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Occurrence and Characterization of *Rhizoctonia* Spp., Causal Agents of Sheath Diseases Isolated from Rice in Lao PDR

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Sheath diseases of rice are major constraints to rice production in Asia. Collection of sheath disease samples were taken during the dry season (January-May), 2015 and the rainy season (June-November), 2016 from several fields in each of two provinces of Lao PDR. The pathogens were isolated and characterized on morphology, pathogenicity and molecular identification. Nineteen isolates of *Rhizoctonia* spp. were studied and found that seven isolates were *R. solani* and twelve isolates were *R. oryzae*. The *R. solani* mycelia on PDA were light brown to brown, sclerotia brown in color, globose to irregular shape. On other hand, *R. oryzae* mycelia on PDA were brown and orange brown, sclerotia were globose to irregular shape, dark brown or light-yellow color. Six isolates of *Rhizoctonia* with different characteristics were selected to identify using molecular technique. Molecular identification was carried out by PCR technique using primers, ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTT ATTGATATGC-3') to amplify PCR products of ITS1-5.8S-ITS2 region of rRNA gene. The PCR products were about 750 base pairs and subjected to base sequence comparison in GenBank database. The result showed that isolates, SMN1-5, SMC1-2 and VNN were *R. solani*, while isolates VHN1-3, SMN1-7 and VHN were *R. oryzae*. Result on pathogenicity evaluation revealed that, the isolate VSV1-2 of *R. oryzae* which inoculated on a sheath produced the largest average lesion area of 303 mm^2, compared to isolate SMN1-5 of *R. solani* with a result lesion area of 191 mm^2. All 19 isolates were pathogenic to rice cultivar (Thadokham 8). The percent study indicates that the *R. solani* can invade both sheath and leaf of rice cultivar (Thadokham 8), however, *R. oryzae* invaded only on sheath. In addition, the occurrence and characterization of *R. oryzae* are reported for the first time in Lao PDR.

Keywords: Occurrence, characterization, *Rhizoctonia*, sheath diseases, rice, Lao PDR.

Sheath diseases caused by *Rhizoctonia solani*, *Rhizoctonia oryzae* and *R. oryzae-sativae* (Common names sheath blight, sheath spot, and aggregate sheath spot, respectively). Having a wide host range, sheath diseases affect many other crops as well as rice[1]. They cause significantly yield losses in many countries[2]. Among them, *R. solani* AG1 IA the causal agent of sheath blight causes the most serious damage[3]. *Rhizoctonia* spp. are soil-borne fungi. They may survive for long periods and over several seasons as sclerotia or mycelia in the soil or in crop debris and spread by water, contaminated tools or plant parts[4]. When conditions are favorable to the pathogen, all the leaves may be blighted and the whole plant die finally. The symptoms of sheath disease caused by *R. oryzae* and *R. oryzae-sativae* are lesions on the leaf sheath very similar to those of *R. solani*,

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with which they can be easily confused[5]. Diagnosis of these diseases by visual observation is extremely difficult and often inaccurate, particularly at the early stages of lesion development[6]. Morphological identification of mycelia is based on *R. oryzae* forming orange to brown, globose sclerotia up to 2 mm in diameter[7], *R. solani* having mycelia which are colorless when young, becoming yellowish brown when older with sclerotia which are superficial, more or less globose but flattened below, color brown to dark brown and measure up to 5mm in diameter[2]. Other means of identification have included testing anastomosis, pectic zymograms, or fatty acids. Recently, molecular techniques have become important tool for fungal identification. Species and sub-species of *Rhizoctonia* have been distinguished successfully by the internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA). This information has been used to generate specific primers for differentiating the closely-related species[5,6]. Up to now there is no reports on the details of sheath diseases of rice and their causal agents in Lao PDR. Therefore, this present study aims to survey and characterize the species of *Rhizoctonia* causal agent of rice sheath diseases in Lao PDR. This leads to the essential information for the strategy of management and control to the diseases needed to improve rice production in Lao PDR.

MATERIALS AND METHODS

Survey and collection of samples

Samples were collected obviously infected rice plants from visual inspection, during the dry season (January-May), 2015 and the rainy season (June-November), 2016 from several fields in each of 2 provinces of the Lao PDR. One province (Sayaboury) is in the north and one (Vientiane Capital) is in the central part of the country.

