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Conformation Techniques for False Smut Pathogen (*U. virens*) on Rice through Cultural and Molecular Tools in Telangana

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ABSTRACT

The study was conducted during period of January to April, 2022 at the Department of Plant Pathology and Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana state, India. The main objective of this study was to investigate cultural and morphological characteristics of *U. virens* isolates on potato sucrose medium (PSA) and their molecular confirmation collected from rice growing regions of Telangana during the *kharif* (rainy season), 2021. The isolates showed varying colony diameters and growth rates, with Uv3 reaching a maximum diameter of 77 mm and a growth rate of 2.85 mm, while Uv11 exhibited a minimum diameter of 40 mm with a growth rate of 1.48 mm. Notably, twenty two isolates showed diverse characteristics, including colony color, elevation, zonation, sectoring, and chlamydospore formation. Cluster analysis revealed the formation of five major groups, each group containing isolates with distinct features and PCR amplification with universal and species-specific primers yielded products of 700 bp and 380 bp, respectively. Phylogenetic tree analysis, indicated, Uv3 and Uv15 belonged to clade X and clade VII, respectively, both comprising Indian isolates. In contrast, Uv4 was grouped under clade II, which included both Chinese and Indian isolates. The proposed technique coupled with information of *U. virens* will immensely contribute to further studies on this fungus and false smut disease in India.

KEYWORDS: Cultural characters, molecular identification, phylogenetic tree, *Ustilaginoidea virens*

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1. INTRODUCTION

Rice is one of the most important and widely cultivated food crops globally. Each year, the world produces 503 million metric tons of rice (Anonymous, 2023a). In India, during 2021-22, rice was grown in 46.38 mha of land, producing 130.29 mt with an average yield of 2.809 t ha^{-1} (Anonymous, 2023b). Telangana, in South India, rice is known as the rice bowl, and rice cultivation area increased from 1.3 mha to 3.2 mha from 1990 to 2020 (Akula et al., 2022). In 2022, Telangana produced 20.22 million metric tons of rice (Anonymous, 2023c). However, rice production faces significant challenges from biotic (pests and diseases) and abiotic (Environmental) stresses, which resulted yield losses ranging from 20% to 50%. Among the various biotic stresses affecting rice production, false smut caused by *Ustilaginoidea virens* (Cooke) Takahashi [teleomorph: *Villosiclava virens* (Tanaka et al., 2008) is one of the most devastating diseases, causing significant quantitative and qualitative losses in grain yield (Rush et al., 2000; Singh and Popaly, 2010).

The first documentation of rice false smut dates back to 1878 in the Tirunelveli district of Tamil Nadu State, India (Cooke, 1878). This fungal pathogen primarily targets rice flower organs, giving rise to smut balls larger in size than rice grains and covered with chlamydospores (Fan et al., 2015). These smut balls undergo a color transformation, starting from white and progressing to pale yellow, yellow, greenish-yellow, or greenish-black over time. During autumn, a wide variation in day and night temperatures promotes the production of dormant structure sclerotia from matured smut balls, which are considered the primary sources of infection for false smut of rice disease. (Yong et al., 2018).

Originally, false smut disease of rice referred as "welcome disease" or "Lakshmi disease" this fungal infection has been considered an auspicious indicator of a bountiful harvest (Duraisamy et al., 2019). Now false smut of rice turn into one of the destructive disease, leading to yield losses of up to 85% (Singh et al., 2014). False smut disease incidence in several states of India was varied from 5% to 80% (Sanghera et al., 2012; Singh et al., 2014; Muniraju et al., 2017; Duraisamy et al., 2019; Thapa et al., 2022). Upadhyay and Singh (2013) reported in different rice varieties yield loss due to false smut disease varying from 4.25 to 20.00%. Apart from causing significant yield losses, *Ustilaginoidea virens* is recognized for producing two mycotoxins, ustiloxins, and ustilaginoidins. These toxins have carcinogenic properties, posing a significant risk to both human and animal health when contaminated rice grains and straw are consumed (Luduena et al., 1994; Shan et al., 2013).

