Cultural, molecular and genetic variability of *Ustilaginoideavirens*causing rice false smut disease

ABSTRACT

All the thirty isolates of *U. virens* showed well defined colonies on PSA medium. The maximum colony diameter found 85.68 mm with growth rate of 2.85 mm in Uv23 isolate and minimum colony diameterfound 10.14 mm with growth rate of 0.33 mm was observed in Uv15 isolate. However, thirty isolates exhibit various cultural and morphological characters like, colony color, growth pattern, elevation and chlamydospores formation. In cluster analysis, two major groups (I), (II) were formed, first major group I contain Uv15 isolate and second major group II contain 29 *U. virens* isolates, further which were dived into two subgroups which are subgroup IIacontain 22 isolates and IIb contain 7 isolates.The PCR amplification was done with species a specific primer which was yielded products of 380 bp and 230 bp respectively. In phylogenetic tree analysis results reveled that, cluster I contain 5 isolates wereas cluster II contain 28 isloates includes china and japan isloates. The MAT1-1-1 primer was amplified a product of 250 bp and contain 18 isolates. Whereas MAT1-2-1 primer was yielded a product of 220 bp and contain 12 isolates. The nucleotide divergence analysis results revealed that numbers of polymorphic sites (s) were found 7, mutations (*Eta*) found 10, nucleotide diversity (*k*) was found 0.76515, tajimas test value was -2.13885. Likewise, for haplotye analysis revealed that total haplotype groups were found (*h*) 10, with haplotype diversity was found (*hd*) 0.4773. Among them hap_1 were major group and contain 24 isolates. Similarly, clear nucleotide variations were observed between Indian isolates and China as well as Japan isolates.The genetic similarity coefficient matrix revealed that, thirty Indian isolates were showed maximum similarity (0.9). Whereas, Uv32 isolate showed 0.6, Uv31 isolate exhibited 0.5 and UA33 isolate found 0.4, these results indicate that genetic variability were observed between the Indian *U. virens* isolates as well as other country of *U. virens* isolates.

Comment [F1]: Re-write. Too much story thereby rendering the Abstract out of context

Keywords: Rice false smut, Cultural variability, Molecular difference, Nucleotide diversity, Haplotypes analysis

1. INTRODUCTION

Rice is the staple food crop in the world. Various biotech and abotech factors affecting rice production, among them isfalse smut of rice caused by*Ustilaginoideavirens* (Cooke.) Takahashi was first reported from Tirunelveli in Tamil Nadu [1] causes significant quantitative and qualitative losses in grain yield [2]. In India, the disease incidence up to 85% and grain yield losses of up to 49% have been reported [3; 4; 5; 6; 7]. The *U. virens* is an ascomycetous fungus produces multinuclear, intracellular and intercellular, homothallic or heterothallic mycelia, which infects booting stage of the rice crop. The symptoms produced by *U. virens* are visible after flowering only, when the fungus transforms individual grains of the panicle into a yellowish smut ball, which changes to yellowish orange, green, olive green and finally to greenish black [5].The *U. virens* produces both stages, ascospores (sexual) and chlamydospores (asexual) in the life cycle [4].

To know the cultural, molecular, genetic variability and population divergence of *U. virens* will be useful information to develop more accurate disease management tool. The *U. virens* isolates were collected from the different geographical locations on various rice varieties have been reported to produce diverse mycelial (colour, growth pattern and size of conidia) characters on culture media [5; 8; 9]. Previously, the genetic diversity of *U. virens* has been studied using different genetic markers such as internal transcribed spacer (ITS) [5; 8; 10], randomly amplified polymorphic DNA [8; 9; 11;12], amplified fragment length polymorphism [10] and single nucleotide polymorphism [13] and also based on whole-genome sequences [14; 15]. Even though, these studies were focused on documenting the disease status and management, whereas the studies on cultural, molecular, genetic and population diversity among the *U. virens* isolates are limited [5; 8;9]. In ascomycetous fungi, sexual reproduction and development are governed by a single locus called mating-type locus 1 (MAT1), which has two idiomorphs, MAT1-1-1 and MAT1-2 -1 [16; 17]. Heterothallic strains contain either MAT1-1 or MAT1-2 idiomorphs at the MAT1 locus, whereas homothallic strains harbour both idiomorphs with different

Comment [F2]: Recast the sentence

arrangements [17; 18]. Mating-type analysis plays an important role in determining the molecular and genetic basis of sexual reproduction and pathogen genetic variation. In this study was described the cultural, morphological, molecular, genetic variability, population divergence and mating type analysis of thirty Indian isolates as well as two China and one Japan isolates of *U. virens* pathogen.

2. MATERIALS AND METHODS

2.1 Collection and isolation of *U. virens* isolates

False smut Infected spikelets were collected from different cultivars of rice growing areas of Indian stats (Fig. 1). The smutted balls were thoroughly washed with distilled water and surface sterilized with 0.1 % mercuric chloride solution for 1 min and subsequently washed three times with sterile distilled water. Using a sterilized needle, a mass of chlamydospores was streaked onto Petri dishes containing potato sucrose agar (PSA) medium under sterile and aseptic conditions. To check bacterial contamination, streptomycin (100 ppm) was incorporated in the medium. The Petri dishes were incubated at 26 ± 2 °C for 2 weeks for fungal growth. A single, isolated colony of the fungus was picked up using a sterilised needle, transferred to the fresh PSA slants, and maintained as a pure culture for further studies [5]. A total thirty isolates were collected and purified. Details of all thirty isolates with respect to varieties and geographical areas were given in Table 1.

Table 1. Isolates of *U. virens* collected from different states of India

Isolate no	Isolate code	Variety	State	Geographical coordination
Uv-1	TNADT	ADT51	Tamil Nadu	11.0140^{o}N, 79.4751^{o} E
Uv-2	TNKNP	BPT5204	Tamil Nadu	12.8185^{o} N, 79.6947^{o} E
Uv-3	TNBS	CO43	Tamil Nadu	11.4792^{o}N, 77.1341^{o}E
Uv-4	TNTNJ	CR1009	Tamil Nadu	10.7870^{o} N, 79.1378^{o} E
Uv-5	TNGDLR	CO43	Tamil Nadu	11.5030^{o} N, 76.4917^{o} E
Uv-6	TNKB	CR1009	Tamil Nadu	10.9602^{o} N, 79.3845^{o} E
Uv-7	TNTY	IWPONNI	Tamil Nadu	10.7905^{o} N, 78.7047^{o} E
Uv-8	TNTVLR	BPT5204	Tamil Nadu	13.1231^{o} N, 79.9120^{o} E
Uv-9	TNTIR	CR1009	Tamil Nadu	10.7661^{o} N, 79.6344^{o} E
Uv-10	TNPBS	IWPONNI	Tamil Nadu	10.9953^{o} N, 76.9165^{o} E
Uv-11	TNWTL	CO43	Tamil Nadu	10.9945^{o} N, 76.9117^{o} E
Uv-12	TNKP	BPT5204	Tamil Nadu	11.3185^{o} N, 77.7106^{o} E
Uv-13	TNARG	CR1009	Tamil Nadu	10.1692^{o} N, 79.0023^{o} E
Uv-14	APNDL	NDLR7	Andhra Pradesh	15.4777^{o} N, 78.4873^{o} E
Uv-15	APVZ	BPT5204	Andhra Pradesh	17.8629^{o} N, 82.1965^{o} E
Uv-16	APPV	BPT5204	Andhra Pradesh	17.2479^{o} N, 81.6432^{o} E
Uv-17	APMND	NDRL7	Andhra Pradesh	15.4709^{o} N, 78.6255^{o} E
Uv-18	APMTR	MTU1010	Andhra Pradesh	16.6269^{o} N, 81.7389^{o} E
Uv-19	APWG	BPT5204	Andhra Pradesh	16.8073^{o} N, 81.5316^{o} E
Uv-20	APELR	BPT5204	Andhra Pradesh	16.7107^{o} N, 81.0952^{o} E
Uv-21	APEG	BPT5204	Andhra Pradesh	16.9666^{o} N, 82.0434^{o} E
Uv-22	APRJY	BPT5204	Andhra Pradesh	17.0005^{o} N, 81.8040^{o} E
Uv-23	APKNL	NLR35559	Andhra Pradesh	15.8281^{o} N, 78.0373^{o} E
Uv-24	APNLR	BPT5204	Andhra Pradesh	14.4426^{o} N, 79.9865^{o} E
Uv-25	TSWGL	Warangal sannalu	Telangana	17.9689^{o} N, 79.5941^{o} E
Uv-26	TSJGL	JGL3838	Telangana	18.7895^{o} N, 78.9120^{o} E
Uv-27	MPJP	JRH124	Madhya Pradesh	23.1858^{o} N, 79.9743^{o} E