Isolation of fungi

An elliptical or oval shape of lesions, with a grayish-green center and brown margin[2] were collected for pure isolation in the laboratory. Leaf lesions were cut into five pieces using a sterile scalpel blade and soaked in 0.5% sodium hypochlorite for 3-5 min, then washed twice in a sterilized distilled water. The leaf materials were dried on sterilized filter paper and placed on potato dextrose agar (PDA) in 9 cm diameter of Petri dishes containing 1g/l streptomycin sulfate. After 48–72 hours of incubation at room temperature (25-30°C), hyphae from the margin of each developing colony were transferred into new dishes[8,9]. The pure cultures were identified base on morphology characteristic of mycelia, hyphae size, color of sclerotia, shape and size, etc., under microscope.

Morphological identification

Morphological identification of fungus species isolate from rice disease was used the slide culture technique. Purified fungal colonies from each isolate were cut by cork borer size about 5 mm and transferred onto to a glass microscope slide with a cover. The sample was incubated at room temperature for 5 days then were examined using light microscope for characteristics of them. The mycelial growth were measured on a compound microscope at 40X magnification. The main hyphal diameter was estimated by measuring 10 main hyphae perpendicular to the longitudinal cell wall. The color, size and shape of sclerotia were measured 10 sclerotia under a dissecting microscope [7,10].

Pathogenicity test

Pathogenicity test of nineteen fungal isolates were evaluated by artificial inoculations with sheath and leaf.

Sheath inoculation

When the rice plants were ten-week-old rice plants (Thadokham 8 variety), a five-day-old, mycelial plug was placed on the rice stem 1 cm below the axil of fully mature leaf and wrapped with parafilm[11]. The symptoms were observed and collected data after seven days of inoculation. Then re-isolation the lesion on sheath was done by followed steps to perform Koch's postulates. In this experiment was set up in completely randomized design (CRD), with 20 treatments and 4 replications. The variance was analyzed and mean comparison was done using Least significant difference (LSD).

Detached leaf inoculation

Four leaves from 10-week-old rice plants were cut into 16-cm-long segments with scissors, and each was placed in a plastic chamber filled bottom with on filter paper moistened with sterilized water. A mycelium plug was then placed on the surface of each leaf near the middle of the leaf segment. As a control treatment, leaf segments from the same cultivar were inoculated with a PDA plug

without mycelia. All leaves were incubated under ambient laboratory conditions. Every 3 days, they were observed and the length of diseased lesions were measured. Disease reactions to *R. solani* isolates were determined, based on the lesion length as resistant (R, <30 mm), moderately resistant (MR, 30-50 mm), moderately susceptible (MS, 51-66 mm), and susceptible (S, <65 mm).[12] Statistic analysis was similar as described previously.

Molecular identification

Identification of *Rhizoctonia* samples based on analysis of ITS1-5.8S-ITS2 rDNA. Six isolates were selected from two groups of *Rhizoctonia* samples based on morphology characteristics and pathogenicity. The fungal was grown into potato dextrose broth and incubated on a shaker at 26 ¯C for 7 days. The mycelia were harvested by filtering through Whatman No. 1 filter paper and freeze-dried. The total genomic DNA extracted using the CTAB method. DNA amplification by Polymerase chain reaction (PCR) using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Amplifications were performed in a total volume of 50 µl using 100 ng of genomic DNA, 0.5 µl of 10 mM deoxynucleotide triphosphate (dNTPs), 2.5 µl of 10X Immo buffer, 1 µl of 50 mM $MgCl_2$, 0.25 µl of 5 unit per µl Immolase Taq DNA polymerase and 2.5 µl of 5 µmol each primer. The mixture was placed into a thermal cycler with the following program: 7 min at 95 ¯C initial denaturation cycles; 1 min at 95 ¯C , 1.30 min at 45 ¯C, 2 min at 72 ¯C , 30 cycles; with a final extension step for 10 min at 72 ¯C. Each PCR product from these amplifications were separated by electrophoresis on 0.8% (w/v) agarose gel mixed with Red safe (TM), and visualized using a UV transilluminator[13]. PCR product were purified and sequenced by Ward Medic, Ltd. The DNA sequences were compared with DNA sequence database of GenBank using nucleotide BLAST program, for sequences aligned using Muscle program.

