International Journal of Agricultural Technology 2024 Vol. 20(2):517-534
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Diversity of *Rhizoctonia solani* causing rice sheath blight in selected rice-based farming systems in Nueva Ecija, Philippines

Bulatao, R.[1], Dela Cruz, A. A.[2], Niones, J. T.[2], Alberto, R. T.[1] and Padilla, C. O.[1*]

[1]Department of Crop Protection, College of Agriculture, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines; [2]Philippine Rice Research Institute, Science City of Muñoz, Nueva Ecija, Philippines.

Bulatao, R., Dela Cruz, A. A., Niones, J. T., Alberto, R. T. and Padilla, C. O. (2024). Diversity of *Rhizoctonia solani* causing rice sheath blight in selected rice-based farming systems in Nueva Ecija. International Journal of Agricultural Technology 20(2):517-534.

Abstract *Rhizoctonia solani* causing sheath blight of rice is one of the most widely spreading diseases of rice. *Rhizoctonia solani* AG1-1A is the distinct anastomosis group of *R. solani* which is known to infect rice plants and is the primary cause of yield reduction. The level of variation and aggressiveness among identified *R. solani* AG1-1A infecting rice in rice-rice, rice-corn, and rice-onion farming systems in Nueva Ecija, Philippines were determined. Of the 30 *R. solani* isolated from the infected sheath of rice, 22 of them belonged to *R.solani* AG1-1A while eight isolates were identified as *R. oryzae.* Despite this prevalence, a low level of genetic variation was observed within *R. solani* AG1-1A isolates. Using VNTR analysis for clustering, it is evident that *R. solani* AG1-1A isolates, obtained from different types of farming systems, exhibit a close genetic relationship. This clustering suggests a shared genetic makeup among isolates from different agricultural contexts. The *R. solani* AG1-1A isolates also demonstrate considerable aggressiveness. Through pathogenicity tests on susceptible test plants (TN-1), the *R. solani* AG1-1A isolates proved their capability to induce severe symptoms, with disease severity ranging from 60% to 100%. This indicates the strong pathogenic potential of the isolated *R. solani* AG1-1A and highlights their impact on rice crops.

Keywords: Diversity, Disease severity, Inoculation, Disease incidence

Introduction

Rice is one of the three major food crops of the world that serves as staple food for about half of the world's population. The global rice production has been estimated to be at the level of 650 million tons with an estimated 156 million hectares of production area. Asia leads the rice industry contributing to about 90% of the world's production, where 75% of the world supply is consumed (FAO, 2008). The demand for rice is expected to increase due to the continuous population growth. By the year 2050, rice production must increase 25% to meet

[*] **Corresponding Author:** Padilla, C. O.; **Email:** celynnepadilla@clsu.edu.ph

the global demand (IRRI, 2017). Although there have been technological advancements and research to improve production, significant losses still occur due to pathogen issues.

Rice sheath blight (RSB) caused by *Rhizoctonia solani* anastomosis group 1 subgroup 1A (AG1-1A) is one of the most common and vicious diseases of rice worldwide (IRRI, 2017). In lowland environments, the symptoms typically manifest at the tillering stage on the leaf sheath at water level, and in upland ecosystems, at ground level. On the leaf sheath and leaves, the pathogen causes elliptical, oval, to irregular, 1-3 cm long, greenish-grey dots. The spot turned grayish-white in the center, with a brown border. When the environment is favorable, the infection quickly spreads to the leaf blades and upper sheaths of the same or nearby tillers. The plant's upper sections have lesions that spread quickly and combine to cover the entire tiller, from the water line to the flag leaf. Larger, somewhat asymmetrical lesions that range in color from greenish gray to white with a brown border develop on the leaf blades, which eventually kill the leaf, tiller, and plant (Bhunkal, 2014). The main reasons for the decrease in yield are the reduction in leaf area, the disease-induced senescence of the leaves, and the young infected tillers (IRRI, 2017).

In the Philippines, this fungal disease poses a serious threat to the effective cultivation of rice during the rainy season, as it targets the majority of farmed high-yielding lowland rice types that are fed by rain. RSB outbreaks are a recurring issue, and controlling them is costly and challenging (Mian *et al.,* 2003). Despite reports of partial genetic resistance to RSB and the identification of the major genes causing resistance (Pan *et al.,* 1999), the disease is still not controlled even by resistance breeding (Cubeta and Vilgalys, 1997; Wu *et al.* 2012). Management strategies such as preventive treatments and the use of antibiotics can be done. However, too much application of fungicides can cause negative effects on the environment. Hence, the development of resistant varieties to control RSB is desirable, but greatly dependent on an understanding of the genetic diversity of the pathogen (Shu *et al*., 2014).

A crucial early step in any study of plant infection, whether it is driven by research or the requirement for quick and precise diagnostics during an epidemic, is the proper identification down to the species level. This aids in decision-making concerning financial loss and expenditure for necessary illness management methods. Recent advances in DNA barcoding technology have significantly changed the epitome of species identification and hold promise for offering a useful, standardized tool for species-level identification that can be applied to ecological research, fungal plant pathogen monitoring, diagnostics, and biodiversity assessment (Kashyap *et al.,* 2017). Accurate identification on *R. solani* targeting rice will help select and implement effective management

practices, such as cultural, physical, and chemical approaches, and even the development of sheath blight resistant rice. An efficient system for the identification of species identification can significantly help in treating fungal diseases and monitor the spatial patterns of fungal distributions. Also, assessing aggressiveness based on the type of farming systems has been a very useful trait for characterizing the diversity among the strains of *R. solani* AG1-1A. In screening for resistance, the geographic distribution of pathogen genotypes is important. The fact that pathogen populations are often geographically sub-structured may only be determined through comprehensive sampling and the application of appropriate genetic markers. A detailed understanding of the pathogen population structure allows for the prediction of the efficacy and resilience of host resistance (Peever *et al.,* 2022). The information generated in the study on the diversity of *R. solani* would be useful for the effective management of the sheath blight disease of rice. Diversity information also helps researchers to breed disease-resistant rice varieties. This study was conducted to determine the diversity of *R. solani* that affects rice growing in different rice-based farming systems in Nueva Ecija, Philippines. This was conducted by determining the level of genetic variations among *R. solani* infecting rice in rice-rice, rice-corn, and rice-onion farming systems and by evaluating the aggressiveness of the selected *R. solani* isolates through a pathogenicity test.