Uv-28	MPJNKV	Kranthi	Madhya Pradesh	23.2072^{o} N, 79.9540^{o} E
Uv-29	KAGVT	BPT5204	Karnataka	15.4319^{o} N, 76.5281^{o} E
Uv-30	DRRPN	BPT5204	Telangana	17.3201^{o} N, 78.3939^{o} E

a).White &yellow-orange smut ball **b).Conidia of *U. virens*** **c) Chlamydosporeof *U. virens***

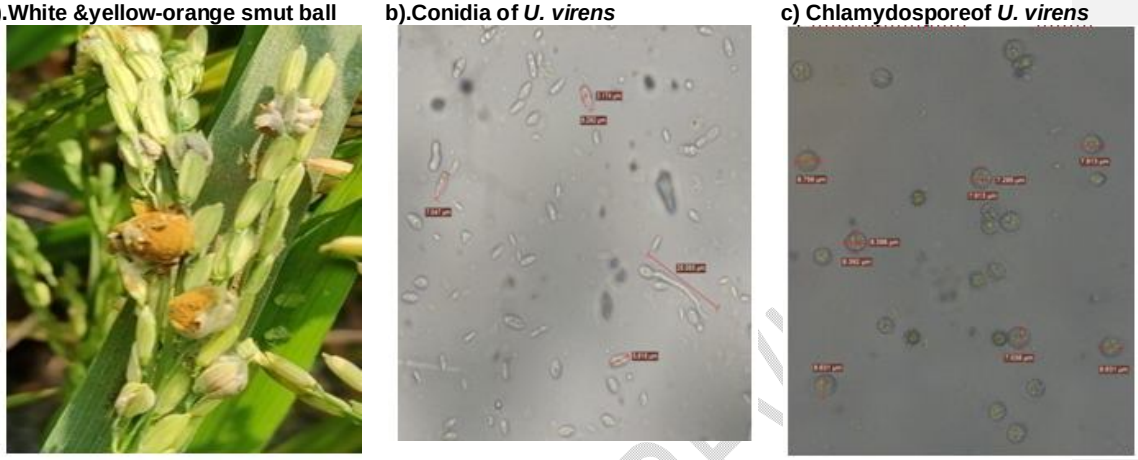

Fig. 1. Typical symptoms showing a) White & yellow-orange smut ball, b) Conidia of *U. virens*& c) Chlamydospore of *U. virens*causing rice false smut disease

2.2Cultural variability among the different isolates of *U. virens*

A total thirty isolates of *U. virens* obtained from diseased samples were studied for morphological variations. Observations on colony diameter, colony colour, growth pattern (elevation and mycelia form), chlamydospore formation and furrow formation were recorded after 15 and 30 days after inoculation. All thirty isolates of *U. virens* were grouped on the basis of their morphological characters using the R software clustering technique [19]. Cultures of *U. virens* obtained on potato sucrose agar (PSA) slants were examined under phase contrast microscope under bright filed (Leica DM 3000) using image analyser software at 40X magnification.

2.3 Fungal DNA extraction, synthesis of primers and PCR profiling

All thirty isolates was grown on PSB for 7 days at 26°C with 125 Rpm for continues shaking in shaker sum incubator after 7 days the mycelial mat was harvested and used for DNA isolation following the protocol described by [20]. The concentration of genomic DNA was measured and quantified using a NanoDropspectro-photometer (Thermo scientific, Wilmington, DC, USA). The specific~~specific~~ primers were used to confirm *U. virens* [5]. The polymerase chain reaction (PCR) mixture (20 µl) consisted of 0.5 µl of 2.5 mM of dNTPs, 10 pmol of each primer(US1-5/US3-3 or US2-5/US4-3). For characterization of mating type analysis the MAT1-1-1 and MAT1-2-1 ideomorphs, oligonucleotide primers published by [21] were designed and synthesized. All oligonucleotides were synthesized using a commercial facility (Eurofins Ltd, Bengaluru, India). Nucleotide sequences of all the primers used were given in Table 2. The PCR profiling for mating type analysis were one unit of Taq polymerase, DNA template (20 ng), initial denaturation at 96°C for 2 min, 30 cycles of amplification (20 s for denaturation at 96°C, 30s for primer annealing at 53°C (for mating type of primers annealing temperature is 56°C) and 30s for extension at 70°C) and one cycle of final extension at 72°C for 7 min. The amplification was carried out on a Thermocycler (Applied Biosystems). PCR-amplified products were analyzed by 1% agarose gel electrophoresis.

2.4 Sequencing and phylogenetic tree constriction

The amplified PCR product of thirty isolates of *U. virens* was sequenced at Barcode Bioscience (Bangalore, India) by sanger'sdideoxy method and sequences were compared with other ribosomal RNA genes of *U. virens* available at NCBI database using BLAST programme. The sequences were submitted to NCBI gene bank and the accession numbers were obtained (Table 2). To assess the variability among the thirty isolates, a phylogenetic tree was constricted with ribosomal RNA sequences using MEGA 7.0 software. For the Phylogenetic study, two isolates from China and one isolate from Japan were also used.

Table 2. List of primers used in this study

Genes	Primer name	Sequence (5'⟶ 3')	Amplification size	Tm (^{o}c)	Reference
Ribosomal rRNA gene	Us1-5 F	CCGGAGGATACAACCAAAAAAACTCT	380bp	53	Zhou et al. (2003)
	Us3-3 R	GCTCCAAGTGCGAGGATAACTGAAT			
	Us2-5 F	CAATGCATGTCTGAGTGGATTTTTG	232bp		
	Us4-3 R	CCAACACCAAGCGCAAGACAGA			
MAT1 locus	MAT1F2	GAAACTCCAACTCAAACGAAGTCG	250bp	56	Fu et al. (2014)
	MAT1R2	GTAAACTTTGGCTATCAACGCCC			
	MAT2F2	GGAGCGACATAATACCGTCAAAGA	220bp		
	MAT2R2	GGGGTGTTTTTCTAAGAGGGCCT			

2.5 Nucleotide variability, haplotypes and genetic similarity of *U.virens* isolates

The nucleotide diversity analysis, a total 33 isolates were studied and out of them two isolates from China and one isolate from Japan. In that, an isolate of *U. albicans*, which causes white false smut disease in China, was also used. All 33 sequences were aligned using the ClustalW multiple alignment method inBioEdit Software 7.2.5 version [22] The nucleotide sequences of 33 isolates were used for the analysis of DNA polymorphism, the number of polymorphic/segregating sites (S), nucleotide diversity (Pi), Theta (per site) from S (Theta-W), the average number of nucleotide differences (k), Tajima's D (D), the number of haplotypes and haplotype diversity using DnaSP v5.10 software [23]. Further, the haplotype data exported from DnaSP v5.10 software was utilized to construct the haplotype tree based on the median-joining algorithm calculation method using software of NETWORK 10.1.1.0 (https://fluxus-engineering.c om/)[24]

