disease varied depending on the cultivars. In irrigated and direct seeded ecosystem of Tungabhadra and upper Krishna project area, the incidence of infected tillers was ranged between 1 to 3.7 per cent in different varieties, the maximum being noticed on varieties like BPT 5204 and RNR 15048. The disease severity of the ecosystem ranged from 1.95 to 14.68 per cent, with the mean disease severity of the ecosystem expressing 6.59 per cent. In hilly upland ecosystem, the intensity of false smut was comparatively higher than TBP and UKP ecosystem. The percentage of infected tillers ranged from 2.17 to 4.72 in rice varieties like Jaya, Abhilash, Thanu and KPR-1 and the disease severity in the ecosystem ranged from 5.50-19.12 per cent with the mean disease severity of 12.27 per cent. Whereas in irrigated Bhadra ecosystem, the disease incidence varied from 1.39 to 4.0 per cent with the mean disease severity of 9.73 per cent but the range of disease incidence was 3.34-26.71 per cent (% infected tillers). A notable disease severity was recorded on BPT-5204 and RNR 15048 varieties. The irrigated Kaveri ecosystem recorded the least mean disease severity of 1.52 per cent (% infected tillers) among the entire surveyed ecosystems. The percentage of infected tillers ranged from 0.82 to 1.90 per cent with the mean disease severity of 4.15 per cent on the varieties like MTU 1001 and Jaya. The coastal ecosystem recorded the disease severity ranged from 2.19-26.99 per cent with the mean disease severity of 19.26 per cent. The disease incidence of the ecosystem ranged from 1.19 to 4.38 per cent (% infected tillers) with the mean per cent infected tillers of 2.93 on the varieties like Narmage, MO-21 and Irga.

The overview of disease severity of different taluks of rice growing ecosystem ranged from 2.00 to 22.99 and 2.10 to 21.42 per cent during *Kharif* 2017 and *Kharif* 2018, respectively. Among the different ecosystems surveyed, the coastal ecosystem recorded the maximum disease severity during 2017 and 2018 and the maximum being recorded in Udupi taluk with 22.99 and 21.42 per cent and which was followed by Karkala taluk with the disease severity of 21.65 and 20.38 per cent. The least disease severity was recorded in Siruguppa taluk of TBP ecosystem with 2.00 per cent and Pandavapura taluk of irrigated Kaveri ecosystem with the disease severity of 2.10 per cent.

The increased disease severity could be credited to mean maximum temperature existed during flowering; intermittent rainfall throughout the cropping season and high humidity that might be favouring the higher disease severity. The cultivation of high yielding and bold seeded varieties like MO-21, Jaya and Narmage by the farmers, congenial weather conditions and admixtures of stored seeds and their usage in the next season for sowing might be contributed for maximum disease severity. The disease was found to be more severe at coastal ecosystem compared to other ecosystems, because the rice growing tracts of coastal ecosystems are low lying areas and receive annual rainfall ranging from 3010.9 to 4694.4 mm resulting in more disease severity. The findings are in agreement with the earlier workers [15], who reported that the incidence by *U. virens* was always greater in the lowlands than at higher altitudes. The disease was found to be more severe in medium duration varieties like Narmage, Irga, MO-21 and Jyothi which was more prevalent and recurring in fields having continuous cultivation of this variety. The continued cultivation of susceptible varieties leads to a build-up of the disease inoculum in the field [16] and false smut disease had been observed in severe form since 2001 in India due to widespread cultivation of high fertilizer-responsive cultivars and hybrids [5].

Isolation and characterisation of false smut pathogen

The isolation of false smut infected samples on PSA resulted in small tiny white coloured colony from the germinated chlamydospores after seven days of incubation (Plate 1A). The maximum numbers of fungal colonies were noticed as yellow smut ball than the green and black coloured smut balls and isolation from the fresh samples has resulted in good colony forming unit than those from refrigerated smut samples. The pure white colony of the fungus was selected and maintained as pure mother culture. The white colonies so produced were fluffy with septate mycelia and produced primary conidia after 15 days of post incubation. Later the fungus gradually changed into yellow to olive green in colour upon maturity, similar to gradual change of colour morphology of smut balls under field conditions (Plate 1B to 1D). The chlamydospores of this fungus were spherical to elliptical with echinulate or warty spore wall and borne