Materials and methods

Sample collection and isolation

The study was conducted at the College of Agriculture, Department of Crop Protection, Central Luzon State University and Molecular Laboratory, Philippine Rice Research Institute, Maligaya, Science City of Muñoz, Nueva Ecija from January 2018 to September 2018.

Isolates were collected from different rice-based farming systems from San Jose City, Science City of Muñoz, and Bongabon, Nueva Ecija. Infected rice plants were uprooted from rice fields. The samples were placed inside plastic bags and brought to the laboratory in a cold container to prevent rapid degradation or senescence of the plant. A portion from the infected sheath leaf of approximately 2-3 mm^2 was cut, surface disinfected with 10% common household bleach for 1-2 minutes, and rinsed in 3 changes of sterile distilled water. Disinfected sections were blot-dried in sterile tissue paper. Four sections of samples were placed equidistantly on a plated Potato Dextrose Agar (PDA) and incubated at room temperature for 5-7 days. A bit of mycelia from the

advancing margin of fungal growth was transferred onto PDA slants and served as a pure culture.

DNA isolation, amplification, and sequencing

Isolates were grown in microtubes with 1 ml potato dextrose broth for 5 days at 27˚C in a mechanical shaker (120 rpm). Samples were centrifuged for 15 minutes to allow the sedimentation of the mycelia. After centrifugation, the liquid broth was discarded and the total genomic DNA was extracted. Universal primers (ITS 1 and ITS 4), forward and reverse primers, were used for each sample. Each PCR product contained 5 µl of forward and reverse primers, 5 µl of sterile distilled water, 5 µl of PCR buffer, 10 µl (mM) diluted DNA, and 25 µl of Taq polymerase. Amplification was carried out in BIO-RAD Thermal Cycler with the following temperature profiles: 1 cycle of 3 min at 94˚C, 34 cycles 30 s at 94˚C, 30 s at 55˚C, and 1 min at 72˚C. Ten microliter PCR products were loaded into 1.5% agarose gels pre-stained with Gel Red. Electrophoresis was performed in 1x TBE buffer at 150 V for 70 min and visualized in a LED transilluminator. PCR products were sent to Apical Scientific Sequencing, Sengalor, Malaysia for purification and sequencing.

ITS-rDNA nucleotide sequence comparison

Similar nucleotide sequences corresponding to at least 98% or higher values of the identified *Rhizoctonia* species were searched and obtained from the National Center for Biotechnology International (NCBI) and the GenBank accession numbers and FASTA sequences were downloaded from EMBL database through the site http://www.ncbi.nlm.nih.gov/Genbank. The aligned sequences were checked for gaps manually and compared with those in the GenBank database using BioEdit Sequence Alignment Editor. Sequences were aligned using the multiple sequence alignment program Clustal X. All genetic distance analyses were established using Molecular Evolutionary Genetic Analysis (MEGA7) software.

Characterization of R. solani through VNTR analysis

The VNTR analysis was conducted using three primers named MR, RY, and GF. The details of the primers are available in Table 1. The amplification of DNA was determined through PCR. A master mix of 7.5 µL reaction was prepared, and 4.5 µL of the master mix was distributed into a 0.2 mL PCR tube. After that, 3 µL of genomic DNA (2.5 ng/µL) of the selected isolates was added

to the tube. To prevent evaporation, a drop of mineral oil was added to each tube. Following this, the VNTR-PCR was performed using the following conditions: 94˚C for 2 min, then 35 cycles of 94˚C for 20 s, 45˚C for 45 s, 72˚C for 2 min, and finally, 72˚C for 5 min. A UV transilluminator was used to expose the gel, and a gel documentation unit was used to capture images of the gel for measuring the bands of amplified DNA fragments (Zakiah, 2016).

Table 1. List of primers and their sequences

VARIABLE NUMBER OF TANDEM REPEAT (VNTR)		
Primer Name	Sequence	Reference
MR	GAGGGTGGCGGTTCT	Zakiah *et al.,* 2016
RY	CAGCAGCAGCAGCAG	Zakiah *et al.,* 2016
GF	TCCTCCTCCTCCTCC	Zakiah *et al.,* 2016

Gel scoring for cluster analysis by NTSYS

Amplified DNA fragments were scored into a binary format by designating "1" for presence and "0" for absence of allele, counted, and recorded according to the position of bands for each of the isolates. The input file was prepared, and cluster analysis was processed using NTSYS-PC (Numerical Taxonomy System Computer 2.10, Rohfl, 2000). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method of the Clustering subroutine SAHN was used to construct the dendrogram.

Pathogenicity test

Thirty-day-old susceptible rice plants, TN-1, were inoculated with a 5mm agar block of *R. solani.* The agar block was placed beneath the leaf sheath and was covered with aluminum foil. Experiments were carried out in 2 replications per isolate. The aluminum foil was removed 3 days after inoculation when typical lesions appeared.

Test plants were evaluated for 7, 14, and 21 days after inoculation (DAI). The evaluation was performed to estimate the aggressiveness of each isolate collected from different rice-based farming systems. This was conducted by determining the percent disease severity, percent relative lesion height, and percent disease incidence. Phenotypic scoring for sheath blight infection was based on the disease severity index for rice sheath blight (Table 2).

Table 2. The phenotypic scoring for rice sheath blight infection

SCALE	DESCRIPTION
0	No lesion
1	Appearance of water-soaked lesions
2	Appearance of necrotic lesion
3	Less than 50% necrosis on the sheath cross sections
4	More than 50% necrosis on the sheath cross sections
5	Necrosis across the entire sheath section resulting in sheath death

Statistical analysis

The experiment was laid out in CRD using Statistical Package for Social Sciences. The differences among the means at 5% level of significance were determined using Tukey's Honest Significance Test (SOFTWARE: SPSS, version 21.0).