3. RESULTS

3.1 Cultural variability of thirty isolates of *U. virens*

The cultural variability of all the thirty isolates of *U. virens* showed well defined colonies on potato sucrose agar (PSA) medium. The maximum colony diameter (85.68 mm) was observed in isolate Uv23, whereas the minimum colony diameter (10.14 mm) was observed in Uv15 after 30 days of incubation. However, when growth rate was calculated Uv23 isolate was exhibited maximum growth rate of 2.85 mm/day and Uv15 isolate was exhibited minimum growth rate of 0.33 mm/day. Initially most of the isolates of *U. virens* in culture medium showed white color colony which later exhibit to different color colonies. However, eighteen isolates viz, Uv3, Uv5, Uv6, Uv8, Uv9, Uv10, Uv11, Uv12, Uv13, Uv16, Uv17, Uv19, Uv21, Uv22, Uv23, Uv28, Uv29, Uv30 were showed white color colonies, Where as Uv2, Uv7, Uv14, Uv20, Uv24, Uv26, Uv27 isolates produce white yellow colonies, likewise Uv25 isolate produce white yellow green colonies, Uv1 and Uv18 isolates exhibit yellow color colonies, Uv4 isolate produce yellow green color and Uv15 isolate showed green color colonies was observed after 30 days of incubation. The isolates also exhibit differed growth patterns like, Uv1, Uv4, Uv15, Uv24, Uv26 isolates exhibit raised elevation and remaining isolates showed flat elevation where as Uv1, Uv3, Uv4, Uv5, Uv6, Uv9, Uv11, Uv12, Uv13, Uv20, Uv22, Uv23, Uv25, Uv30 isolates were exhibits circular growth patterns. Similarly, Uv2, Uv7, Uv8, Uv10, Uv14, Uv15¸ Uv16, Uv17, Uv18, Uv19, Uv21, Uv24, Uv26, Uv27, Uv28, Uv29 isolates produced irregular growth patterns on culture media. Likewise, some of the isolates were produce chlamydospores on culture media viz, Uv1, Uv2, Uv4, Uv14¸ Uv18, Uv20¸ Uv25, Uv26¸ Uv27, Uv29 and rest of the isolates was not produce chlamydospores. (Table 3, Fig. 2)

Table 3. Cultural and morphological variability of thirty isolates of *U.virens*

Isolate	Colony color	Growth pattern		Chlamydospore formation	Furrow formation	Radial growth (mm) after 15 days	Radial growth (mm) after 30 days	Average growth rate/ day (mm)
		Elevation	Mycelia form					
Uv-1	Yellow	Raised	Circular	Yes	+ + +	12.75	25.17	0.83
Uv-2	White yellow	Flat	Irregular	Yes	No	35.25	79.26	2.64
Uv-3	White	Flat	Circular	No	No	14.28	31.42	1.04
Uv-4	Yellow green	Raised	Circular	Yes	+	12.35	26.65	0.88
Uv-5	White	Flat	Circular	No	No	35.47	76.58	2.55
Uv-6	White	Flat	Circular	No	No	25.36	48.75	1.62
Uv-7	White yellow	Flat	Irregular	No	No	18.89	38.47	1.28
Uv-8	White	Flat	Irregular	No	No	32.47	68.65	2.28
Uv-9	White	Flat	Circular	No	No	35.38	75.58	2.51
Uv-10	White	Flat	Irregular	No	No	31.56	65.17	2.17
Uv-11	White	Flat	Circular	No	No	29.48	60.35	2.01
Uv-12	White	Flat	Circular	No	No	31.65	62.14	2.07
Uv-13	White	Flat	Circular	No	No	15.68	34.78	1.15
Uv-14	White yellow	Flat	Irregular	Yes	No	32.66	68.47	2.28
Uv-15	Green	Raised	Irregular	No	+ +	5.04	10.14	0.33
Uv-16	White	Flat	Irregular	No	No	36.40	73.56	2.45
Uv-17	White	Flat	Irregular	No	No	25.38	55.49	1.84
Uv-18	Yellow	Flat	Irregular	Yes	+ +	36.37	74.17	2.47
Uv-19	White	Flat	Irregular	No	No	37.56	77.46	2.58
Uv-20	White yellow	Flat	Circular	Yes	No	22.78	45.37	1.51
Uv-21	White	Flat	Irregular	No	No	23.71	48.87	1.62
Uv-22	White	Flat	Circular	No	No	26.08	55.74	1.85
Uv-23	White	Flat	Circular	No	No	43.72	85.68	2.85
Uv-24	White yellow	Raised	Irregular	No	No	33.56	67.45	2.24
Uv-25	White yellow green	Flat	Circular	Yes	No	22.34	52.47	1.74
Uv-26	White yellow	Raised	Irregular	Yes	No	24.15	48.78	1.62
Uv-27	White yellow	Flat	Irregular	Yes	No	23.47	51.77	1.72
Uv-28	White	Flat	Irregular	No	No	22.70	43.28	1.44
Uv-29	White	Flat	Irregular	Yes	No	24.17	58.47	1.94
Uv-30	White	Flat	Circular	No	No	41.87	80.14	2.67
CD(0.05						1.17	1.12	

Fig. 2. Cultural and morphological variability among the thirty isolates of *U.virens*

3.2 Cluster analysis for among the *U. virens* isolates

To study the cultural variability among the *U.virens*isolates characters *viz*, colony color, elevation, mycelial form, chlamydospore formation, furrow formation and radial growth rate were evaluated in all the thirty isolates by cluster analysis and dendrogram was generated using R software (Version 4.2.0) (Fig. 3). The results revealed that significant variation was observed with respect of colony color, elevation, mycelia form chlamydospore formation and furrow formation. These characters were coded as number and then scored. Two major groups (I, II) were formed, in which the first major group (I) include one isolate (Uv15) the second major group (II) included a total of 29 isolates, which were dived further into two subgroups, subgroup (IIa) included 22 isolates (Uv23, Uv10, Uv24, Uv8, Uv14, Uv18, Uv2, Uv30, Uv16, Uv19, Uv5, Uv9, Uv11, Uv12, Uv29, Uv17, Uv22, Uv25, Uv27, Uv26, Uv6, Uv21) and second subgroup (IIb) included 7 isolates (Uv1, Uv4, Uv3, Uv13, Uv7, Uv20, Uv28), (Table 4)

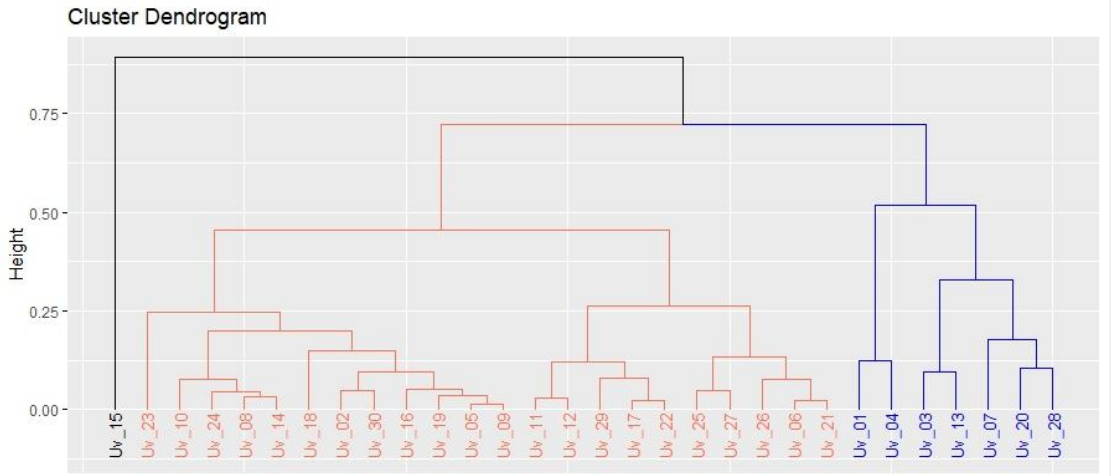

Fig. 3. Dendrogram generated based on cultural variability of thirty isolates *U. virens*using R software (V 4.2.0)

Table 4. Grouping of thirty isolates of *U. virens*based on the cultural variability characters

Group	Sub-group	Name of the isolates
1	1	Uv15
2	2a	Uv23, Uv10, Uv24, Uv8, Uv14, Uv18, Uv2, Uv30, Uv16, Uv19, Uv5, Uv9, Uv11, Uv12, Uv29, Uv17, Uv22, Uv25, Uv27, Uv26, Uv6, Uv21
	2b	Uv1, Uv4, Uv3, Uv13, Uv7, Uv20, Uv28

3.3 Molecular variability of *U. virens* isolates

All the thirty isolates of *U.virens*were subjected to PCR analysis for molecular conformation. The genomic DNA was isolated from all the isolates and PCR amplification was done with false smut specific primers *viz,* US1-5/US3-3 and US2-5/US4-3 and the specific primers amplified from Lane 1 to Lane 30 a products of 380 bp (Fig. 4a) and 230 bp (Fig. 4b), respectively.