laterally on minute sterigmata on radial hypha. These chamydospores germinated by short germ tube and produced conidiophores bearing minute conidia at the tapering apex (Plate 1E & 1F). Totally 20 isolates were made from five rice growing ecosystems of Karnataka and maintained as pure culture. The pathogenicity of the isolated fungus was confirmed on rice variety BPT 5204 by artificially injecting and spraying the tillers at booting and flowering stage with the conidial suspension. The inoculated plant through injection method resulted in typical whitish to yellow coloured smut balls on the panicles at 20 days post inoculation. *U. virens* infected the young ovary of the individual spikelets and transformed them into large velvety green balls. The smut balls were yellow in colour and later turned to olive green and to greenish black. The present findings are in accordance with earlier workers [9], who developed a modified method of artificial inoculation by injecting the conidial inoculum of the fungus. The pathogenicity has been developed [5] by injecting 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 ℃ for 2 days and then at 26 ℃ for 5 days and produced smut ball after 15 days of inoculation. In the present study also, the injection of conidia produced whitish to yellow colour smut balls and typical false smut disease was developed at 20 days post inoculation.

Only few reports are available on the isolation of the *U. virens* due to its slow growth and the presence of more contaminant fungi in the smut balls. Since the importance had been given to false smut disease in the recent years and the isolation of *U. virens* procedure has been standardized by many scientists. Culture media such as potato sucrose agar (PSA), potato dextrose agar (PDA), yeast peptone potato dextrose agar (YPPDA) and XBZ agar *etc*., were reported for the isolation of pathogen from false smut disease infected samples by many workers [10]. Presently, isolation using PSA and its identity was confirmed using morphological and cultural descriptions based on previous reports described by many recent authors [17, 18].

Among 20 isolates, five representative isolates of the surveyed rice ecosystems were studied for cultural characters on five different media as mentioned in material and methods. Among the different media tested (Table 2 and Plate 2), PSA recorded the mean colony diameter of 56.05 mm at 30 days of post inoculation and statistically superior to all other media. Among the different media tested, least colony growth was noticed in corn meal agar with mean colony diameter of 34.10 mm. The five representative isolates of different ecosystem tested on these solid media showed differential preference for utilization of nutrient media. The isolate Uv-12 recorded maximum mycelial growth of 43.90 mm and was on par with Uv-14 (43.60 mm) and Uv-15 (43.55 mm). The minimum colony diameter was recorded by Uv-20 with 41.55 mm. The interaction between different isolates and their growth on solid media indicated that, the isolate Uv-12 recorded significantly highest colony diameter (71.75 mm) in PSA medium whereas minimum colony diameter was recorded by Uv-12 (27.25 mm) on corn meal agar. All the other isolates recorded variable mycelial growth in different media.

Colony developed from the typical chlamydospore germination showed white fluffy mycelium on PSA after seven days of inoculation which was similar to that of the previous reports [5]. All organisms require specific compounds for their growth and metabolism and fungi are no exception. The elements supplied through artificial nutrient media may not be favourable for all fungi. As a result, some fungi showed good growth only on some media. Hence, the present study was carried out to identify the best media for growth of different isolates of false smut fungus collected from different ecosystems of Karnataka. Our findings are supported by earlier workers [19] who found that among the solid media tested, PSA supported the fastest mycelial growth. As per the study [20], PSB was found to be optimal media to promote conidial production of *U. virens*. Similar observations were also made by many other scientists [21, 22]. Further, the sucrose content in PSA was suitable for the pathogen growth and the highest dry weight of mycelia was produced [23].

The morphological identification of fungal pathogen was done based on cultural characteristics and descriptions of *U. virens* [24, 25] and molecular confirmation of the fungus was done based on ITS regions. The DNA was isolated from the *U. virens* mycelium by the CTAB method. The primer pair's ITS-1 and ITS-4 amplified a product at 600-700 bp (Plate 3). The NCBI-BLAST of sequences confirmed the fungus and showed 91-98% sequence identity with *U. virens*. The sequences were deposited at NCBI Gen Bank, Maryland, USA and accession numbers were obtained (MN340255, MN340256, MN340257, MN340258, MN340259, MN340260, MN340261, MN340262, MN340263, MN340264, MN340265, MN340266, MN340267, MN218699, MN218700, MN218701, MN218702, MN218703 and MN218704).

The results were in agreement with scientists[17] who observed sequence length of Uv2 (Bulandshahr, Uttar Pradesh) and Uv3 (Haridwar, Uttarakhand) with 645 and 634 bp respectively and the identity was 91-99 per cent. It is thus indicated that identity of the fungus could be confirmed by the sequencing PCR products of ITS regions using primers.