U. virens exhibits diverse colony and chlamydospore characteristics in culture media, varying across geographical rice growing regions and cultivars (Sharanabasav et al., 2021; Rani and Sharma, 2018; Baite et al., 2014; Khedkar et al., 2018; Savitha et al., 2022). Chlamydospores exhibit spherical to elliptical shape with a thick, double-walled structure (Ladhalakshmi et al., 2012). Colonies of *U. virens* in cultural media initially exhibit a white color, then transition to yellow, ultimately developing a velvety green appearance (Bag et al., 2021; Manu et al., 2017). Species specific primers US1-5/US3-3 and US2-5/US4-3 for *U. virens* were used by Zhou et al. (2003), Fu et al. (2014) and Sekhar et al. (2022) in molecular detection. Based on available literature, our research work was planned to study the morphological, cultural, and molecular techniques to identify variation among the *U. virens* isolates from Telangana state.

2. MATERIALS AND METHODS

Current study on cultural and molecular confirmation of *U. virens* isolates was conducted during period of January to April, 2022 at the Department of Plant Pathology and Institute of Biotechnology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India.

2.1. Isolation of U. virens from smut balls

A total of 22 samples showed typical false smut symptoms were collected from different regions of Telangana state during *kharif* 2021. Collected smut balls were washed, surface-sterilized with 4% NaOCl for 30 seconds, and rinsed three times with sterilized distilled water. Spore suspension prepared from surface sterilized smut balls streaked onto PSA (potato sucrose agar) in Petri dishes and incubated at 26±1°C for seven days. After incubation, small colonies with white or green-colored mycelium were formed, and further purified using the hyphal tip method for subsequent analysis (Baite et al., 2014; Baite and Sharma, 2015).

2.2. Morphological study

The cultures were identified according to descriptions given by Sharma and Joshi (1975), Verma and Singh (1988) and Baite and Sharma, 2015. Twenty-seven days after incubation, morphological characters such as surface color, sectoring elevation, mycelial margin, reverse pigmentation, and chlamydospore production were recorded for *U. virens* isolates grown on PSA. The size of Chlamydospore was measured for all the isolates. Initially, imaging was conducted at 10x magnification using a binocular microscope (LM 52 1704), and later measurements were performed using Tcapture software.

2.3. Molecular study

In this study, *U. virens* isolates were cultured in potato sucrose broth to obtain mycelium for DNA extraction using the CTAB method with slight modifications from

Murray and Thompson's protocol (1980). Initially, 0.3 to 0.5 g of mycelium from each isolate was ground into a fine powder using liquid nitrogen and mixed with 1000 µl of extraction buffer (100 mM Tris HCl, pH 8.0, 20 mM EDTA, 1.4 mM NaCl). After a 45-minute incubation at 65 °C, the mixture was centrifuged for 15 minutes at 12000 g at room temperature, and the supernatant was treated with chloroform:isoamyl (24:1) alcohol for DNA precipitation. The resulting supernatant was collected, and chilled isopropanol (volume equal to the supernatant) was added for DNA precipitation. After centrifugation at 12000 g for 15 minutes at 4°C, the supernatant was discarded, and the DNA pellet was washed with 200 µl of 70% ethanol, followed by centrifugation at 5000 g for 5 minutes. Later, 50 µl of 1x TE buffer was added to dissolve the DNA, which was then stored at -20°C for subsequent analysis. For molecular confirmation, the Internal Transcribed Spacer (ITS) region was targeted using ITS-1F and ITS-4R primers (White et al., 1990). The polymerase chain reaction (PCR) mixture, totaling 10 µL in volume, consisted of 5 µl of Takara master mix, 1 µl of each primer (ITS-1F/ITS-4R) at a concentration of 2.5 Pico moles, 1 µl of nuclease-free water, and 2 µl of genomic DNA from the *U. virens* isolate, with a concentration of 50 nanograms. The PCR conditions included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 1 minute, and concluding with an elongation step lasting 7 minutes at 72°C. The amplified PCR products were separated on agarose gel electrophoresis and visualized using a gel documentation system. Additionally, species pecific primers US1-5(CCGGAGGATACAACCAAAAAAACTCT)/ US3-3(GCTCCAAGTGCGAGGATAACTGAAT) were used to confirm the identity as a *U. virens* pathogen (Zhou et al., 2003; Fu et al., 2014; Sekhar et al., 2022). Sequences of PCR products of ITS regions (Uv3, Uv4, and Uv15) were compared to the NCBI Gene Bank database and submitted for public access.