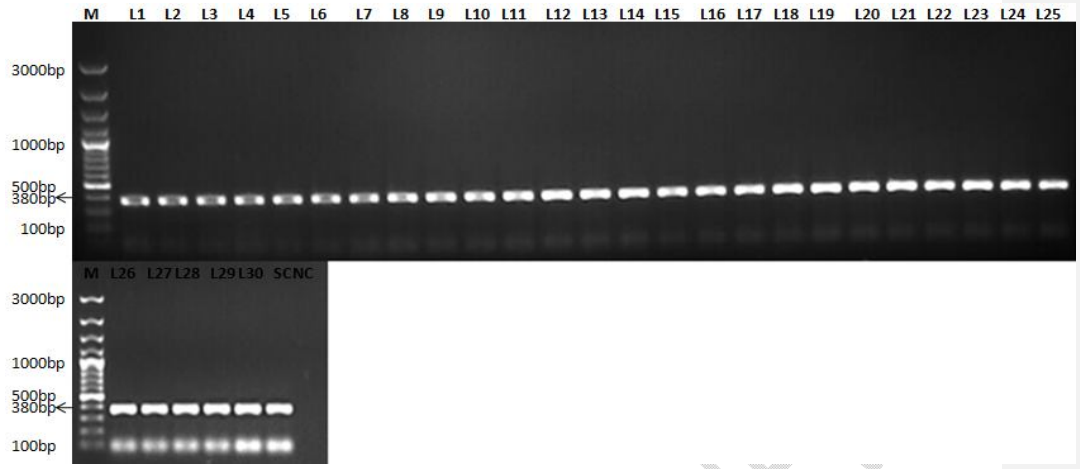

Fig.4a. Molecular conformation of*U.virens*using species specific primer (US1-5/US3-3) amplified at 380 bp.M-3000bp marker; Lane 1-30 different isolates of *U.virens*; NC- negative control.

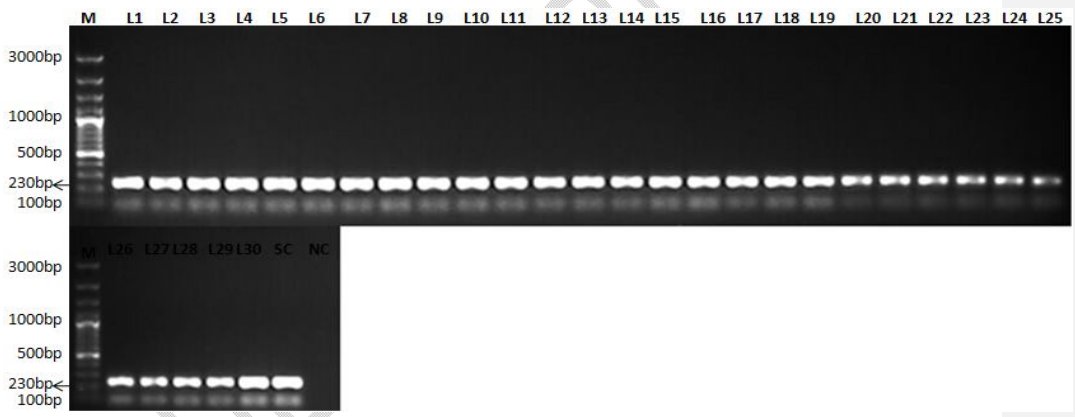

Fig.4b. Molecular conformation of *U.virens*using species specific primer (US2-5/US4-3) amplified at 230 bp.M-3000bp marker; Lane 1-30 different isolates of *U.virens*; NC- negative control.

3.4 Phylogenetic analysis for *U. virens* isolates

The sequenced product of 30 isolates were analysed using BLAST programme in NCBI. Then, all sequenses were submited to NCBI GenBank and obtaind the accession numbers (Table 5). The phylogenetic tree was constructed with 33 isolates among them two isolates from china one isolate from japan retrived from NCBI.The results reveled that two clusters was formed, cluster I and cluster II, cluster I contain 5 isolates (Uv3, Uv10, Uv14, Uv19, Uv23), wereas cluster II contain 28 isloates includes china and japan isloates (Uv1, Uv2, Uv4, Uv5, Uv6, Uv7, Uv8, Uv9, Uv11, Uv12, Uv13, Uv15, Uv16, Uv17, Uv18, Uv20, Uv21, Uv22, Uv24, Uv25, Uv26, Uv27, Uv28, Uv29, Uv30, Uv-china, Uv-japan and UA-china (causing white false smut in rice), (Fig. 5).

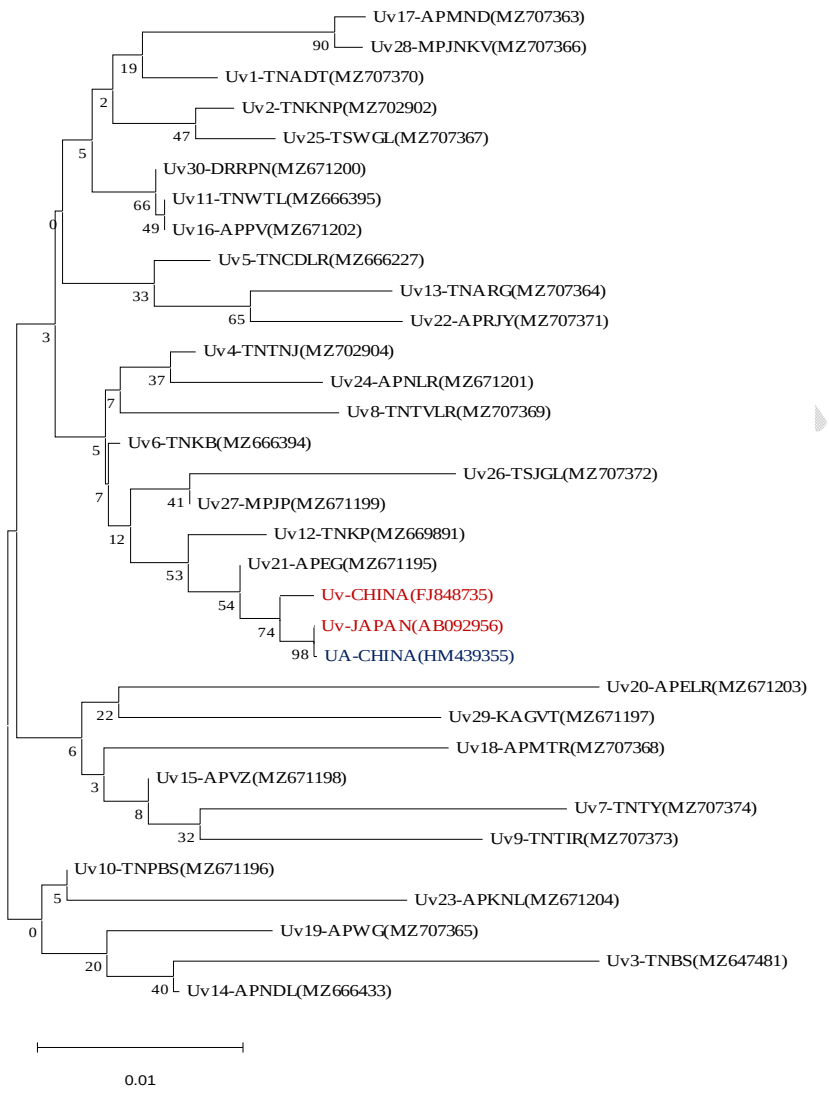

Fig. 5.Phylogenetic tree were constricted based on nucleotide sequences of ribosomal RNA gene of thirty three isolates using mega 7 software with 1000 bootstrip replications. Red color represents Uv-FJ848735, Uv-AB092956, wereas blue color is *U. albicans* (UA-HM439355)sequence retrieved from NCBI database and remaining all were study isolates.