Molecular diversity analysis of isolates of *U. virens* using RAPD markers

The genetic variation was studied among 11 representative isolates of *U. virens* collected from five different rice ecosystems by RAPD-PCR using five random operon decamer primers in pair wise combination. The RAPD-PCR amplification results revealed the polymorphism between the isolates and representative RAPD profile is depicted (Plate 4). The size of the amplified products ranged from 290-3400 bp. Relationship among the isolates were evaluated by cluster analysis of the data based on similarity matrix, where the Jaccard similarity coefficient values of the isolates of *U. virens* were from 0.00 to 0.79 and the higher similarity value of 0.79 was observed between Uv-9 (Boppanahalli) and Uv-12 (Ponnampet), whereas the lesser similarity had been observed between Uv-1 and Uv-2 isolates at 0.00 similarity coefficient. The dendrogram was generated by using UPGMA method and all the 11 isolates were grouped into two major clusters (Fig. 2). The cluster I was comprised of 10 isolates and the isolate Uv-2 (Kasabe camp) from direct seeded rice showed complete divergence from cluster I and formed separate clade. The cluster I was further divided into two sub clusters at 0.06 similarity values, where the sub cluster I included Uv-9 (Boppanahalli) and Uv-12 (Ponnampet) clustered in one clade and showed divergence from Uv-1(Burdipad) in the same sub cluster. Further, the sub cluster II was divided into sub cluster IIa and IIb. The sub cluster IIa

comprised of five isolates *viz*., Uv-6 (ARS, Gangavathi), Uv-19 (Holehonnur), Uv-14 (Andaru), Uv-15 (Kumsi) and Uv-20 (VC, Farm). Again in sub cluster IIa, the isolates Uv-6 and Uv-19 clustered in one clade and showed divergence from Uv-14, which formed separate clade in the same sub cluster IIa. The isolates Uv-15 and Uv-20 formed another cluster in the sub cluster IIa. The sub cluster IIb comprised of Uv-16 (Nittur) and Uv-18 (Hulaginahole) isolates and showed divergence from the sub cluster IIa by indicating variability among the isolates.

Little information is available on variability in *U. virens* isolated from infected grains of rice. Knowledge of genetic mechanisms underlying the variability in pathogen is almost invariably achieved through the use of molecular markers which serve to distinguish one species or isolate from another. Two of the key factors determining the choice of markers are the level of discrimination required and the technology available. The present study was in agreement with the work carried out by previous researchers [26], they analysed 110 isolates of *U. virens* sampled from North China, samples showed genetic diversity of 0.305, which had approximately equally distributed within and among populations, whereas the genetic diversity was 0.458 among isolates from Beijing and which showed an extremely high level of genetic differentiation among the 55 isolates. Polymorphic information content (PIC) value ranged from 0.130 for primer LC-77 to 0.438 for primer LC-78. Average PIC value was 0.306. Genetic diversity of the eight isolates of *U. virens* was studied by random amplified polymorphic DNA (RAPD) marker using nine primers which showed a considerable level of genetic variation. The dendrogram showed two main clusters; cluster I comprised of five isolates (UV1, UV5, UV8, UV6 and UV7), while Cluster- II consisted of three isolates (UV2, UV3 and UV4) [17].

In the present study, though major clusters supported the geographical origin of the isolates, but the critical analysis does not support the exact geographical origin and genetic property of the isolates. This signifies that *U. virens* isolates widely distributed in the rice growing ecosystem and which could be responsible for spread and survival of the pathogen. The RAPD based phylogenetic tree revealed that the isolates collected from similar locations exhibited less variability and belonged to the same cluster while those collected from the farther distance showed higher variability and grouped into separate clusters.