2.4. Data analysis

A binary matrix was constructed pair wise, with the presence or absence of determined morphological characters scored as 1 and 0, respectively and were analyzed using NTSYSPC (version 2.02) software (Jamshidi and Jamshidi, 2011). Jaccard similarity coefficients were utilized to cluster the matrices, generating a dendrogram through the SHAN clustering program, which employed the UPGMA (un-weighted pair group method with arithmetic averages) method, aimed to group similar isolates based on shared morphological characteristics. Molecular phylogenetic analysis of three sequenced isolates (Uv3, Uv4I and Uv15) with 48 sequences available in NCBI database was performed using the maximum likelihood method (Sharanabasav et al., 2021; Sekhar et al., 2022) using MEGA 11 (Tamura et al., 2021) and interactive Tree of Life (iTOL) software (Letunic and Bork, 2016).

3. RESULTS AND DISCUSSION

3.1. Morphological study of U. virens

During sample collection, the morphology of smut balls varies at different stages of development. In the early stages, smut balls were covered with a white mycelial mat. In later stages, upon the release of conidia (chlamydospores), they turned yellowish or orange, and then velvety greenish to dark in color (Figure 1) Under a compound microscope, chlamydospores of the collected samples showed a two-walled structure with a round or globular shape. Echinulation was not prominent (Figure 2). The size of chlamydospores varied among different isolates, ranging from 4.36 to 6.84 µm. *Uv16* exhibited the largest chlamydospores, while *Uv21* had smallest (Table 1). The results align with the chlamydospore morphology described by Verma and Singh (1988). Furthermore, the research by Baite et al. (2017) corroborates our findings, as they reported a similar size range of 4.20–6.54 µm for chlamydospores. On the other hand, Fu et al. (2022) presented contrasting results, indicating a smaller size range of 1.01–1.33 µm. This discrepancy underscores the variability in reported chlamydospore sizes potentially influenced by factors such as geographical location, environmental conditions, or genetic differences among isolates.

Figure 1: Symptoms of false smut on rice grains. a: White mycelial growth protruding out from the gap between lemma and palea, b: White mycelial mat over the surface of the smut ball, c: Release of chlamydospores upon the burst of the mycelial mat, d-f: Smut balls transitioning from yellowish or orange color to velvety greenish or dark in color

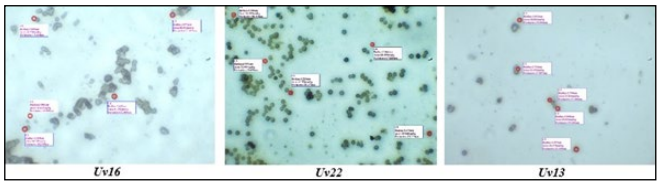

Figure 2: *Ustilaginoidea virens* chlamydospores viewed under compound microscope at 10X magnification

3.2. Isolation and cultural characters

Ustilaginoidea virens isolates showed a very slow growth rate, typically requiring a period of 5 to 7 days for the

Table 1: Chlamydospores size (µm) of false smut samples

Isolate	Place of collection (village, mandal, district)	Chlamydospore size (µm)
Uv1	Anjamanpur, Navipet,Nizamabad	5.40±0.38*
Uv2	Armoor, Armoor, Nizamabad	5.64±0.32
Uv3	Morthad,Morthad, Nizamabad	5.28±0.22
Uv4	Rudrur, Rudrur,Nizamabad	5.44±0.17
Uv5	Arapeta, Metpalli, Jagtial	4.66±0.27
Uv6	Kondapur, Narsapur, Medak	4.98±0.12
Uv7	Shivampet, Shivampet, Medak	5.52±0.16
Uv8	Annaram, Ramareddy, Kamareddy	5.06±0.25
Uv9	Rajaram, Mallial, Jagtial	4.94±0.24
Uv10	Namilikonda, Kodimyala, Jagtial	5.22±0.12
Uv11	Gargul, Kamareddy, Kamareddy	5.54±0.23
Uv12	Chandapur, Thadwai, Kamareddy	5.16±0.17
Uv13	Kurnavalli, Thallada, Khammam	5.98±0.27
Uv14	Gokinepally, Mudigonda, Khammam	5.54±0.12
Uv15	Rajendranagar, Rajendranagar, Rangareddy	5.96±0.23
Uv16	Venkampalli, Gangadhara, Karimnagar	6.84±0.23
Uv17	Yangal, Chendurthi, RajannaSiricilla	5.68±0.23
Uv18	Kandi, Kandi, Sangareddy	5.10±0.22
Uv19	Rajakkapeta, Dubbaka, Siddipet	5.68±0.18
Uv20	Kuntiramannagudem, Suryapet, Suryapet	5.90±0.23
Uv21	Parkal, Parkal, Warangal Rural	4.36±0.31
Uv22	Chelgal, Jagtial, Jagtial	6.36±0.20
CD (p<0.05)		0.51
SEm±		0.18