Results

Isolate identification and characterization

Thirty *R. solani*-like isolates were collected from sheath blight-infected rice plants from three farming systems in Nueva Ecija, Philippines, namely: rice-corn, rice-rice, and rice-onion. Ten isolates each from San Jose City, Muñoz, and Bongabon were collected. The DNA sequence search was performed using the BLAST standard nucleotide-nucleotide basic local alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST/). Twenty-two isolates were categorized as *R. solani* AG1-1A and 8 were *R. oryzae* (Table 3). Out of the 22 isolates, five were from San Jose City, ten from Science City of Muñoz, and seven were from Bongabon. The obtained sequences of *R. solani* AG1-1A were found to be 76-100% similar to the identified *R. solani* AG1-1A in Genbank. On the other hand, SJCPAL 3 RC, SJCC 3 RC, SJCC 4 RC, SJCPM 2 RC, SJCPM 3 RC, BONGM3 RO, BONGM4 RO, and BONGP3 RO were matched to *R. oryzae* in Genbank, and well supported by 95-100% bootstrap.

Phylogenetic analysis

Twenty-two isolates of identified *R. solani* were grouped through MEGA software using phylogenetic analysis (Figure 1). The evolutionary history was inferred using the UPGMA method and evolutionary distances were computed using the p-distance method (Saitou and Nei, 1987). The phylogenetic tree was divided into two major clades: the out-group, *C. gloeosporiodes,* and the in-group which consists of *R. solani* grouped as AG1-1A. Based on Figure 1, the majority

of the *R. solani* isolates did not group directly on the AG1-1A aside from eight isolates. SJCC1 RC, SCMM2 RR, SCMBAN3 RC, BONGPM1 RO, BONGPM2 RO, BONGP1 RO, SJCPM1 RC, and SCMBS4 RR group together on the same cluster with the two *R.solani* AG1-1A references.

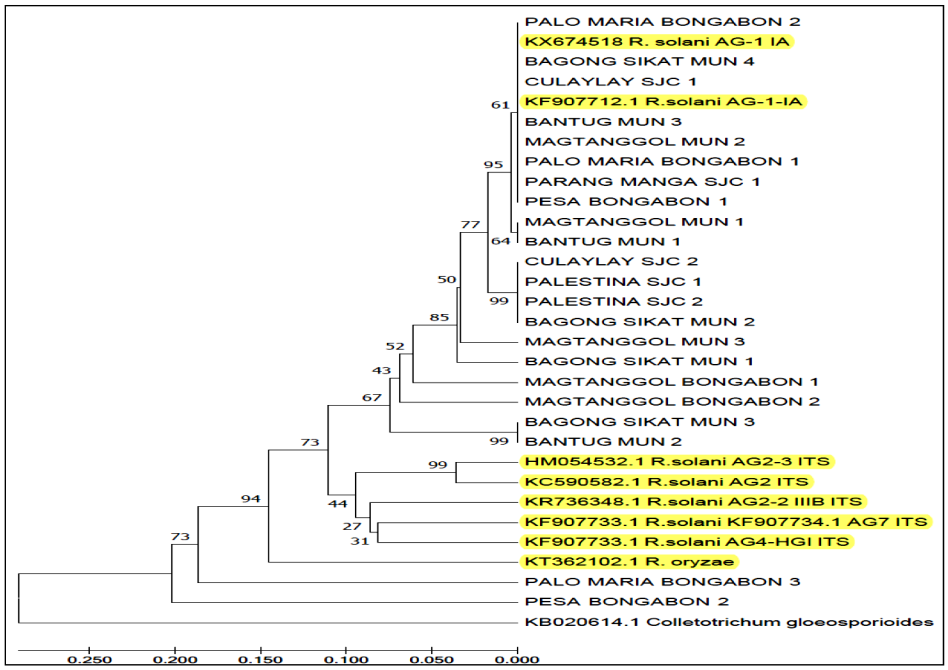

Figure 1. Evolutionary relationships of taxa. Phylogenetic tree based on UPGMA method illustrating the relation of *R. solani* AG1-1A. Genetic distance among clades was indicated by a bar. Highlighted names are sequences downloaded from NCBI/Genbank, to serve as a control, of known species and anastomosis groups, to help with classification

Identified *R. solani* AG1-1A isolates did not grouped in other reference anastomosis groups; however, BONGPM3 RO and BONGP2 RO did not cluster in AG1-1A. It was observed that the maximum similarity (%) of BONGPM3 RO and BONGP2 RO are 81% and 76%, respectively (Table 3).

Characterization of R. solani through VNTR analysis

Based on sequence analysis, identified *R. solani* AG1-1A isolates were further genotyped by PCR using VNTR analysis and pathogenicity test. Three VTNR primers MR, RY, and GF (Table 1). With the conditions optimized, the

three primers amplified multiple polymorphic DNA fragments (Zakiah *et al.,* 2016) (Figures 2-4). The UPGMA dendrogram was generated based on the similarity values calculated from the total data set shown in Figure 5. Based on a cluster dendrogram prepared according to PCR products obtained in different isolates from diverse farming systems and using three different sets of primers, all 22 isolates of *R.solani* have been grouped into two major clusters at a similarity coefficient of 0.57.

R. solani AG1-1A isolates were clustered into two in which Cluster I contained 16 isolates and Cluster II was comprised of six isolates. Based on the result, isolates SCMBAN1 from Muñoz and SJCC1 from San Jose City and SCMM3 from Muñoz and SJCC2 from San Jose City, from cluster I, are similar with 0.88 similarity coefficient. However, the same was observed from cluster II where isolates SCMBS4 and SCMM2 both from Muñoz had 0.88 similarity coefficients as well. Cluster I was further subdivided into two sub-groups at a similarity coefficient of 0.618. The first subgroup consists of 11 isolates while the second subgroup consists of five isolates. Similarly, cluster II was also subdivided into two subgroups at a similarity coefficient of 0.65, with three isolated for each subgroup.