3.5 Mating type analysis of *U. virens* isolates

The determining the sexuality of pathogen, conducted the mating type of analysis among the *U.virens* isolates. All thirty isolates of genomic DNA was isolated and PCR were performed with mating type specific primers *viz,* MAT1-1-1 and MAT1-2-1. The MAT1-1-1 primer was amplified a product of 250 bp and contain 18 isolates (Uv1, Uv2, Uv3, Uv4, Uv6, Uv8, Uv11, Uv12, Uv14, Uv15, Uv19, Uv21, Uv23, Uv25, Uv26, Uv28, Uv29, Uv30), whereas MAT1-2-1 primer was yielded a product of 220 bp and contain 12 isolates (Uv5, Uv7, Uv9, Uv10, Uv13, Uv16, Uv17, Uv18, Uv20, Uv22, Uv24, Uv27), (Table 5, Fig. 6a & 6b)

Table 5.GeneBank accession numbers for ribosomal RNA gene present in *U. virens*andMating type studies of thirty isolates of *U.virens*

Isolate no.	Gen bank Accession numbers	Mating type	
		MAT1-1-1	MAT1-2-1
Uv-1	MZ707370	Yes	No
Uv-2	MZ702902	Yes	No
Uv-3	MZ647481	Yes	No
Uv-4	MZ702904	Yes	No
Uv-5	MZ666227	No	Yes
Uv-6	MZ666394	Yes	No
Uv-7	MZ707374	No	Yes
Uv-8	MZ707369	Yes	No
Uv-9	MZ707373	No	Yes
Uv-10	MZ671196	No	Yes
Uv-11	MZ666395	Yes	No
Uv-12	MZ 669891	Yes	No
Uv-13	MZ707364	No	Yes
Uv-14	MZ666433	Yes	No
Uv-15	MZ671198	Yes	No
Uv-16	MZ671202	No	Yes
Uv-17	MZ707363	No	Yes
Uv-18	MZ707368	No	Yes
Uv-19	MZ707365	Yes	No
Uv-20	MZ671203	No	Yes
Uv-21	MZ671195	Yes	No
Uv-22	MZ707371	No	Yes
Uv-23	MZ671204	Yes	No
Uv-24	MZ671201	No	Yes
Uv-25	MZ707367	Yes	No
Uv-26	MZ707372	Yes	No
Uv-27	MZ671199	No	Yes
Uv-28	MZ707366	Yes	No
Uv-29	MZ671197	Yes	No
Uv-30	MZ671200	Yes	No

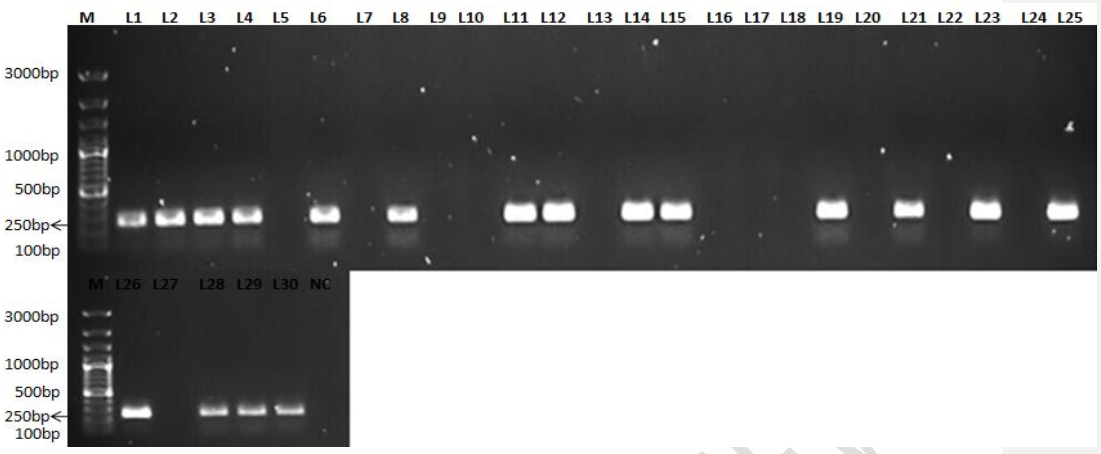

Fig.6a.Determining the mating type of *U.virens* using primer MAT 1-1-1 amplified at 250bp. M-3000bp marker; Lane1,2,3,4,6,8,11,12,14,15,19,21,23,25,26,28,29,30 isolates were presence of *U.virens* DNA; NC- negative control.

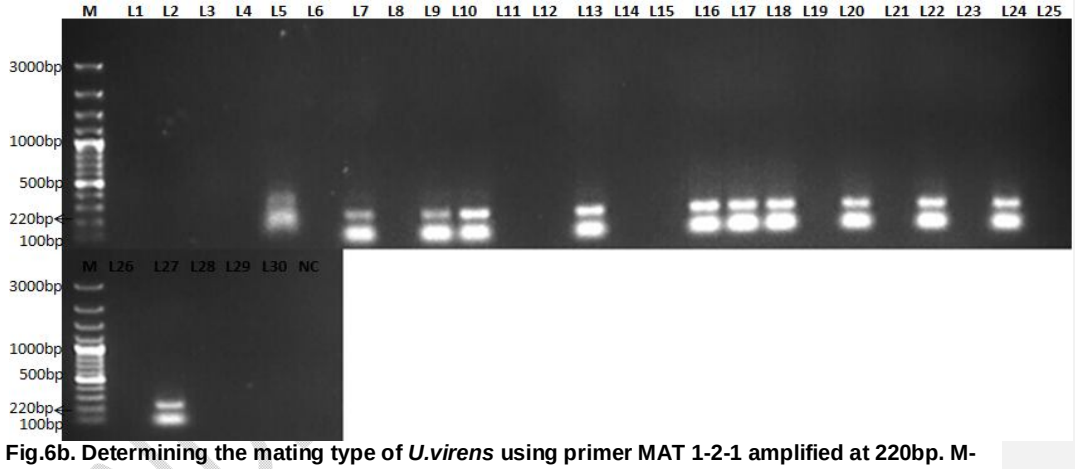

Fig.6b. Determining the mating type of *U.virens* using primer MAT 1-2-1 amplified at 220bp. M-3000bp marker; Lane 5,7,9,10,13,16,17,18,20,22,24,27 isolates were presence of *U.virens* DNA; NC- negative control.

3.6 Nucleotide diversity among the *U. virens* isolates

The nucleotide diversity among the 33 sequences of *U. virens* were analysed using DnaSP software version 5.10.01 (Julio Rozas et al., 2010), (developed by Universitat de Barcelona). The number of sites were analysed 686, among them excluding sites with gaps/missing data were 267. The number of pairwise comparisons were found 528, in this average number of sites were analysed 343.01 with differences of 8.106 and nucleotide diversity (*Pi*) were fond 0.02356. The tajimastest analysis were revealed that number of polymorphic sites (s) were found 7, mutations (*Eta*) were 10, nucleotide diversity (*k*) were 0.76515, tajimas test value was -2.13885 and stastiscal significance were found $P < 0.05$.