Table 1: A preliminary survey on different parameters of false smut of rice in different rice growing ecosystems of Karnataka

Sl. No.	Ecosystem	Taluk	Surveyed villages	Variety	Infected tillers (%)			Infected grains (%)			Disease Severity (%)		
					Range	Mean	SEM	Range	Mean	SEM	Range	Mean	SEM
1	Irrigated & DSR ecosystems of TBP and UKP command	Raichur Sindhanur Manvi Gangavathi Siruguppa Hospet Shahpur	Burdipad, Yapaldinni, Kasabe camp Chandrabanda Gorlati Hanumapur Neermanvi Mustoor, Siddapur ARS, Gangavathi Vaddarahatti Uddihal, Ulenoor Boggur, Malapur Devalapur Jeeriganur Doranahalli Gonal	BPT 5204 RNR 15048 RNR 15048 GNV-05-01 GVT 6201 Kaveri sona Nellur sona	1-3.70	2.50	0.18	1-4.7	2.47	0.195	1.95-14.68	6.59	0.893
2	Hilly upland	Sirsi Dharwad Virajpet	Bommanahalli Boppanahalli Kalakeri, Holtikoti Ponnampet	Jaya Abhilash Thanu KPR-1	2.17-4.72	3.32	0.503	2.13-4.12	3.58	0.368	5.50-19.12	12.27	2.654
3	Irrigated Bhadra	Tarikere Shivamogga Channagiri Davanagere Harihara Harapanahalli Bhadravati	Bargenahalli Basavapur Kumsi, Kudli Rudrapura Somlapur Hulikatte Jarikatte Hulaginahole Malebennur Ditur, Nittur Agasanahalli Holehonnur	Jaya MTU 1010 BPT 5204 Kaveri sona Sonal Sriram sona RNR 15048 JGL1798 Super Aman	1.39-4.00	2.66	0.182	2-7.4	3.53	0.422	3.34-26.71	9.73	1.659
4	Irrigated Kaveri	Mandya Maddur Pandavapura Malavalli	VC Farm Sowdenahalli Gejjalagere Maddur Devarahalli Banasamudra Byadarahalli	Thellhamsa Thanu MTU 1001 Jaya	0.82-1.93	1.52	0.163	1.25-5.00	2.76	0.489	2.41-8.65	4.15	0.942
5	Coastal	Brahmavara	Agradahalli Charitharu	MO-21 Irga	1.19-4.38	2.93	0.514	1.84-10.3	6.38	1.120	2.19-26.9	19.26	3.684

		Udupi Karkala	Varamballi Anjaru, Andaru Jarkal	Champaka Jaya, Narmage Irga				6			9		

Table 2: Growth response of five representative isolates of *U. virens* on different solid media

Ecosystems	Taluk	Place of collection	Isolates	Colony Diameter (mm)					
				Medium					
				Czapek Dox Agar	Potato Dextrose Agar	Malt Extract Agar	Corn Meal Agar	Potato Sucrose Agar	Mean
Irrigated TBP and UKP command	Raichur	Burdipad	Uv-1	59.75	44.50	35.25	32.25	42.00	42.75
Hilly upland	Virajpet	Ponnampet	Uv-12	41.50	41.50	37.50	27.25	71.75	43.90
Coastal	Karkala	Andaru	Uv-14	35.00	49.00	36.75	36.75	60.50	43.60
Irrigated Bhadra	Shivamogga	Kumsi	Uv-15	33.25	60.00	29.25	40.00	55.25	43.55
Irrigated Kaveri	Mandya	VC Farm	Uv-20	46.25	41.25	35.25	34.25	50.75	41.55
			Mean	43.15	47.25	34.80	34.10	56.05	

	Isolates (I)	Media (M)	Interaction effect (I X M)
S.Em±	0.22	0.22	0.50
C.D. at 1%	0.65	0.65	1.46