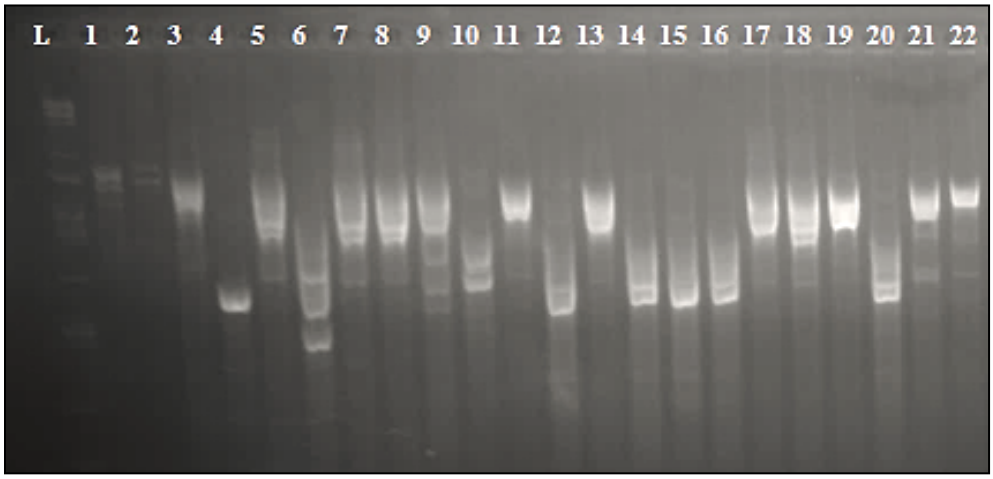

Figure 2. Variable number of tandem repeat (VNTR) fingerprinting patterns of total genomic DNA of *R. solani* isolates with primer MR. 1kb ladder; Lanes 1 to 22 (isolates)

Table 3. List of samples with the corresponding type of farming system and isolates identified using NCBI BLAST

Sample name	Place of collection	Type of farming system	Isolate code	Homology	Max Similarity (%)	Accession no.
Palestina 1	San Jose City	Rice-corn	SJCPAL1 RC	*Rhizoctonia solani strain 2773*	99	KU696484.1
Palestina 2	San Jose City	Rice-corn	SJCPAL2 RC	*Rhizoctonia solani isolate GDHZ12*	99	KF053535.1
Palestina 3	San Jose City	Rice-corn	SJCPAL3 RC	*Rhizoctonia oryzae isolate VC 241*	95	KT362102.1
Culaylay 1	San Jose City	Rice-corn	SJCC1 RC	*Rhizoctonia solani strain AG 1-IA isolate XS3-01*	97	MG397063.1
Culaylay 2	San Jose City	Rice-corn	SJCC2 RC	*Thanatephorus cucumeris*	97	AB195928.1
Culaylay 3	San Jose City	Rice-corn	SJCC3 RC	*Rhizoctonia oryzae isolate VC 241*	99	KT362102.1
Culaylay 4	San Jose City	Rice-corn	SJCC4 RC	*Rhizoctonia oryzae isolate VC 140*	96	KT362087.1
Parang Manga 1	San Jose City	Rice-corn	SJCPM 1 RC	*Rhizoctonia solani strain RRS9*	95	KF570303.1
Parang Manga 2	San Jose City	Rice-corn	SJCPM 2 RC	*Rhizoctonia oryzae isolate VC 51*	100	KT362129.1
Parang Manga 3	San Jose City	Rice-corn	SJCPM 3 RC	*Rhizoctonia oryzae isolate VC 51*	95	KT362129.1
Bantug 1	Muñoz	Rice-rice	SCMBAN1 RR	*Rhizoctonia solani isolate Hz-1*	99	EU152872.1
Bantug 2	Muñoz	Rice-rice	SCMBAN2 RR	*Rhizoctonia solani isolate Ms-2*	98	EU152868.1
Bantug 3	Muñoz	Rice-rice	SCMBAN3 RR	*Rhizoctonia solani strain AG 1-IA isolate WLP2-01*	100	MG397056.1
Bagong Sikat 1	Muñoz	Rice-rice	SCMBS1 RR	*Rhizoctonia solani strain HRBdo*	96	KF959725.1
Bagong Sikat 2	Muñoz	Rice-rice	SCMBS2 RR	*Rhizoctonia solani isolate RMPM23*	96	JF701749.1

Bagong Sikat 3	Muñoz	Rice-rice	SCMBS3 RR	*Rhizoctonia solani isolate Ms-2*	98	EU152868.1
Bagong Sikat 4	Muñoz	Rice-rice	SCMBS4 RR	*Rhizoctonia solani AG-1 IA isolate RUP02*	99	KX674518.1
Magtanggol 1	Muñoz	Rice-rice	SCMM1 RR	*Rhizoctonia solani isolate RMPM13*	99	JF701748.1
Magtanggol 2	Muñoz	Rice-rice	SCMM2 RR	*Rhizoctonia solani strain AG 1-IA isolate XS5-01*	100	MG397062.1
Magtanggol 3	Muñoz	Rice-rice	SCMM3 RR	*Rhizoctonia solani isolate SVPRS18*	95	KU933591.1
Magtanggol 1	Bongabon	Rice-onion	BONGM1 RO	*Rhizoctonia solani strain AG 1-IA isolate YJWC2-01*	96	MG397065.1
Magtanggol 2	Bongabon	Rice-onion	BONGM2 RO	*Rhizoctonia solani strain HRBhw*	93	KF959720.1
Magtanggol 3	Bongabon	Rice-onion	BONGM3 RO	*Ceratorhiza oryzae-sativae strain CBS 439.80*	99	MH861282.1
Magtanggol 4	Bongabon	Rice-onion	BONGM4 RO	*Rhizoctonia oryzae isolate VC 241*	98	KT362102.1
Palo Maria 1	Bongabon	Rice-onion	BONGPM1 RO	*Rhizoctonia solani isolate Gx-46*	90	EU152869.1
Palo Maria 2	Bongabon	Rice-onion	BONGPM2 RO	*Rhizoctonia solani isolate RMPM23*	98	JF701749.1
Palo Maria 3	Bongabon	Rice-onion	BONGPM3 RO	*Rhizoctonia solani strain RRS9*	81	KF570303.1
Pesa 1	Bongabon	Rice-onion	BONGP1 RO	*Rhizoctonia solani AG-1 IA isolate RKH*	100	KX674524.1
Pesa 2	Bongabon	Rice-onion	BONGP2 RO	*Rhizoctonia solani strain RJUdp*	76	KF959686.1
Pesa 3	Bongabon	Rice-onion	BONGP3 RO	*Rhizoctonia oryzae isolate VC 241*	99	KT362102.1