*Standard deviation values

formation of a small, white, yellow, or olive green colony from germinating chlamydospores on PSA (Figure 3a). The individual colonies were subsequently isolated and maintained as pure cultures. The formation of a large colony required approximately 15 to 27 days (Figure 3b-d). The morphology of these colonies was characterized by milky white, yellow, and olive green colors, with fluffy or compact mycelium. These morphological characteristics align with the findings of previous studies of Baite et al. (2014), where *U. virens* was reported to change the mycelial color from white to yellow on PSA petriplates and similar results were mentioned by Baite et al. 2015. Lin et al. (2018) reported

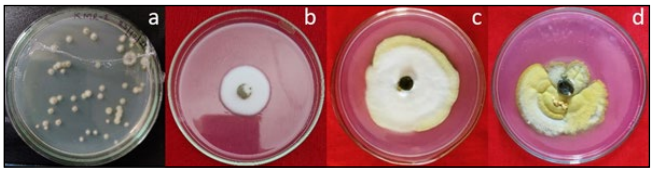

Figure 3: a: Small colonies from germinating chlamydospores at 5 days after incubation (DAI), b: Milky white mycelia at 15DAI, c: 27 days old mycelium turning into yellow, d: Mycelium producing chlamydospores at center at 27 DAI

that, rice false smut pathogen was a slow-growing, required one to two weeks to form large colonies.

3.3. Mycelial growth pattern of U. virens isolates

Twenty-two isolates at 27 DAI showed varied mycelial growth, ranging from 40 mm to 77 mm in diameter (Table 2). The maximum mycelial growth was observed in Uv3 (77 mm), followed by Uv19 (76.46 mm), Uv21 (72.66 mm), with the minimum mycelial growth observed in Uv11 (40 mm), Uv16 (43.66 mm), and Uv18 (43.66 mm). The mycelial growth rate per day ranged from 1.48 mm (Uv11) to 2.85 mm (Uv3) (Figure 4). These results align with a previous study by Savita et al., 2022, which reported colony diameters ranging from 42 mm to 71.75 mm. Similarly, Sekhar et al., 2022, observed *U. virens* colony diameter spanning from 10.14 mm to 85.68 mm, with growth rates of 0.33 mm to 2.85 mm. Bait et al., 2014, reported a mean diameter ranging from 25 to 40 mm.

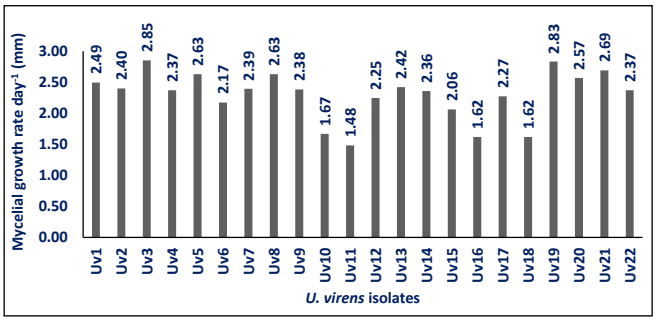

Figure 4: Variability in mycelial growth rate day^{-1} (mm) of different *U. virens* isolates