3.7 Haplotypes of *U. virens* isolates

The haplotypes analysis among the 33 sequencesincludes two sequences from china and one sequence from japan were analysed using DnaSPsoftware version 5.10.01. The number of sites were

analysed 686, among them total number of sites (excluding sites with gaps/missing data) were found 267. A total haplotype groups were found (*h*) 10, whereas haplotype diversity were (*hd*) 0.4773. among them hap_1 were major group and contain 24 isolates (Uv1-MZ707370, Uv2-MZ702902, Uv4-MZ702904, Uv5-MZ666227, Uv6-MZ666394, Uv7-MZ707374, Uv8-MZ707369, Uv9-MZ707373, Uv11-MZ666395, Uv12-MZ669891, Uv13-MZ707364, Uv14-MZ666433, Uv16-MZ671202, Uv17-MZ707363, Uv19-MZ707365, Uv21-MZ671195, Uv22-MZ707371, Uv24-MZ671201, Uv27-MZ671199, Uv28-MZ707366, Uv30-MZ671200, Uv31-FJ848735, Uv32-AB092956, UA33-HM439355), followed by hap_2 (Uv3-MZ647481), hap_3 (Uv10-MZ671196), hap_4 (Uv15-MZ671198), hap_5 (Uv18-MZ707368), hap_6 (Uv20-MZ671203), hap_7 (Uv23-MZ671204), hap_8 (Uv25-MZ707367), hap_9 (Uv26-MZ707372) and hap_10(Uv29-MZ671197), (Table 6). A total 10 haplotypes groups distribution were representation in a pictorial tree (Fig.7), using PopARTsoftware version 1.70 developed by Allan Wilson centre imaging evolution initiative.

Table 6. Haplotype Distribution among the thirty three isolates of *U. virens*

Haplotype group	Number of isolates	Isolates distribution
Hap_1	24	Uv1-MZ707370, Uv2-MZ702902, Uv4-MZ702904, Uv5-MZ666227, Uv6-MZ666394, Uv7-MZ707374, Uv8-MZ707369, Uv9-MZ707373, Uv11-MZ666395, Uv12-MZ669891, Uv13-MZ707364, Uv14-MZ666433, Uv16-MZ671202, Uv17-MZ707363, Uv19-MZ707365, Uv21-MZ671195, Uv22-MZ707371, Uv24-MZ671201, Uv27-MZ671199, Uv28-MZ707366, Uv30-MZ671200, Uv31-FJ848735, Uv32-AB092956,UA33-HM439355.
Hap_2	1	Uv3-MZ647481
Hap_3	1	Uv10-MZ671196
Hap_4	1	Uv15-MZ671198
Hap_5	1	Uv18-MZ707368
Hap_6	1	Uv20-MZ671203
Hap_7	1	Uv23-MZ671204
Hap_8	1	Uv25-MZ707367
Hap_9	1	Uv26-MZ707372
Hap_10	1	Uv29-MZ671197

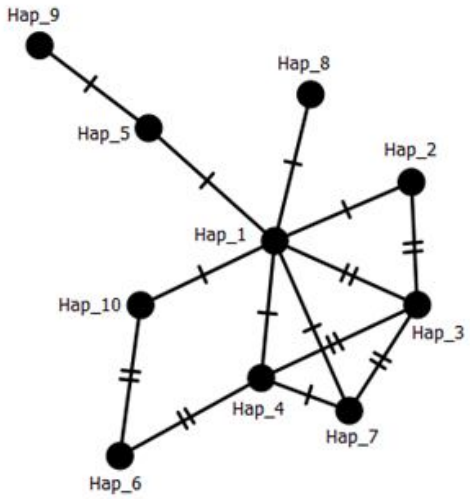

Fig.7. Haplotypes group'sdistribution among the thirty three*U. virens* isolates and the tree represents distance between among the each group.

3.8 Multiple sequence alignment and genetic similarity coefficient matrix among the *U. virens* isolate

A total 33 sequence were analysed for nucleotide variability of*U. virens* isolates using the ClustalW multiple alignment method of BioEdit Software 7.2.5 version. The results revealed that, clear nucleotide variations were observed between Indian isolates and China (Uv31-FJ848735, UA33-HM439355) as well as Japan (Uv32-AB092956) isolates. The genetic similarity coefficient matrix revealed that, thirty Indian isolates were showed maximum similarity (0.9). Whereas, Uv32-AB092956 showed (0.6), Uv31-FJ848735 showed (0.5) and UA33-HM439355 found (0.4), these results indicate that genetic variability were observed between the Indian *U. virens* isolates as well as other country *U.virens*isolates(Table7).

Table 7. Genetic similarity coefficient matrix for thirty three *U. virens* sequences based on ribosomal RNA gene sequencing and red colorrepresents were Uv31, Uv32 & Uv33 isolates wereother countryobtained from NCBI Gen Bank