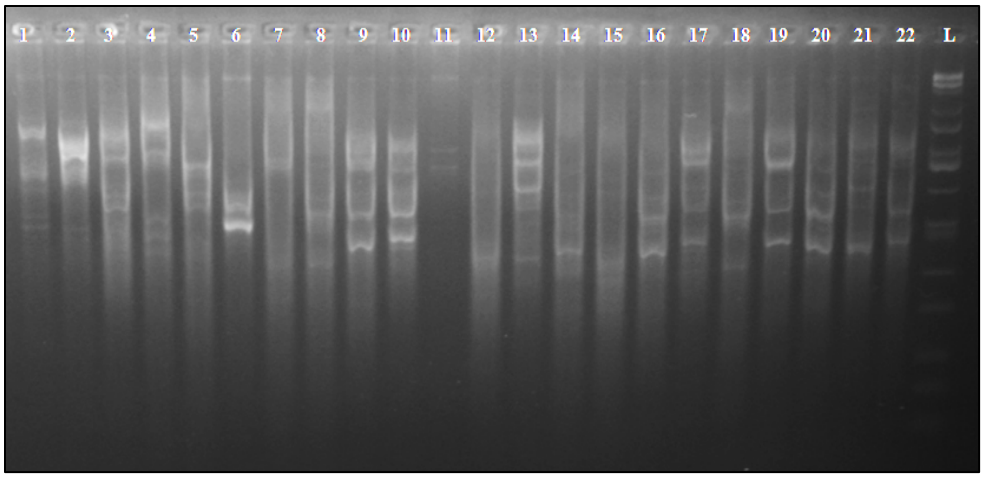

Figure 3. Variable number of tandem repeat fingerprinting (VNTR) patterns of total genomic DNA of *R. solani* isolates with primer RY. 1kb ladder; Lanes 1 to 22 (isolates)

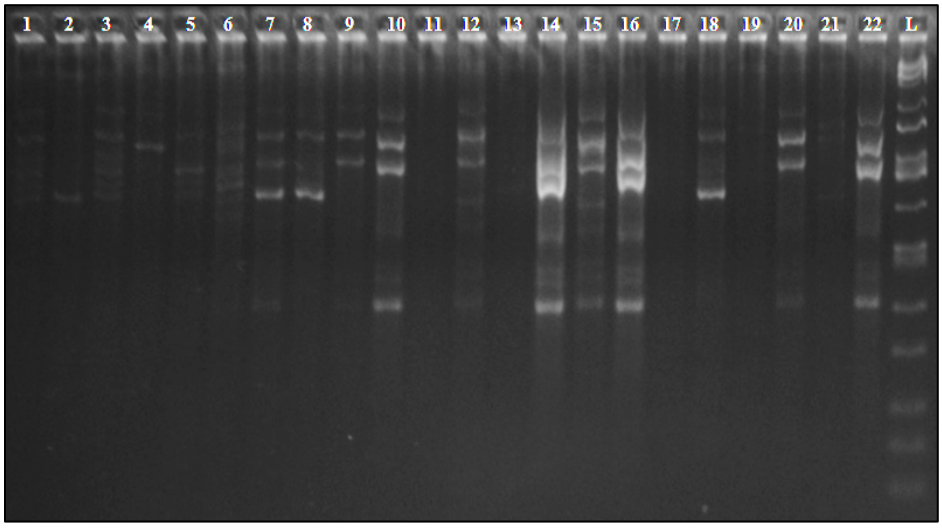

Figure 4. Variable number of tandem repeat (VNTR) fingerprinting patterns of total genomic DNA of *R. solani* isolates with primer GF. 1kb ladder; Lanes 1 to 22 (isolates)

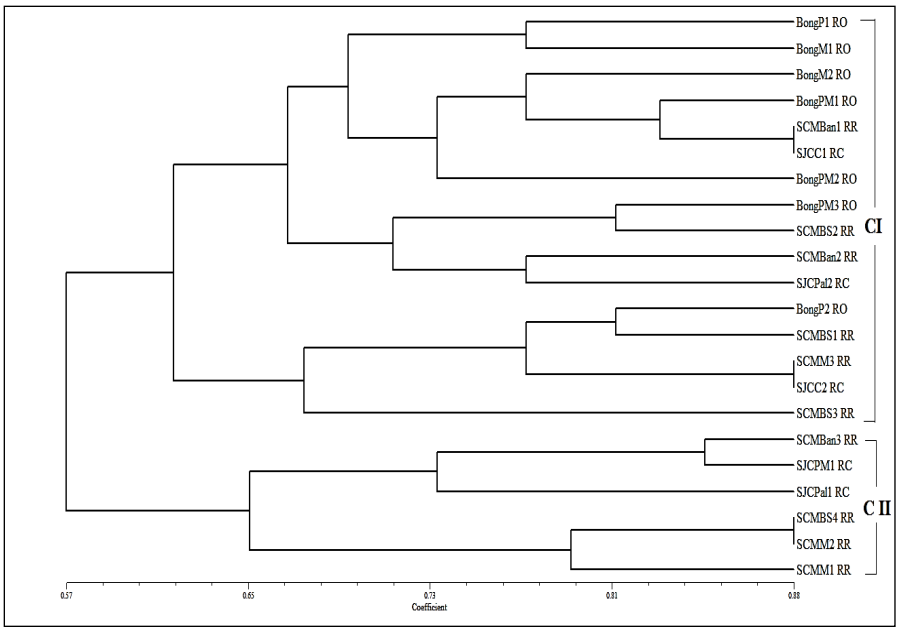

Figure 5. Un-weighted pair group method with arithmetic mean (UPGMA) dendrogram of *R. solani* constructed with NTSYSpc 2.0 using cluster analysis based on presence and absence of bands

Pathogenicity test

The evaluation of 22 isolates of *R. solani* was carried out on susceptible rice cultivar (TN-1) under screen house condition. Nine tillers for every sample replication were observed. All isolates of *R. solani* produced lesion on leaf, sheaths and stems after inoculation on susceptible rice. Typical symptoms were observed showing irregular shape dark brown spot in the rice plants (Figure 6).