RESULTS

Morphological characteristics

Nineteen fungal isolates from rice with sheath symptom of *Rhizoctonia* were cultured and divided into two groups based on morphological characteristics. First group consisted of isolates SMN1-7, VSV1-1, VSV1-2, VSV1-3, VHN1-1, VHN1-2, VHN1-3, VHN1-4, SSN1-2, SSD1, VSH1-2, and VSD1 (Table 1). The colonies of this group were white color then becoming yellowish brown and grew slowly on PDA media (Fig. 1 a). The width of hyphae ranges from 4-7 µm, branching at right angle with a slight constriction at the point of branching and presence of a septum (Fig. 1 b). The sclerotia were formed in formless masses arranged in pattern. The size of sclerotia ranged from 0.4-2 mm, globose to irregular shape, rough and found pale brown color (Fig. 1 c). These isolates were identified to *Rhizoctonia oryzae* based on morphological characteristics. The second group was identified to *Rhizoctonia solani* included isolates SMN1-1, SMN1-2, SMN1-4, SMN1-5, SMN1-6, SMC1-2, and VNN (Table 1), which showed rapid growth and brown colony on PDA (Fig. 2 a). Hyphal width ranged from 8 µm to 12 µm, branching near distal septum (Fig. 2 b). Sclerotia were compacted masses of small hyphal cells with thin cell wall. Pigmentation of sclerotia were brown, which changed from white to brown color after formed 30 hours. Sclerotia were globose to irregular shape and flatted below (Fig. 2 c). The texture of sclerotia were rough with sunken and were ranged from 0.86-4 mm.

Pathogenicity test

The pathogenicity tests were performed by inoculation the pathogen on sheath and leaves of Thadokham 8 rice variety. The symptoms developed by 7 of 19 isolates of tested fungi, *R. solani* showing water-soaked oval shape spots. Measurement of sheath lesion was average length of 9.3-38.3 mm. including, the light green center of lesion and surrounded by dark brown margin then the spots were coalesced on sheath. Symptom caused by *R. oryzae* expressed the lesion in oval shape with length 9.3-56 mm, the center showed straw color and surrounded with a reddish-brown border. Statistical analysis was significant in comparison among isolates. The symptom caused by isolate VSV1-2 of *R. oryzae* was larger lesion on sheath average 56 mm and lesion area 303 mm^2 compared to isolate SMN1-5 of *R. solani* with a result lesion area of 191 mm^2. In addition, on the number of infected lesion per hill of isolates from *R. solani* was more than infection from isolate *R.*

Table 1. Identification fungal isolates of sheath diseases based on morphological characteristics on PDA media within 14 days