The growth characteristics of 22 isolates of *U. virens* were examined at 27 DAI, revealing distinct patterns in mycelial development (Table 2). The isolates showed variability in elevation, with 11 isolates displayed flat growth and remaining 11 (Uv1, Uv2, Uv3, Uv6, Uv7, Uv8, Uv10, Uv12, Uv14, Uv16 and Uv21) exhibited fluffy mycelium. Mycelial sectoring (noticeable regions within mycelium) was observed in specific isolates (Uv18, Uv20, Uv6, Uv12, Uv14 andUv16). Zonation, characterized by concentric rings of growth, was noticed in certain isolates (Uv3, Uv18, Uv6, Uv12, Uv14, Uv16 and Uv7). Circular growth was observed in numerous isolates (Uv4, Uv9, Uv15, Uv19, Uv1, Uv10,

Table 2: Cultural characters of different *U. virens* isolates on PSA media

Isolate	Sectoring	Zonation	Elevation	Growth form	Mycelial margin	Surface color	Reverse pigmentation	Chlamydospore production	Mycelial growth (mm)
Uv1	No	No	Raised	Circular	Entire	White	White-light brown	Yes	67.34 ±2.31*
Uv2	No	No	Raised	Circular	Filiform	White-yellow	White-yellow	No	64.80 ±2.75
Uv3	No	Yes	Raised	Irregular	Entire	White-yellow	Yellow-green	Yes	77.00 ±3.33
Uv4	No	No	Flat	Circular	Filiform	White-green	Yellow-green	Yes	64.00 ±2.65
Uv5	No	No	Flat	Irregular	Filiform	White	White-green	No	71.00 ±1.92
Uv6	Yes	Yes	Raised	irregular	Filiform	White	Light brown	No	58.66 ±0.69
Uv7	No	Yes	Raised	Circular	Entire	White	White-yellow	No	64.66 ±1.34
Uv8	No	No	Raised	Irregular	Entire	White	light brown-yellow	No	71.00 ±3.14
Uv9	No	No	Flat	Circular	Filiform	White-yellow	Yellow-green	Yes	64.34 ±1.57
Uv10	No	No	Raised	Circular	Entire	White	White-yellow	No	45.00 ±1.50
Uv11	No	No	Flat	Irregular	Entire	White	Green	No	40.00 ±1.44
Uv12	Yes	Yes	Raised	Circular	Entire	White-yellow	Yellow-green	Yes	60.66 ±0.77
Uv13	No	No	Flat	Circular	Entire	White-yellow	Light brown-yellow	No	65.34 ±1.53
Uv14	Yes	Yes	Raised	Irregular	Filiform	White-yelow	Yellow-green	No	63.66 ±1.03
Uv15	No	No	Flat	Circular	Filiform	White-yellow-green	Yellow-green	Yes	55.66 ±2.46
Uv16	Yes	Yes	Raised	Irregular	Filiform	White-yellow	Light brown-yellow	Yes	43.66 ±0.43
Uv17	No	No	Flat	Circular	Filiform	white-yellow	yellow-green	No	61.34 ±2.38
Uv18	Yes	Yes	Flat	Irregular	Filiform	White-yellow	Yellow-	Yes	43.66 ±1.69
Uv19	No	No	Flat	Circular	Entire	White	Yellow-green	No	76.46 ±1.31
Uv20	Yes	No	Flat	Circular	Entire	White-green	Green	Yes	69.34 ±0.19
Uv21	No	No	Raised	Irregular	Filiform	White-yellow	White-yellow-green	No	72.66 ±1.24
Uv22	No	No	Flat	Irregular	Entire	White-yellow	Yellow-green	Yes	64.00 ±2.42
CD (p<0.05)									3.18
SEm±									1.11

*Standard deviation values

Uv2, Uv20, Uv7, Uv13, Uv17 andUv12), contrasted with irregular growth patterns seen in the remaining isolates. The mycelial margins varied, with some isolates displaying filiform margins (Uv2, Uv17, Uv21, Uv18, Uv14, Uv4, Uv9, Uv6, Uv5 and Uv16), while others showed entire mycelial margins. The surface color of the colonies varied among isolates. Eight isolates (Uv19, Uv10, Uv11, Uv8, Uv7, Uv6, Uv5 and Uv1) exhibited a white surface, eleven isolates (Uv13, Uv12, Uv3, Uv22, Uv2, Uv17, Uv21, Uv18, Uv9, Uv16 and Uv14) displayed white-yellow, two isolates (Uv20, Uv4) showed white-green, and one isolate (Uv15) displayed white-yellow-green in cultural media.