Disease severity at 7 DAI showed that the isolate with the highest mean percentage was SCMBS3 (32.22%) while seven isolates namely: SJCPAL1 RC, SCMM1 RR, SCMM2 RR, SCMBAN1 RR, SCMBAN2 RR and SCMBAN4 RR did not show any lesions (Figure 7). However, at 14 DAI, all isolates were capable of infecting the susceptible rice variety and showed more severe symptoms. The highest mean was observed at SJCC 1 (82.22%) where necrosis across the entire leaf section was evident (Figure 8). Whereas the lowest mean was SJCPAL2 RC (21.11%) which indicates the least aggressive isolate. On 21 DAI, only three out of 22 isolates namely SJCC1 RC, SCMBS1 RR, and SCMBS2 RR showed 100% aggressiveness that resulted to leaf and sheath death. Overall, no significant differences were observed among 22 isolates at 7, 14, and 21 DAI.

Figure 6. Rice plant infected by rice sheath blight disease: (A) base sheath, (B) leaf and (C) leaf sheath

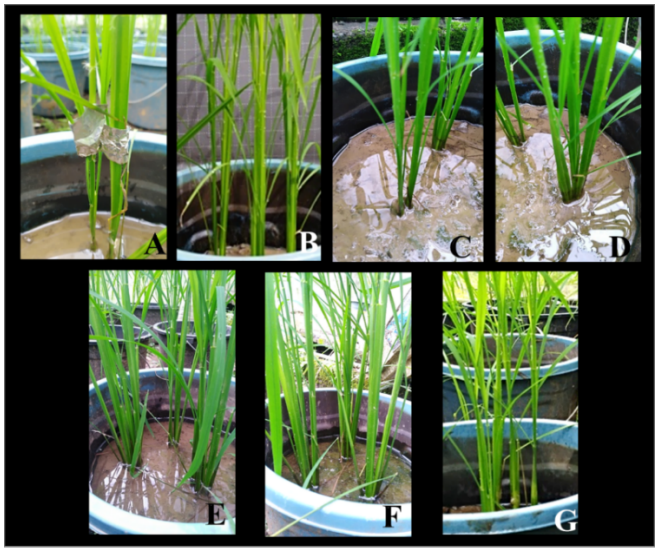

Figure 7. Isolates that did not show symptoms 7 days after inoculation. (A) SJCPAL 1 RC, (B) SJCPAL 2 RR, (C) SJCM 1 RR, (D) SCMM 2 RR, (E) SCMBAN 1 RR, (F) SCMBAN 2 RR, and (G) SCMBS 4 RR

Similarly, no significant differences were observed in relative lesion height and disease incidence at 7, 14, and 21 DAI on plants inoculated with the isolates. As shown in Table 7, SCMBS3 RR (8.31%) has the highest mean relative lesion height at 7 DAI, while seven isolates did not show any symptoms. At 14 DAI, SCMM3 RR (29.50%) had the highest mean and the lowest was SCMM2 RR (3.11%). On the other hand, SJCC1 RC and SCMM3 RR both obtained the highest relative lesion height which was 34.59% at 21 DAI while SCMM2 RR (3.11%) and SJCPAL1 RC (9.1%) showed the lowest at 14 and 21

DAI, respectively. In terms of disease incidence, SJCC1 RC showed consistency of having the highest mean at all observation day. Although seven isolates did not affect the tillers at 7DAI, while SJCPAL2 RC obtained the lowest mean at 14 and 21 DAI with 5.56% and 55.56%, respectively.

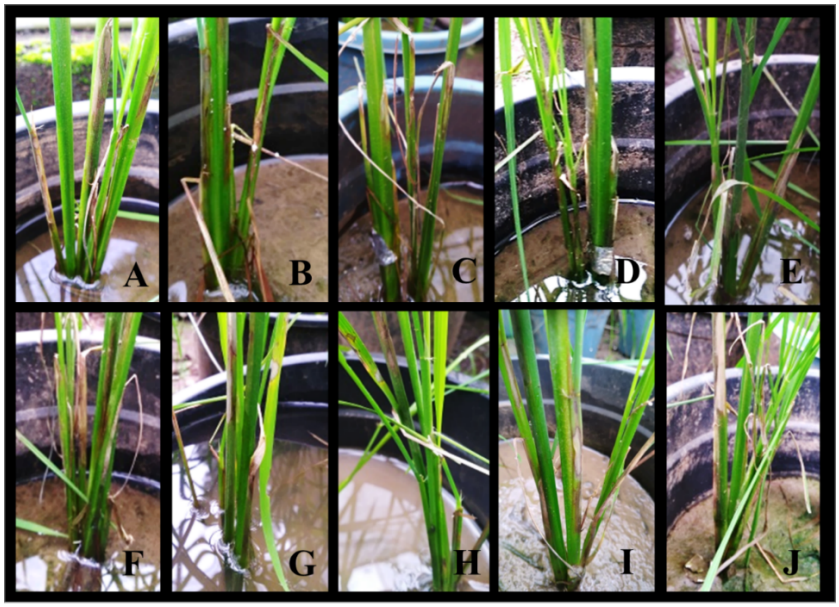

Figure 8. Isolates that showed 80-100% infection 14 and 21days after inoculation. (A) SJCC 1 RC, (B) SCMBS 1 RR, (C) SCMBS 2 RR, (D) SJCPM 1 RC, (E) SCMM 3 RR, (F) SCMBAN 1 RR, (G) SCMBAN 3 RR, (H) SCMBS 3 RR, (I) BONGP 1 RO, (J) BONGP 2 RO

Discussion

Rice sheath blight was always associated with *R. solani*. However, several studies described the association of *R. circinata* var. *zeae, R. circinata* var. *oryzae, R. oryzae-sativae,* and *R. circinata* var. *circinata,* with the rice sheath blight complex in South America. In addition, *R. oryzae-sativae* are capable of inducing RSB symptoms in rice using a cross-pathogenicity assay, albeit with lower intensity than *R. solani* AG1-1A (Molina *et al.,* 2016). Identification of causal organism is essential for selecting and implementing effective management practices. Fungal verification through molecular approach allows the differentiation of the *R. solani* and *R. oryzae* isolates. Species identification through molecular technique offers the most accurate method of determining fungal isolates since identification based on cultural and morphological method often leads to wrong identification.