Isolate	Hyphal Diameter (µm)	Colony color	Sclerotium					Identification
			Diameter (mm)	Shape	Color	Distribution	Conglomeration	
SMN 1-7	4.27-5.42	Light brown	0.38 - 0.93	Globose-irregular	Dark brown-gray	Coalescence	Frequently	*Rhizoctonia oryzae*
VSV 1-1	3.97-5.44	Brown	0.51 - 0.88	Globose-irregular	Dark brown	Coalescence	Frequently	*Rhizoctonia oryzae*
VSV 1-2	4.1-6.09	Light brown	0.58-1.09	Globose-irregular	Brown	Concentrate ring	Frequently	*Rhizoctonia oryzae*
VSV 1-3	4.03-5.62	Brown	0.5 - 0.96	Globose-irregular	Brown	Concentrate ring	Frequently	*Rhizoctonia oryzae*
VHN 1-1	4.45-5.92	Brown	0.39 - 1.00	Globose-irregular	Dark brown-gray	Concentrate ring	Frequently	*Rhizoctonia oryzae*
VHN 1-2	3.72-6.96	Orange brown	0.89-1.84	Globose	Dark brown-gray	Coalescence	Frequently	*Rhizoctonia oryzae*
VHN 1-3	4.27-5.77	Light brown	0.97-1.69	Globose	Pale brown	Coalescence	Frequently	*Rhizoctonia oryzae*
VHN	4.21-6.84	Brown	0.48 - 1.14	Globose	Dark brown	Concentrate ring	Frequently	*Rhizoctonia oryzae*
SSN 1-2	4.21-6.24	Orange brown	0.49-1.12	Globose	Dark brown	Scattered	Frequently	*Rhizoctonia oryzae*
SSD 1	4.08-6.43	Brown	0.56 - 1.35	Globose	Dark brown	Coalescence	Frequently	*Rhizoctonia oryzae*
VSH 1-2	4.04-6.22	Brown	0.44 - 0.85	Globose-irregular	Brown	Coalescence	Frequently	*Rhizoctonia oryzae*
VSD 1	4.8-6.83	Brown	0.51 - 1.11	Globose	Brown	Coalescence	Frequently	*Rhizoctonia oryzae*
SMN1-1	8.02 - 11.71	Light brown	1.25 - 3.36	Globose-irregular	Brown	Scattered	Rare	*Rhizoctonia solani*
SMN1-2	6.69-9.41	Brown	1.5- 4.2	Globose	Brown	Scattered	Rare	*Rhizoctonia solani*
SMN1-4	6.47 - 9.81	Light brown	0.86 - 4.00	Globose-irregular	Brown	Scattered	Rare	*Rhizoctonia solani*
SMN1-5	6.64 - 10.76	Brown	1.25 - 3.82	Globose-irregular	Brown	Scattered	Rare	*Rhizoctonia solani*
SMN1-6	6.32 - 9.15	Brown	1.23 - 4.04	Globose	Brown	Scattered	Rare	*Rhizoctonia solani*
SMC1-2	6.65-10.25	Brown	1.45 - 2.36	Globose	Brown	Scattered	Rare	*Rhizoctonia solani*
VNN	6.74 - 8.79	Brown	1.11 - 3.94	Globose	Brown	Scattered	Rare	*Rhizoctonia solani*

Table 2. Length, lesion area, number of tillers and infected tillers including height of rice Thadokham 8 cultivar inoculated with different isolates of *Rhizoctonia* spp. on sheath

Rhizoctonia spp.	Rice cultivar Thadokham 8				
Isolates	Lesion (mm)	Lesion area (mm^2)	No. infected tiller	No. of Tiller	Plant height (cm)
VHN1-2	35 bc	171.67 bcd	1.66 cde	26.6 cd	107 a
VSD1	25 bcd	138.3 bcd	1.66 cde	3 bc	86 gh
VHN1-1	34 bc	198.3 ab	2.33 cd	3 bc	100.33 abcd
VSV1-3	21 bcd	102.6 bcd	1 ef	2.66 cd	97.33 bcde
VSV1-2	56.6 a	303.3 a	2 cde	3 bc	105 ab
SSD1	10 de	26.6 d	1 ef	3 bc	90.33 efg
SSN1-2	16.6 cde	83.3 cd	1.33 de	3 bc	86.67 fgh
VSH1-2	33 bc	180 bc	1.33 de	3 bc	106.67 a
VHN1-3	30 bc	150 bc	2.33 cd	3.33 b	92.67 defg
VHN	26 bcd	101.3 bcd	1 ef	4 a	95.69 cdef
VSV1-1	9.3 de	28 d	1 ef	3 bc	95 cdefg
SMN1-7	21.6 bcd	108.3 bcd	1.66 bc	3 bc	107.67 a
SMN1-1	25 bcd	105 bcd	3.66 ab	3 bc	100.33 abcd
SMN1-5	38.3 ab	191.6 abc	3.66 ab	2.66 cd	96.67 bcde
SMN1-2	25 bcd	125 bc	4 a	2.33 d	103 abc
SMN1-6	16.6 cde	83.3 cd	4 a	3 bc	101 abcd
VNN	31.6 bc	148.3 bc	4 a	3 bc	86.33 gh
SMN1-4	19.3 bcd	86 bcd	4.66 a	3 bc	108 a
SMC1-2	9.3 de	28 d	2.66 bc	2. 66 cd	90 efg
Control	0 e	0 f	0 f	3 bc	76.67 h
F-test	**	**	**	*	**
C.V. (%)	18.79	18.15	12.27	5.83	5.67

** Significant difference at P <0.01 by LSD
* Significant difference at P <0.05 by LSD
Means followed by the same letter (s) in a column are not significantly different (P <0.05 by LSD).