Seq->	Uv1	Uv2	Uv3	Uv4	Uv5	Uv6	Uv7	Uv8	Uv9	Uv10	Uv11	Uv12	Uv13	Uv14	Uv15	Uv16	Uv17	Uv18	Uv19	Uv20	Uv21	Uv22	Uv23	Uv24	Uv25	Uv26	Uv27	Uv28	Uv29	Uv30	Uv31	Uv32	UA33
Uv1	ID	0.952	0.936	0.960	0.955	0.957	0.944	0.955	0.944	0.896	0.949	0.729	0.958	0.911	0.891	0.949	0.938	0.939	0.955	0.910	0.865	0.958	0.939	0.960	0.958	0.949	0.955	0.936	0.947	0.946	0.537	0.609	0.493
Uv2	0.952	ID	0.935	0.988	0.960	0.994	0.932	0.974	0.941	0.909	0.988	0.732	0.971	0.918	0.901	0.988	0.921	0.935	0.927	0.918	0.869	0.963	0.929	0.991	0.963	0.974	0.985	0.921	0.941	0.957	0.534	0.605	0.490
Uv3	0.936	0.935	ID	0.935	0.936	0.935	0.946	0.925	0.969	0.888	0.941	0.743	0.935	0.921	0.878	0.941	0.943	0.944	0.952	0.910	0.882	0.938	0.955	0.932	0.944	0.919	0.930	0.946	0.960	0.941	0.542	0.614	0.497
Uv4	0.960	0.988	0.935	ID	0.958	0.994	0.927	0.977	0.938	0.909	0.982	0.735	0.974	0.921	0.901	0.982	0.921	0.927	0.930	0.912	0.872	0.971	0.927	0.997	0.963	0.980	0.991	0.918	0.935	0.949	0.535	0.607	0.491
Uv5	0.955	0.960	0.936	0.958	ID	0.955	0.930	0.947	0.936	0.891	0.963	0.729	0.952	0.903	0.883	0.963	0.927	0.928	0.930	0.905	0.865	0.952	0.928	0.955	0.944	0.944	0.949	0.925	0.930	0.941	0.535	0.606	0.491
Uv6	0.957	0.994	0.935	0.994	0.955	ID	0.929	0.977	0.943	0.912	0.982	0.735	0.974	0.921	0.903	0.982	0.924	0.930	0.930	0.912	0.872	0.966	0.929	0.997	0.966	0.980	0.991	0.924	0.941	0.951	0.535	0.607	0.491
Uv7	0.944	0.932	0.946	0.927	0.930	0.929	ID	0.922	0.966	0.901	0.930	0.743	0.933	0.918	0.891	0.930	0.954	0.963	0.969	0.913	0.880	0.933	0.957	0.929	0.947	0.922	0.929	0.952	0.957	0.941	0.540	0.612	0.496
Uv8	0.955	0.974	0.925	0.977	0.947	0.977	0.922	ID	0.930	0.896	0.969	0.724	0.969	0.908	0.891	0.969	0.908	0.919	0.925	0.899	0.860	0.963	0.914	0.974	0.958	0.969	0.971	0.908	0.928	0.935	0.533	0.604	0.489
Uv9	0.944	0.941	0.969	0.938	0.936	0.943	0.966	0.930	ID	0.896	0.935	0.745	0.941	0.921	0.886	0.935	0.952	0.963	0.963	0.916	0.885	0.944	0.974	0.941	0.952	0.927	0.935	0.955	0.977	0.946	0.544	0.616	0.499
Uv10	0.896	0.909	0.888	0.909	0.891	0.912	0.901	0.896	0.896	ID	0.904	0.707	0.899	0.898	0.964	0.904	0.890	0.904	0.899	0.917	0.843	0.904	0.891	0.912	0.904	0.906	0.912	0.890	0.901	0.903	0.520	0.589	0.477
Uv11	0.949	0.988	0.941	0.982	0.963	0.982	0.930	0.969	0.935	0.904	ID	0.735	0.974	0.921	0.896	1.000	0.921	0.930	0.927	0.918	0.872	0.966	0.924	0.980	0.957	0.963	0.974	0.921	0.935	0.951	0.535	0.607	0.491
Uv12	0.729	0.732	0.743	0.735	0.729	0.735	0.743	0.724	0.745	0.707	0.735	ID	0.737	0.756	0.702	0.735	0.737	0.744	0.746	0.713	0.847	0.736	0.753	0.735	0.733	0.728	0.735	0.735	0.740	0.740	0.518	0.588	0.475
Uv13	0.958	0.971	0.935	0.974	0.952	0.974	0.933	0.969	0.941	0.899	0.974	0.737	ID	0.918	0.891	0.974	0.924	0.927	0.936	0.912	0.870	0.980	0.930	0.971	0.974	0.960	0.966	0.924	0.938	0.952	0.535	0.606	0.491
Uv14	0.911	0.918	0.921	0.921	0.903	0.921	0.918	0.908	0.921	0.898	0.921	0.756	0.918	ID	0.885	0.921	0.913	0.910	0.919	0.915	0.890	0.910	0.916	0.921	0.921	0.910	0.921	0.913	0.916	0.921	0.547	0.620	0.502
Uv15	0.891	0.901	0.878	0.901	0.883	0.903	0.891	0.891	0.886	0.964	0.896	0.702	0.891	0.885	ID	0.896	0.887	0.893	0.888	0.906	0.836	0.891	0.880	0.903	0.891	0.898	0.901	0.885	0.888	0.895	0.516	0.584	0.473
Uv16	0.949	0.988	0.941	0.982	0.963	0.982	0.930	0.969	0.935	0.904	1.000	0.735	0.974	0.921	0.896	ID	0.921	0.930	0.927	0.918	0.872	0.966	0.924	0.980	0.957	0.963	0.974	0.921	0.935	0.951	0.535	0.607	0.491
Uv17	0.938	0.921	0.943	0.921	0.927	0.924	0.954	0.908	0.952	0.890	0.921	0.737	0.924	0.913	0.887	0.921	ID	0.938	0.944	0.896	0.872	0.919	0.946	0.924	0.933	0.910	0.923	0.994	0.943	0.932	0.535	0.607	0.491
Uv18	0.939	0.935	0.944	0.927	0.928	0.930	0.963	0.919	0.963	0.904	0.930	0.744	0.927	0.910	0.893	0.930	0.938	ID	0.952	0.910	0.883	0.933	0.955	0.930	0.933	0.924	0.924	0.935	0.957	0.935	0.544	0.616	0.499
Uv19	0.955	0.927	0.952	0.930	0.930	0.930	0.969	0.925	0.963	0.899	0.927	0.746	0.936	0.919	0.888	0.927	0.944	0.952	ID	0.915	0.883	0.938	0.961	0.930	0.944	0.919	0.924	0.941	0.961	0.952	0.544	0.616	0.499
Uv20	0.910	0.918	0.910	0.912	0.905	0.912	0.913	0.899	0.916	0.917	0.918	0.713	0.912	0.915	0.906	0.918	0.896	0.910	0.915	ID	0.853	0.910	0.913	0.912	0.924	0.901	0.909	0.896	0.924	0.929	0.524	0.594	0.481
Uv21	0.865	0.869	0.882	0.872	0.865	0.872	0.880	0.860	0.885	0.843	0.872	0.847	0.870	0.890	0.836	0.872	0.872	0.883	0.883	0.853	ID	0.867	0.893	0.872	0.870	0.865	0.872	0.869	0.880	0.877	0.612	0.693	0.561
Uv22	0.958	0.963	0.938	0.971	0.952	0.966	0.933	0.963	0.944	0.904	0.966	0.736	0.980	0.910	0.891	0.966	0.919	0.933	0.938	0.910	0.867	ID	0.933	0.969	0.971	0.960	0.963	0.919	0.938	0.941	0.535	0.606	0.491
Uv23	0.939	0.929	0.955	0.927	0.928	0.929	0.957	0.914	0.974	0.891	0.924	0.753	0.930	0.916	0.880	0.924	0.946	0.955	0.961	0.913	0.893	0.933	ID	0.929	0.938	0.916	0.924	0.943	0.966	0.944	0.547	0.620	0.502
Uv24	0.960	0.991	0.932	0.997	0.955	0.997	0.929	0.974	0.941	0.912	0.980	0.735	0.971	0.921	0.903	0.980	0.924	0.930	0.930	0.912	0.872	0.969	0.929	ID	0.963	0.982	0.994	0.921	0.938	0.949	0.535	0.607	0.491
Uv25	0.958	0.963	0.944	0.963	0.944	0.966	0.947	0.958	0.952	0.904	0.957	0.733	0.974	0.921	0.891	0.957	0.933	0.933	0.944	0.924	0.870	0.971	0.938	0.963	ID	0.952	0.957	0.935	0.949	0.952	0.535	0.606	0.491
Uv26	0.949	0.974	0.919	0.980	0.944	0.980	0.922	0.969	0.927	0.908	0.963	0.728	0.960	0.910	0.898	0.963	0.910	0.924	0.919	0.901	0.865	0.960	0.916	0.982	0.952	ID	0.982	0.908	0.924	0.935	0.533	0.604	0.489
Uv27	0.955	0.985	0.930	0.991	0.949	0.991	0.929	0.971	0.935	0.912	0.974	0.735	0.966	0.921	0.901	0.974	0.923	0.924	0.924	0.909	0.872	0.963	0.924	0.994	0.957	0.982	ID	0.921	0.932	0.943	0.535	0.607	0.491
Uv28	0.936	0.921	0.946	0.918	0.925	0.924	0.952	0.908	0.955	0.890	0.921	0.735	0.924	0.913	0.885	0.921	0.994	0.935	0.941	0.896	0.869	0.919	0.943	0.921	0.935	0.908	0.921	ID	0.946	0.932	0.534	0.605	0.490
Uv29	0.947	0.941	0.960	0.935	0.930	0.941	0.957	0.928	0.977	0.901	0.935	0.740	0.938	0.916	0.888	0.935	0.943	0.957	0.961	0.924	0.880	0.938	0.966	0.938	0.949	0.924	0.932	0.946	ID	0.952	0.540	0.612	0.496
Uv30	0.946	0.957	0.941	0.949	0.941	0.951	0.941	0.935	0.946	0.903	0.951	0.740	0.952	0.921	0.895	0.951	0.932	0.935	0.952	0.929	0.877	0.941	0.944	0.949	0.952	0.935	0.943	0.932	0.952	ID	0.539	0.611	0.494
Uv31	0.537	0.534	0.542	0.535	0.535	0.535	0.540	0.533	0.544	0.520	0.535	0.518	0.535	0.547	0.516	0.535	0.535	0.544	0.544	0.524	0.612	0.535	0.547	0.535	0.535	0.533	0.535	0.534	0.540	0.539	ID	0.882	0.894
Uv32	0.609	0.605	0.614	0.607	0.606	0.607	0.612	0.604	0.616	0.589	0.607	0.588	0.606	0.620	0.584	0.607	0.607	0.616	0.616	0.594	0.693	0.606	0.620	0.607	0.606	0.604	0.607	0.605	0.612	0.611	0.882	ID	0.809
UA33	0.493	0.490	0.497	0.491	0.491	0.491	0.496	0.489	0.499	0.477	0.491	0.475	0.491	0.502	0.473	0.491	0.491	0.499	0.499	0.481	0.561	0.491	0.502	0.491	0.491	0.489	0.491	0.490	0.496	0.494	0.894	0.809	ID