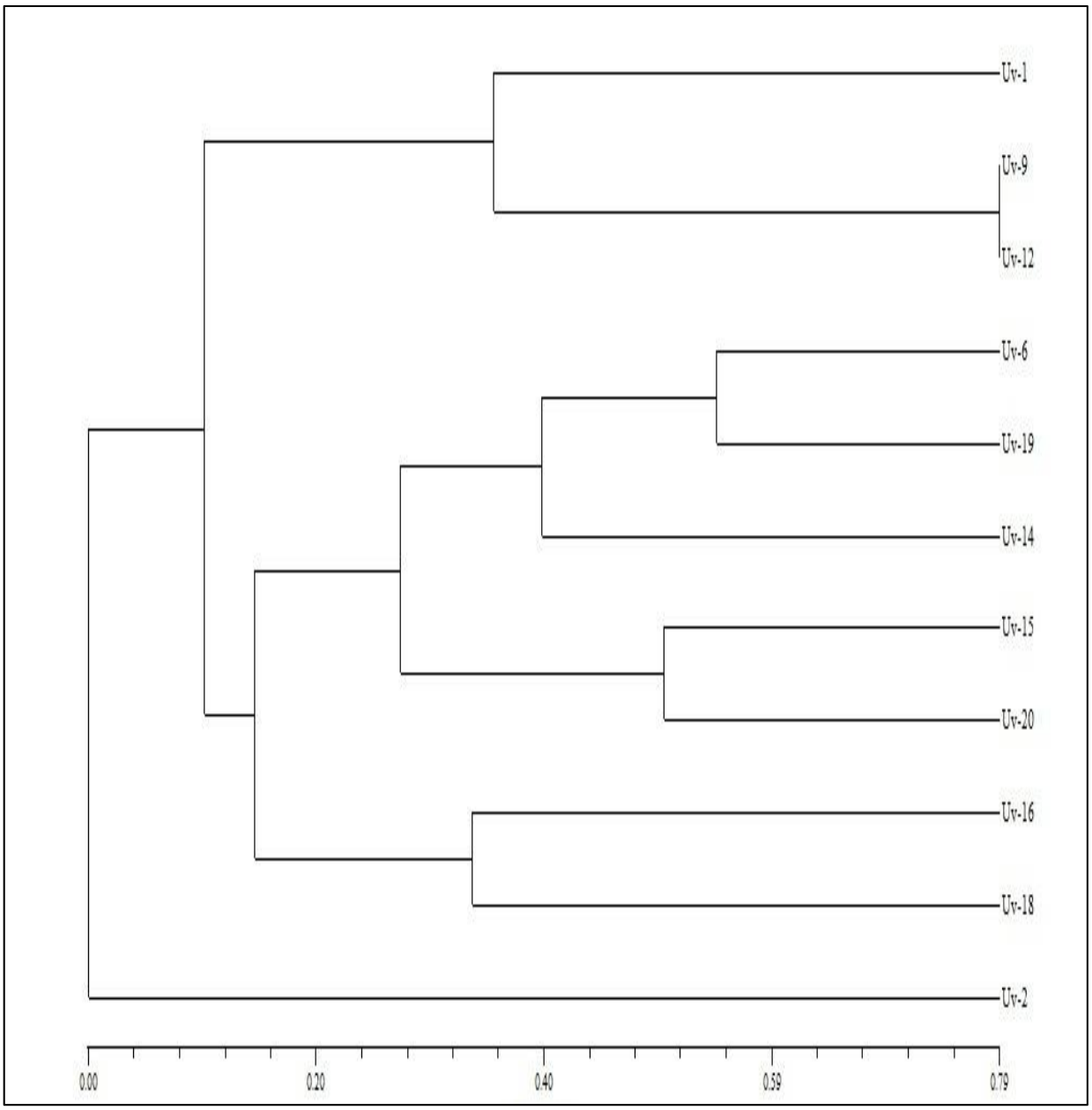

Fig 1: Dendrogram depicting the similarity of *U. virens* isolates based on RAPD loci