Table 3. Lesion length, and disease reaction of rice leaf (Thadokham 8) inoculated with different isolates of *Rhizoctonia solani*

Isolates	Lesion length (mm)	Disease reaction
Rhizoctonia solani (SMN1-1)	45	MR
Rhizoctonia solani (SMN1-2)	30.6	MR
Rhizoctonia solani (SMN1-4)	29.3	R
Rhizoctonia solani (SMN1-5)	35	MR
Rhizoctonia solani (SMC1-2)	38.3	MR
Rhizoctonia solani (VNN)	32.3	MR
Rhizoctonia solani (SMN1-6)	28.3	R
F-test	NS	
C.V. (%)	21.88	

NS = non significance
Resistance (R, <30 mm)
Moderately resistant (MR, 30-50 mm)
Moderately susceptible (MS, 51-65 mm)
Susceptible (S, >65 mm)

oryzae. Average 4 lesions per hill was significantly difference in statistic at P<0.05. However, isolates from *R. oryzae* were not infected on leaves (Table 2).

In contrast on leaf, only isolates from of *R. solani,* which tested on leaves, the symptoms were irregular-shape, gray-green in the center, then cover by white abundant mycelia and white sclerotia then turned to brown color similar to sclerotia on the PDA. Measurement of the lesion revealed that the isolate SMN1-1 caused lesion length 45 mm larger than caused by other 6 isolates. On disease reactions, 5 isolates (SMN1-1, SMN1-2, SMN1-5, SMC1-2, VNN) of *R. solani* gave rise to rice plants responded to the pathogens as moderately resistant, 2 isolates (SMN1-4, SMN1-6) as resistance (Table 3). Nevertheless, the lesion length of rice leaves in comparison among isolates of *R. solani* was not statistically different.

Table 4. Similarity of six selected isolates of *Rhizoctonia* compared to sequence data of GenBank accession number

Isolates	Morphological identification	Geographic origin	Similarity	GenBank accession number	Identity (%)
SMN1-5	*R. solani*	Sayabouly province	*R. solani* strain RSR8	KF570302	98
SMC1-2	*R. solani*	Sayabouly province	*R. solani* strain AG1-1AKA	KJ577141	99
VNN	*R. solani*	Vientiane capital	*R. solani* isolate RASC8	JF701711	97
VHN1-3	*R. oryzae*	Vientiane capital	*R. oryzae* isolate VC241	KT362102	99
SMN1-7	*R. oryzae*	Sayabouly province	*R. oryzae* isolate VC241	KT362102	99
VHN	*R. oryzae*	Vientiane capital	*R. oryzae* isolate VC51	KT362129	99

Molecular identification

Rhizoctonia isolates were divided previously into 2 groups based on morphological characteristics. Six isolates of *Rhizoctonia* were selected to identify by molecular technique for more accurately confirm. The PCR amplification of internal transcribe spacer (ITS1-5.8S-ITS2) of rDNA region, the fragments obtained were about 750 base pairs for all isolates (Fig. 3). All sequences were subjected to compare base sequences in GenBank database using BLAST program. The result showed that; isolates SMN1-5, SMC1-2 and VNN were similar to *R. solani* strain RSR 8 (KF570302) with 98% identity, *R. solani* strain AG1-1AKA (KJ577141) with 99% identity and *R. solani* isolates RASC8 (JF701711) with 97% identity, respectively (Table 4). The DNA sequences of *Rhizoctonia* isolate VHN1-3 was found similar to *R. oryzae* isolate VC 241 (KT362102) with 99% identity. The sequence data of *Rhizoctonia* isolates SMN1-7 and VHN had 99% similarity to *R. oryzae* isolate VC241 (KT362102) and *R. oryzae* isolate VC51 (KT 362129), respectively (Table 4). DNA sequences of ITS-5.8S-ITS2 of individual isolate from *Rhizoctonia* six isolates aligned with DNA sequences from database of GenBank.