Table 4. Disease severity, relative lesion height, and disease incidence at 7, 14 and 21 days after inoculation

Isolate code	Disease severity (%) DAI			Relative lesion height (%) DAI			Disease incidence (%) DAI		
	7	14	21	7	14	21	7	14	21
SJCPAL1 RC	0	34.44	64.44	0	4.63	9.1	0	11.11	57.50
SJCPAL2 RC	0	21.11	66.67	0	3.43	10.11	0	5.56	55.56
SJCC1 RC	30	82.22	100	5.54	15.74	34.59	66.67	88.33	100
SJCC2 RC	4.44	37.78	74.44	0.84	4.97	16.46	11.11	11.11	88.33
SJCPM 1 RC	21.11	55.56	87.78	2.96	6.7	21.05	50	50	72.50
SCMM1 RR	0	36.67	67.77	0	5.47	18.71	0	50	74.17
SCMM2 RR	0	23.33	73.33	0	3.11	14.69	0	61.11	79.17
SCMM3 RR	24.44	64.44	94.44	5.5	29.50	34.59	38.89	38.89	76.67
SCMBAN1 RR	0	73.33	92.22	0	13.38	27.90	0	61.11	75.00
SCMBAN2 RR	0	50.00	77.78	0	7.82	27.21	0	50	83.33
SCMBAN3 RR	2.22	38.89	93.33	0.14	6.08	18.07	5.56	50	87.78
SCMBS1 RR	23.33	64.44	78.89	5.64	9.95	32.25	38.89	38.89	74.17
SCMBS2 RR	30	53.33	86.67	4.33	6.91	21.62	61.11	61.11	69.13
SCMBS3 RR	32.22	65.56	86.67	8.31	13.13	28.95	50	50	78.57
SCMBS4 RR	0	43.33	78.89	0	6.26	15.66	0	44.44	57.78
BONGM1 RO	28.89	57.78	78.89	4.64	11.76	19.23	50	50	75.00
BONGM2 RO	21.11	43.33	71.11	4.17	5.80	11.96	38.89	38.89	80.83
BONGPM1 RO	3.33	48.89	78.89	0.35	7.46	18.95	11.11	11.11	74.17
BONGPM2 RO	11.11	45.56	76.67	1.53	6.52	15.12	27.78	27.78	64.80
BONGPM3 RO	22.22	38.89	67.76	4.78	9.06	14.73	38.89	38.89	89.17
BONGP1 RO	21.11	62.22	90.00	4.06	12.55	21.68	44.44	44.44	78.06
BONGP2 RO	17.78	50.00	96.67	2.93	8.78	14.96	27.78	27.78	56.94

According to Parmeter (1970), several researchers have already examined the differences between isolates of *R. solani* AG-1 from various geographic locations. *Rhizoctonia solani* is a well-recognized fungus that consists of many many races, forms or groups of various isolates that differs in pathogenicity, morphology in the culture and/or physiology (Bonman, 1992). However, in the case of Australian (Duncan *et al.,* 1993) and Philippines (Pascual *et al.,* 2000) isolates, genetic relatedness related to origin was reported. Conversely, Toda *et al.* (1999) indicated that the isolated pathogen come from the same geographical origin or host plants were not always genetically related. For example, in this study, SJCPAL 1 and SJCPAL 2 both from rice-corn type of farming system did not fall on the respective subgroups proving the above statement. Clustering analysis of fungal species with small sample size can affect the groupings of *R. solani* isolates. In order to determine more dominant and complex groupings of isolates, larger number of isolate populations must be considered.

Isolates collected from rice sheath blight-infected lesions were genetically different although it showed low level of genetic variation. *R. solani* isolates collected from the same farming system did not necessarily clustered in the same group. It was observed that some isolates with different type of farming systems shared the same sub-groups while some isolates from same type of farming system did not fall under similar sub-groups. The result is due to a wide host range of *R. solani* present in the rice growing areas in the Philippines. This fungus is a soil-borne pathogen which capable of inducing symptoms in rice including AG1-1A and AG 8, which can also severely infect corn and onion under favorable condition. Also, the opportunity of the cross-species infection of the pathogen may be common, and the prevalence of the pathogen could be substantially enhanced by its spatial overlap with alternate hosts (Wang *et al.* 2015) which contributed to the spread of pathogen in rice-based farming systems in Nueva Ecija, Philippines. For instance, *R. solani* AG1-1A causes banded leaf and sheath blight on maize, which is considered an emerging disease problem in rice-corn farming system. *R. solani* from rice and weeds can also infect onion as an alternate host plant (Gonzalez-Vera *et al.,* 2010).

Virulence and aggressiveness of *R. solani* isolates collected from three different farming systems did not significantly differ. Isolates showed high level of aggressiveness on susceptible check based on disease severity index which ranges from 60% to 100%, indicating that all isolates were capable of inducing prominent and severe symptoms at 21 DAI. In this study, the results suggests that aggressiveness and virulence is not correlated with geographical location. As per the findings of Wang *et al*. (2015), the movement of *R. solani* AG1-1A was likely to be arbitrary, caused by various environmental factors and human activities,

such as irrigation water and farm equipment. These could have played a role in the dissemination of the pathogen. The adoption of cropping systems defined by high planting density, frequent applications of nitrogenous fertilizers, and intensive mono-cropping has led to an increase in sheath blight occurrence in recent years in rice-growing countries. These agricultural methods, together with the pathogen's saprophytic nature and broad host range, as well as the planting of susceptible yet high-yielding types, have caused the fungus to spread, establish itself, and survive throughout all rice-producing areas (Singh *et al*, 2002).