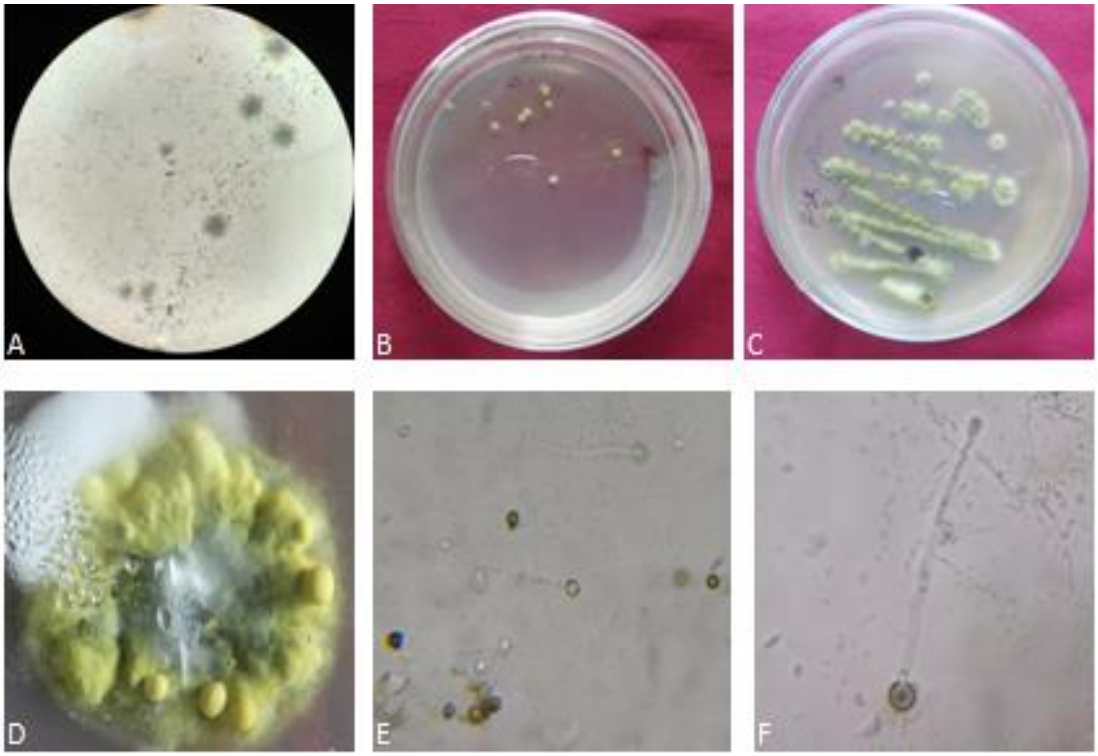

Plate 1: Growth of *U. virens* isolate (Uv-6) on the PSA media, A. Chlamydospore germination B. Pure white colony C. Yellowish green colony D. Chlamydospore formed culture E&F. Germinated conidiophores bearing minute conidia at the tapering apex

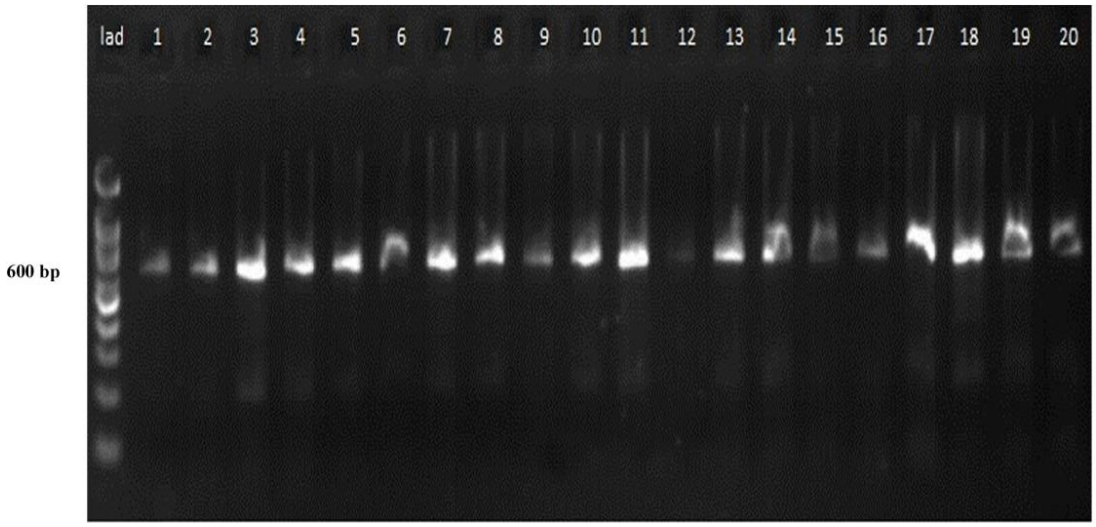

Plate 2: Polymerase chain reaction (PCR) amplification of internal transcribed spacer (ITS) regions of *Ustilaginoidea virens* with universal ITS primers (ITS 1 and ITS 4). Lane M – 100 bp DNA marker; lanes 1 to6 - *U. virens* isolates collected from TBP and UKP ecosystem, Uv-9 to Uv-12 from hilly upland, Uv-13 and Uv-14 from coastal ecosystem, Uv-15 to Uv-19 from irrigated Bhadra and Uv-20 from Kaveri command ecosystems of Karnataka

Conclusion

The present study depicted the information on the status of disease severity in the surveyed ecosystems and which would helps us to identify the 'hot spots' in different rice growing ecosystems of Karnataka and also essential for designing a strong, viable and ecosystem specific management strategy. We also demonstrated the successful isolation of the fungus on the artificial media and its artificial inoculation on rice plants and confirmed the identity of the pathogen through cultural, morphological and molecularly. The study on RAPD variability of isolates collected from rice growing regions of Karnataka revealed the existence of diversity and signifies that *U. virens* isolates widely distributed in the rice growing ecosystem and which could be responsible for spread and survival of the pathogen.

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