DISCUSSION

The causal agents of the disease were isolated and found that among the 19 isolates, they were divided into 2 groups of characteristics with the difference on color of mycelia on culture media and sclerotia. For the first group, they belonged to

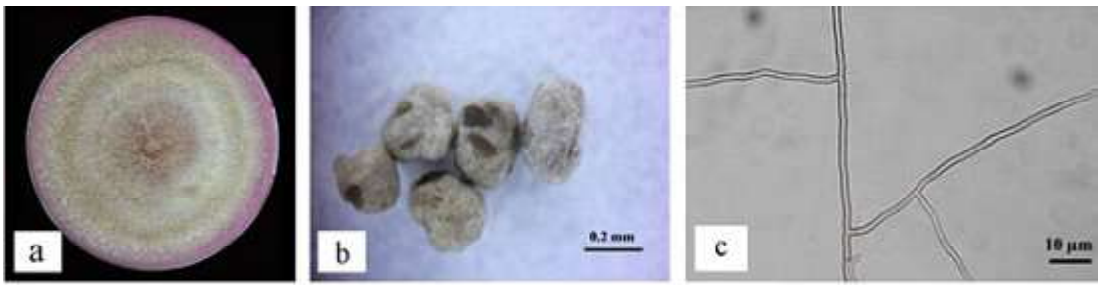

Fig. 1. Morphology characterize of *R. oryzae* (a) Culture on PDA, (b) Sclerotia (c) Mycelia

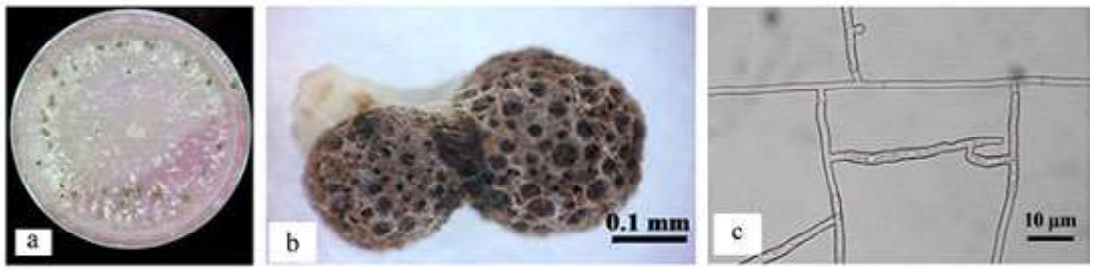

Fig 2. Morphology characterize of *R. solani* (a) Culture on PDA, (b) Sclerotia, (c) Mycelia

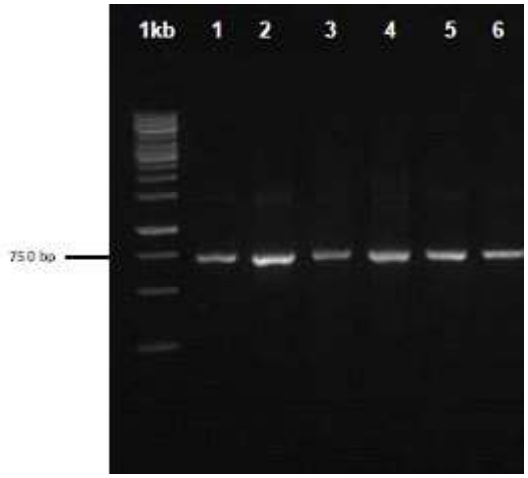

Fig. 3. PCR products of ITS1-5.8S-ITS2 of rDNA from six isolates of *Rhizoctonia* using primer ITS5 and ITS4, SMN1-5, SMC1-2, VNN, VHN1-3, SMN1-7, VHN (Lanes 1-6, respectively), DNA marker 1 Kb DNA ladder plus

R. solani, which mycelia colourless, turn to brown[14]. Furthermore, the mycelia of these species grew very fast to cover on lid of the PDA plate within 48 hours at the 27 $^{-}$C[15]. In the previous study, it was reported that the optimum temperature range was 28-31 $^{-}$C[2]. Furthermore, *R. solani* colony color varied from brown to dark brown and sclerotia brown to dark brown[1], sclerotical shape was globose to subglobose (round to flat on one side), large (1-4 mm). It is correlated in this study that the sclerotia isolated from paddy field in Lao PDR were brown color, global shape and flat on one side, large 0.86-4 mm in diameter. *R. solani* grew on PDA white to light tan when young, but after 3 weeks ranged from brown to dark brown with concentric rings of dark and light mycelium. Sclerotia were few to many, 0.5 to 2.0 mm in size. Individual sclerotia often coalesced into large clumps. Mature sclerotia were tan to light brown and scattered randomly over the agar surface and hypha 7.28 – 8.74 µm[16], which correlated with this study that hyphae measured in diameter were 8-12 µm. The sclerotia of *R. oryzae* on PDA were formed masses and irregular to globes[3]. The result in this study revealed the hyphae in diameter 4-7 µm, sclerotia globose to irregular in shape and often produced in formless masses arranged in a pattern. Sclerotial diameter were 0.4-2 mm, white when young turn to pale brown color. The most obvious differences between *R. oryzae* and *R. solani* are found in culture. The sclerotial masses of *R. oryzae* are indefinite size and shape, but is generally a color shade of salmon. Normally, the sclerotia of *R. solani* are large and round and more regular in shape and generally brown and mycelium is superficial in culture 6-10 µm in width[17]. The information obtained from this present study, at least provided the morphological characteristics of hypha and sclerotia of both *R. solani* and *R. oryzae* in Lao PDR.