The molecular approach showed that isolates from different rice-based farming systems in Nueva Ecija, Philippines were closely related and the pathogenicity test helped to identify the aggressiveness among the isolates. Results indicated that all isolates were highly virulent which were capable of inducing severe symptom on susceptible check and there is no significant difference in the level of aggressiveness among isolates. Since application of fungicides can adversely affect the environment, developing resistant cultivar against this disease could potentially solve the problem, yet no germplasm with high-level resistance has been obtained due to its wide host range. Pathogen diversity information will help researchers to breed effective disease resistant rice variety. However, migration and spread of *R. solani* in a certain field can be lessen by applying cautious disease management such as cleaning of farm equipment, altering and using different method of irrigation system, planting crops that were seldom or not infected by any AGs of *R. solani*. Biological and chemical controls are more effective when used in combination with good cultural practices. Understanding genetic relatedness of *R. solani* AG1-1A isolates from different type of farming system will enhance more effective disease management techniques.

Acknowledgements

This study will not be possible without the generous support of the Department of Agriculture-Biotechnology Scholarship Program and the Philippine Rice Research Institute.

References

Bhunkal, N. (2014). Studies on sheath blight of rice (*Oryza sativa* L.) caused by Rhizoctonia solani Kühn. College of Agriculture CCS Haryana Agricultural University Hisar – 125 004, Haryana, India.

Bonman, J. M. (1992). Durable Resistance to Rice Blast Disease: Environment Influences. Euphytica. 63:115-123.

Cubeta, M. A. and Vilgalys, R. (1997). Population Biology of the Rhizoctonia Solani Complex. Population Genetics of Soilborne Fungal Plant Pathogens, 87:480-84. The American Phytopathological Society. Web. 4 Oct. 2011. The American Phytopathological Society.

Duncan, S., Barton, J. E., and O'Brien, P. A. (1993). Analysis of variation in isolates of Rhizoctonia solani by random amplified polymorphic DNA assay. Mycological Research, 97:1075-1082.
Food and Agriculture Organization [FAO] (2008). Species description *Oryza sativa* L. Date Retrieved from http://www.fao.org/ag/AGP/AGPC/doc/ GBASE/data/pf000274.htm
Gonzalez-Vera, A. D., Bernardes-de-Assis, J., Zala, M., McDonald, B. A., Correa-Victoria, F., Graterol-Matute, E. J. and Ceresini, P. C. (2010). Divergence between sympatric rice-and maize-infecting populations of *Rhizoctonia solani* AG-1 IA from Latin America. Phytopathology, 100:172-182.
International Rice Research Institute [IRRI]. (2017). Rice Knowledge Bank. Rice Sheath Blight Disease Symptoms. Retrieved from http://www.knowledgebank.irri.org/training/ factsheets/pestmanagement/diseases/item/sheath-blight.
Kashyap, P. L., Rai, P., Kumar, S., Chakdar, S. and Srivastava, A. (2017). DNA Barcoding for Diagnosis and Monitoring of Fungal Plant Pathogens. Molecular Markers in Mycology (pp.87-122). DOI:10.1007/978-3-319-34106-4_5
Molina, L. M. R., MESA, E. C., Pereira, D. A. S., Herrera, M. R. S. and Ceresini, P. C. (2016). *Rhizoctonia solani* AG-1 IA infects both rice and signalgrass in the Colombian Llanos. Pesquisa Agropecuária Tropical, 46:65-71.
Mian, M. S., Stevens, C. and Mia, M. A. T. (2003). Diversity of the rice blast pathogen *Pyricularia grisea* from Bangladesh analysed by DNA fingerprinting. Bangladesh Journal of Plant Pathology, 19:81-85.
Pan, X. B., Rush, M. C., Sha, X. Y., Xie, Q. J., Linscombe, S. D., Stetina, S. R. and Oard, J. H. (1999). Major gene, nonallelic sheath blight resistance from the rice cultivars Jasmine 85 and Teqing. Crop Science, 39:338-346.
Parmeter, J. R. (1970). *Rhizoctonia solani*, Biology and Pathology. London, UK: University of California, University of California Biology and Pathology. (http://eol.org/pages/11446730/details).
Pascual, C. B., Toda, T., Raymondo, A. D. and Hyakumachi, M. (2000). Characterization by conventional techniques and PCR of *Rhizoctonia solani* isolates causing banded leaf sheath blight in maize. Plant Pathology, 49:108-118.
Peever, T. L., Zeigler, R. S., Dorrance, A. E., Correa-Victoria, F. J. and Martin, S. S. (2022). Virulence Variation in Pathogen Populations. Phytopathology News.
Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4:406-425.
Shu, C. W., Zou, C. J., Chen, J. L., Tang, F., Yi, R. H. and Zhou, E. R. (2014). Genetic diversity and population structure of Rhizoctonia solani AG-1 IA, the causal agent of rice sheath blight, in South China, Canadian Journal of Plant Pathology, 36:179-186.
Singh, V., Singh, U. S., Singh, K. P., Singh, M, Kumar, A. (2002). Genetic diversity of *Rhizoctonia solani* isolated from rice: Differentiation by morphological characteristics, pathogenecity, anastomosis behaviour and RAPD fingerprinting. J Mycol Plant Pathol, 32:332-344.
Toda, T., Hyakumachi, M., Suga, H., Kageyama, K., Tanaka, A. and Tani, T. (1999). Differentiation of Rhizoctonia AG-D isolates from turfgrass into subgroups I and II based on rDNA and RAPD analyses. European Journal of Plant Pathology, 105:835-846.
Wang, L. L., Liu, L. M., Hou, Y. X., Li, L. and Huang, S. W. (2015). Pathotypic and genetic diversity in the population of Rhizoctonia solani AG 1-IA causing rice sheath blight in China. Plant Pathology, 64:718-728.
Wu, W., Huang, J., Cui, K., Nie, L. and Wang, Q. (2012). Sheath blight reduces stem breaking resistance and increases lodging susceptibility of rice plants. Field Crops Research, 128: 101-108.
Zakiah, R. (2016). Morphological and Genetical Variability among *Rhizoctonia solani* Isolates Causing Sheath Blight Disease of Rice. Science Direct, 23:42-50.

(Received: 24 March 2023, Revised: 17 January 2024, Accepted: 17 March 2024)