4. DISCUSSION

The thirty isolates of *U. virens* showed well defined colonies on PSA medium. The maximum colony diameter found 85.68 mm with growth rate of 2.85 mm in Uv23 isolate and minimum colony diameter found 10.14 mm with growth rate of 0.33 mm was observed in Uv15 isolate. However, thirty isolates exhibit various cultural and morphological characters like, colony color, growth pattern, elevation and chlamydospores formation. Similar morphological characteristics of the pathogen were described by [5]. Meanwhile, maximum (n=25) isolates showed flat elevation, only five isolates was exhibit raised elevation, likewise sixteen isolates produced irregular margin and remaining (n=14) were exhibited circular margin after 30 days of incubation. These results are agreement with [5; 8]. In cluster analysis, two major groups (I), (II) were formed, first major group I contain Uv15 isolate and second major group II contain 29 *U. virens* isolates, further which were dived into two subgroups which are subgroup IIa contain 22 isolates and IIb contain 7 isolates. The results of the experiment were in accordance with the finding of [8; 9], who studied 35 isolates and 12 isolates respectively and observed different characters like growth pattern, growth rate, colony color., and grouped them in different clusters. The PCR amplification was done with species specific primers which were yielded products of 380 bp and 230 bp respectively. Similar results were observed, [5], who studed 9 isolates and confirmed as *U. virens* using the specific primers of US1-5/US3-3 and US2-5/US4-3. In phylogenetic tree analysis results reveled that, cluster (I) contain 5 isolates wereas cluster (II) contain 28 isloates includes china and japan isloates.Similar results were observed by [25], who studied 71 *U. virens* isolates for phylogenetic analysis among them, 54 isolates was grouped into clusters (I) and 7 isolates were groued in cluster (II). The MAT1-1-1 primer was amplified a product of 250 bp and contain 18 isolates. Whereas, MAT1-2-1 primer was yielded a product of 220 bp and contain 12 isolates. Our findings were similar with [21; 25], who studied mating type of analysis using the MAT1-1-1 and MAT1-2-1 locus primers. The nucleotide divergence analysis results revealed that numbers of polymorphic sites (s) were found 7, mutations (*Eta*) found 10, nucleotide diversity (*k*) was found 0.76515, tajimas test value was -2.13885. The experiment results were accordance with [25], who studied 61 isolates and observed nucleotide diversity *(k)* 0.9163. Likewise, for haplotye analysis revealed that total haplotype groups were found (*h*) 10, with haplotype diversity was found (*hd*) 0.4773. Among them hap_1 were major group and contain 24 isolates. The similar results were observed [25], who studied 61 isolates and observed 42 haplotypes. Similarly, clear nucleotide variations were observed between Indian isolates and China as well as Japan isolates. The genetic similarity coefficient matrix revealed that, thirty Indian isolates were showed maximum similarity (0.9). Whereas, Uv32 isolate showed 0.6, Uv31 isolate exhibited 0.5 and UA33 isolate found 0.4.

5. CONCLUSION

We conclude that this study revealed, cultural, morphological variations, nucleotide diversity and population divergence were exhibited among the *U. virens* Indian isolates along with *U. virens* of other county isolates. This study was very useful for the understand the diversity of the rice false smut pathogen at different geographical areas meanwhile, adapt the better management strategies to control the rice false smut disease in rice ecosystem.

REFERENCES

1. CookeMC. Some extra-European fungi. Grevillea. 1878; 7, 3.
2. Rush MC, Shahjahan AKM, Jones JP, Groth DE. Outbreak of false smut of rice in Louisiana. Plant Dis. 2000; 84, 100.
3. Dodan DS, Singh R. False smut of rice: present status. Agricultural Reviews-Agricultural Research Communications Centre, India. 1996; 17, 227–240.
4. Biswas A. False smut disease of rice: a review. Env Ecol. 2001; 19, 67 –83.
5. Ladhalakshmi D, Laha GS, Singh R, Karthikeyan A, Mangrauthia SK, Sundaram RM, Thukkaiyannan P, Viraktamath, BC. Isolation and characterization of *Ustilaginoideavirens* and survey of false smut disease of rice in India. Phytoparasitica.2012; 40, 171–176.
6. Kumari S, Kumar J. Evaluation of yield losses and management practices of false smut in rice (*Oryzasativa*). Indian Phytopathol. 2015; 68, 45 –49

7. Muniraju KM, Pramesh D, Mallesh SB, Mallikarjun K, Guruprasad GS. Disease severity and yield losses caused by false smut disease of rice in different rice ecosystems of Karnataka. Int J Microbiol Res. 2017; 9, 955–958.
8. Baite MS, Sharma RK, Devi TP, Sharma P, Kamil D. Morphological and molecular characterization of *Ustilaginoideavirens* isolates causing false smut of rice in India. Indian Phytopathol. 2014; 67, 222–227.
9. Rani R, Sharma VK. Diversity analysis of northern Indian isolates of *Ustilaginoideavirens*. Indian Phytopathol. 2018; 71, 33 –42.
10. Zhou YL, Izumitsu K, Sonoda R, Nakazaki T, Tanaka E, Tsuda M,Tanaka C. PCR-based specific detection of *Ustilaginoideavirens* and *Ephelis japonica*. J Phytopathol. 2003; 151, 513–518.
11. Yongli Z, Jinjuan F, Chaozhen Z, Xiaozhou L, Shu W, Kaijun Z. Preliminary analysis of genetic diversity and population structure of *Ustilaginoideavirens*. ActaPhytopathologicaSinica. 2004; 34, 442–448.
12. Ping TX, Jian-Wei S, Er-Ming L, Nian-Xi L, Jin-Hui, W, Qi-Ming X. Analysis of genetic diversity of *Ustilaginoideavirens* in Hunan. J Hunan AgricultUniv. 2008; 34, 694–697.
13. Sun X, Kang S, Zhang Y, Tan X, Yu Y, He H, Zhang X, Liu Y. Genetic diversity and population structure of rice pathogen *Ustilaginoideavirens* in China. PLoS One. 2013; 8, e76879.
14. Zhang Y, Zhang K, Fang A, Han Y, Yang J, Xue M, Bao J, Hu D.Specific adaptation of *Ustilaginoideavirens* in occupying host florets revealed by comparative and functional genomics. Nat Commun. 2014; 5, 1–12.
15. Pramesh D, Muniraju KM, Prasanna Kumar MK, Mahesh HB, Shankargouda I,Manjunath C. Complete genome sequence of the rice false smut pathogen *Ustilginoideavirens* UV-GVT strain from India. 2018; 3rd ARRW International Symposium, Feb 6-9, Cuttack, India.
16. Turgeon BG, Yoder OC. Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet Biol. 2000; 31,1 –5.
17. Yu JJ, Sun WX, Yu MN, Yin XL, Meng XK, Zhao J, Huang L, Huang L. Characterization of mating-type loci in rice false smut fungus *Villosiclavavirens*. FEMS Microbiol Lett.2015; 362, p.fnv014.
18. Nelson MA. Mating systems in ascomycetes: a romp in the sac. Trends Genet. 1996; 12, 69 – 74.
19. Rohlf JF. NTSYS-PC. Numerical taxonomy and multivariate analysis system, Version 1.8. Exeter Software, 1998; New York
20. Murray MG, Thompson W. Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 1980; 8:4321–4325
21. Fu R, Yin C, Deng Q, Li P, Zheng A. Mating type loci analysis indicates that *Villosiclavavirens*, the causal agent of false smut disease of rice, is a homothallic fungus. Trop Plant Pathol. 2014; 39, 203–209.
22. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/ 98/NT. Nucleic Acids Symp Ser. 1999; 41, 95-98.
23. Librado P, Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25, 1451–1452.
24. Bandelt HJ, Forster P,Rohl A. Median-joining networks for inferring intraspecific phylogenies. MolBiolEvol. 1999; 16, 37.
25. Sharanabasav H, Pramesh D, Prasannakumar MK, Chidanandappa E, Yadav MK, Ngangkham U, Parivallal B, Raghavendra BT, Manjunatha C, Sharma SK, Karthik N. Morpho-molecular and mating-type locus diversity of *Ustilaginoideavirens*: an incitant of false smut of rice from Southern parts of India. Journal of Applied Microbiology. 2021; ISSN 1364-5072.