Interestingly, we found in this study that the larger lesion area sheath was obtained from VSV1-2 isolate of *R. oryzae*. As well, *R. oryzae* was weakly virulent on rice and lesion remain limited to the outer rice sheaths and the fungus does not invade to the culm[15]. Besides, the virulence of some *Rhizoctonia* groups can be changed in response to temperature[7]. As well as that *R. oryzae* was mildly virulent to avirulent at 10 °C and moderately virulent at 20 °C. Conversely, *R. solani* AG-8 was highly virulent at 10 °C, but mildly virulent at 20 °C[18]. Furthermore, the rice variety cultivars was resulted in resistant or susceptible to sheath disease[19]. The isolates of *Rhizoctonia* spp. collected from Lao PDR were mostly pathogenic to rice Thadokham 8 variety (a popular sticky rice improved in LPDR). This indicated the diversification of the sheath disease in Lao PDR, and the study on it is needed for disease control.

Molecular detection techniques are accurate results rapidly enough to be useful for disease management decisions. PCR with specific primer has been used for detection and identification of pathogenic fungi, and the internal transcribed spacer (ITS) regions of the rDNA have been successfully used to generate specific primer capable of differentiating many closely related fungal species[20]. So, the result on molecular of this study can be one more confirm to characterized of *Rhizoctonia* into 2 spices as *R. solani* and *R. oryzae*. By using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTT ATTGATATGC-3') amplification of ITS1-5.8S-ITS2 region of rDNA. The ITS1-5.8S-ITS2 region was amplified with primers ITS1 and ITS4 then a BLAST search of the NCBI GenBank database with the only sequence generated gave 98% similarity to *Rhizoctonia solani* AG2-3[21]. Other, amplification of the nuclear

rDNA ITS region including the 5.8S rDNA was performed with primer ITS4 and ITS5 shown the result in *R. solani* was similarity in nucleotide sequence 97% (NCBI GenBank)[22]. Furthermore, amplification of the 5.8S ribosomal DNA and part of the ITS regions, using the designed primers in combination with the general fungal primers ITS1F and ITS4B can be identification *R. solani* species complex[23]. Including, Sequencing of the IST (internal transcribed spacer) region of ribosomal DNA showed that isolates were closely related 99-100% sequence of *R. solani* [24].

CONCLUSION

In this study, based on morphological characteristics the *Rhizoctonia* infected rice in Lao PDR was divide into 2 groups as of *R. solani* and *R. oryzae,* the similar point in culture mycelia are white mycelium of initiation then changed the color, hypha with septation, including branching at right angle. Obvious morphological differences between *R. solani* and *R. oryzae* found in culture media, the sclerotial masses of *R. oryzae* are of indefinite size and shape, orange to brown. The sclerotia of *R. solani* are large and round and more regular in shape and generally brown and mycelium is superficial. On pathogenicity test all of 19 isolates are pathogenic to rice cultivar Thadokham 8. The difference of 2 groups is that *R. solani* can invade sheath and leaves, however, *R. oryzae* invade only sheath of rice. Moreover, the PCR amplification of ITS1-5.8S-ITS2 of rDNA was performed, the PCR products obtained were about 750 base pairs for all 6 selected isolates. The sequence bases of PCR products were 97-99% similarity compared to the GenBank database. *R. oryzae* is reported for the first time on the occurrence and characterization in Lao PDR.

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