The reverse pigmentation of the colonies also varied, with three isolates (Uv7, Uv2 andUv10) showing white-yellow, Uv1 displaying white-light brown, Uv5 exhibiting white-green, Uv21 showing white-yellow-green, Uv18 displaying yellow, and three isolates (Uv8, Uv13 and Uv16) having yellow-light brown. Uv6 exhibited light brown, while nine isolates (Uv19, Uv4, Uv12, Uv3, Uv22, Uv17, Uv9, Uv15 and Uv14) displayed yellow-green reverse pigmentation, and two isolates (Uv11 and Uv20) showed yellow-green.

The results are in agreement with the studies conducted by Khedkar et al. (2018), who reported that the colony of *U. virens* initially displayed a hyaline color, later transitioning to orange, greyish, and ultimately brown to black. Chlamydospores formed on pseudomorphs started as smooth spheres with an orange-yellow hue, maturing into dark brown, echinulate structures with a rough surface and a diameter of 5 to 6 µm. Rani and Sharma (2018) stated that the initial colony color in most isolates of *U. virens* was white, which gradually transformed to yellow and eventually to green. The isolates also exhibited differences in growth patterns, ranging from appressed and fluffy to less fluffy and raised. Sekhar et al. (2022) also reported similar morphological characteristics of the false smut pathogen, such as colony color, growth pattern, elevation, mycelial margin, and chlamydospore formation in various groups of *U. virens* isolates.

3.4. Cluster analysis

U. virens isolates were clustered into five groups based on their morphological similarity, at similarly coefficient of 0.69 (Figure 5). Group I comprised six isolates (Uv1, Uv7, Uv10, Uv8, Uv11, Uv19); Group II consisted of eight isolates (Uv2, Uv17, Uv13, Uv4, Uv9, Uv15, Uv22, Uv20); Group III included five isolates (Uv3, Uv12, Uv14, Uv16, Uv18); Group IV had two isolates (Uv5, Uv21); and Group V contained a single isolate, Uv6. This grouping indicated that isolates from the same region or district did not necessarily cluster together. This implies that, *U. virens* isolates exhibit morphological variability both within and among regions. The result of the experiment was consistent with the findings of Baite et al. (2014), who reported that twelve isolates of *U. virens* were grouped into three clusters based on colony characteristics. Rani and Sharma (2018), using morphological and cultural characters, identified two clusters among 35 isolates of *U. virens*. Sekhar et al. (2022) revealed that cluster analysis of 30 *U. virens* isolates formed two groups based on cultural and morphological characteristics such as colony color, growth pattern, elevation, and chlamydospore formation.

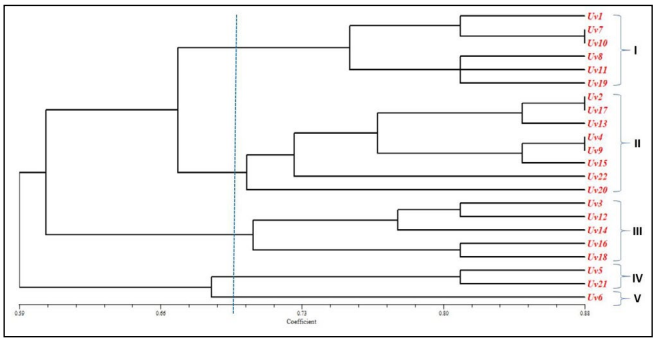

Figure 5: Dendrogram of 22 isolates of *U. virens* generated by unweighted pair group method arithmetic mean (UPGMA) analysis of morphological characters

3.5. Molecular study

PCR amplification using universal primers ITS-1F and ITS-4R produced amplicon of approximately 700 bp for the ITS region in the genomic DNA of 22 *Ustilaginoidea virens* isolates (Figure 6a). Additionally, false smut-specific primers US1-5/US3-3 generated a 380-bp product for each isolate (Figure 6b), confirming that all isolates were identified as false smut pathogen (*U. virens*). The identity of the representative isolates (Uv3, Uv4 and Uv15) was also confirmed by sequencing PCR products of ITS regions using ITS1 and ITS4 primers, revealing sequence identities of up to 98.51%, 99.54%, and 99.66% with *U. virens*, respectively. The obtained sequences were submitted to Gen Bank, NCBI, and accession numbers were assigned as OR512524 for Uv3, OR483808 for Uv4 and OR461676 for Uv15.

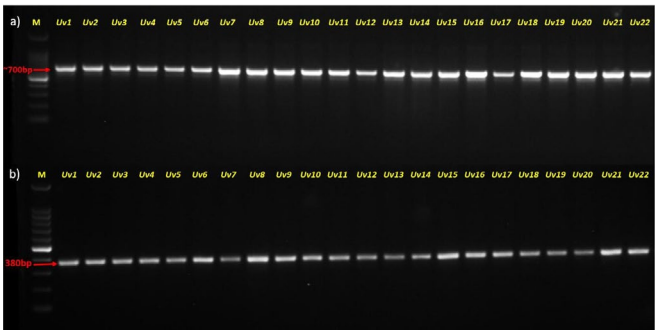

Figure 6: a: PCR amplification of ITS region of *U. virens* isolates using universal primers (ITS1F/ITS4R); b: Molecular conformation of *U. virens* isolates using species specific primer (US1-5/US3-3) amplified at 380 bp.M-100bp marker

3.6. Phylogenetic analysis of U. virens isolates

A total of 51 *U. virens* isolates sequences were downloaded from the NCBI database, including three representative isolates from this experiment (Uv3, Uv4, and Uv15). The phylogenetic tree was generated using the maximum likelihood method with MEGA 11 software and iTOL software. The resulting tree revealed a diverse clustering pattern, with the 51 isolates forming 12 distinct clades (Figure 7). Notably, our representative isolate *Uv3* was positioned within the X clade, alongside three other Indian isolates. *Uv4* was grouped into clade II, sharing this cluster with four Indian isolates and one from China. On the other hand, *Uv15* was situated in clade VII, where all isolates belonged to the Indian region. A similar study was conducted by Sekhar et al. (2022), who reported that the phylogenetic tree analysis of 30 isolates of *U. virens* revealed two clusters. Sharanabasav et al. (2021) performed a phylogenetic analysis among 61 isolates of *U. virens* based on internal transcribed sequences, revealing two clusters; however, most isolates (n=54) were grouped in Cluster I, indicating a common ancestral origin.

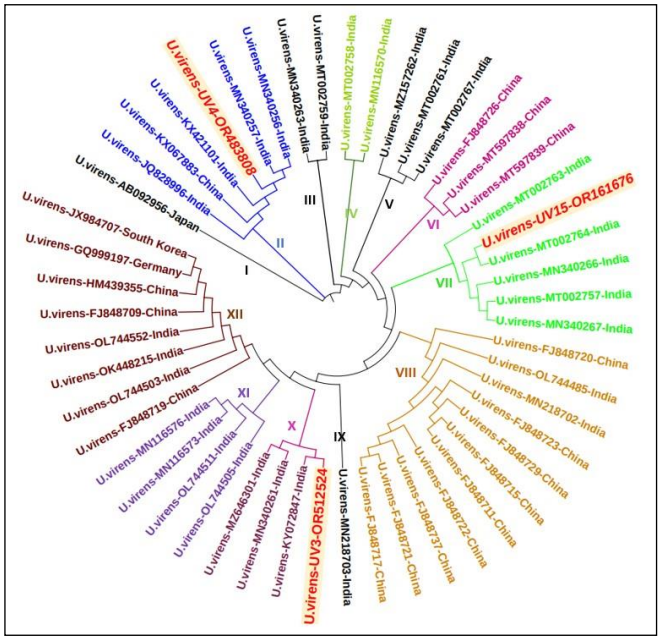

Figure 7: Molecular phylogenetic tree of *U. vires* isolates by maximum like hood method

4. CONCLUSION

Examination of twenty-two isolates of *U. virens* revealed significant variations in colony diameters, growth rates, and morphological features. The formation of distinct clusters highlighted the diversity among isolates. Additionally, PCR analysis using universal and species-specific primers facilitated the identification and molecular confirmation of isolates. The phylogenetic tree analysis revealed the genetic relationships, with Uv3 and Uv15 clustering with Indian isolates in clade X and clade VII, respectively, while Uv4 grouped with both Chinese and Indian isolates in clade II.

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