Malays. Appl. Biol. (2019) 48(1): 1–6

In vitro TOLERANCE OF RICE PATHOGEN *Rhizoctonia solani* BY ORCHID MYCORRHIZAL FUNGI ISOLATED FROM ORCHIDS IN BRIS, SETIU WETLANDS

NURUL ALIAA*, SITI NORADILA, A.G., ZAITI, M.Z. and FATIN SYAKIRAH, S.

School of Fundamental Science, Universiti Malaysia Terengganu,
21030 Kuala Nerus, Terengganu, Malaysia
**E-mail: nurul.aliaa@umt.edu.my*

Accepted 16 January 2019, Published online 20 March 2019

ABSTRACT

Rice (*Oryza sativa*) is a major staple food that feeds more than half of the human population in the world. However, rice production faces major loss in yield due to rice diseases including Rice Sheath Blight (RSB), which is caused by a soil-borne fungal pathogen, *Rhizoctonia solani*. Interestingly, the same pathogenic *R. solani* in rice has also been isolated from orchids and is known to form a vital symbiotic relationship, called orchid mycorrhiza. Thus, this study aims at isolating and identifying the orchid mycorrhizal fungi (OMF) present in roots of orchids at beach ridges interspersed with swales (BRIS), Setiu Wetlands and to determine their antagonistic activity towards pathogenic *R. solani* through dual culture bioassay. Mycorrhizal fungi were isolated from the roots of *Phalaenopsis pulcherrima*, *Bromheadia finlaysoniana* and *Dendrobium crumenatum.* Three isolates were identified through morphological identification to be *Rhizoctonia* species namely A, B and C along with one fungal isolate as *Daldinia* species. *In vitro* antagonistic test against pathogenic *R. solani* showed *Rhizoctonia* species (B) displayed the strongest anti-fungal activity with 60.46% of Percentage Inhibition of Radial Growth (PIRG) value followed by *Rhizoctonia* species (C) with 59.9%, *Rhizoctonia* species (A) with 51.02% and *Daldinia* species with 39.80%. This preliminary investigation shows the potential of orchid mycorrhizal fungi to be developed as a biocontrol agent.

Key words: BRIS, orchid, orchid mycorrhiza, *Rhizoctonia solani*

INTRODUCTION

Rice (*Oryza sativa*) is a staple food for more than half of the world population, especially to those who live in Asia. Although rice production is gradually increasing worldwide, paddy farming is still exposed to Rice Sheath Blight (RSB) disease, which is caused by the soil-borne pathogen, *Rhizoctonia solani*. *R. solani* causes wilt and death of plants through the formation of expanding necrotic lesions from infected areas with loss of chlorophyll and stimulated cell death (Taheri & Tarighi, 2011). In Eastern Asia, RSB affects approximately 15 to 20 million ha of irrigated rice and has caused yield losses up to 6 million tons of rice per year (Rice Knowledge Bank, 2017). Remarkably, the same pathogenic *R. solani* in rice has also been isolated from orchids and is known to form a vital symbiotic relationship, called orchid mycorrhiza (Mosquera-Espinosa *et al*., 2013). Mycorrhiza is a symbiotic association that is essential for one or both partner, between fungi and the roots of a living plant, which is responsible for nutrients transfer (Brundrett, 2004). There are classically two types of mycorrhiza; ectomycorrhiza and endomycorrhiza, based on the location where interaction happens in the cell. However, orchid mycorrhiza is exclusively occurring in orchids where it is essential to facilitate seed germination as the seeds of orchids are very minute and contain minimal stored food reserves (Rasmussen, 2009). In this interaction, fungal hyphae grow into host tissues and form specific coiled structures called pelotons within the cortical cells.

Orchids are one of the largest plant families that are able to survive at various environments. There are orchids that live in Sub-Antartic region (*Nematoceras dienemum*), on the mountain (*Disa uniflora*), under the ground (*Rhizantella gardnerii*) and even in harsh environments such as Beach Ridges Interspersed Swales (BRIS) soil in Setiu Wetlands, Terengganu (Jamilah *et al*., 2016). BRIS

* To whom correspondence should be addressed.

is an extreme habitat with low fertility, nutrients and water retention capacity, making it undesirable for agricultural purposes as more than 90% of BRIS is composed of sand (Lah *et al*., 2011). It is thought that this highly adaptive capability of orchids may be due to its interaction with their orchid mycorrhizas.

Thus, this study aims at isolating and identifying the orchid mycorrhizal fungi (OMF) present in roots of orchids in BRIS, Setiu Wetlands, and determine their antagonistic activity towards pathogenic *R. solani* through dual culture bioassay. It is important to identify the fungi that form mycorrhizas with orchids and the specificity of these relationships to understand how orchids interact with their environment (Bougoure *et al.,* 2009). A better understanding of this relationship may provide essential knowledge in overcoming the rice disease thus maintaining the sustainability of rice production in Malaysia.

MATERIALS AND METHODS

Sample Collection

Sample collection was carried out at Setiu Wetlands (BRIS area) as epiphytic and terrestrial orchids have been reported to be present there. Nine plots were selected randomly including both epiphytic (*Dendrobium crumenatum*) and terrestrial orchids (*Phalaenopsis pulcherrima* and *Bromheadia finlaysoniana*).

Isolation and Identification of Orchid Mycorrhizal Fungi

Isolation of OMF was done according to Warcup and Talbot (1967). Root samples were washed under running tap water then cut into 1 cm sections and surface sterilized. The sections were then incubated onto 3.9% (w/v) Potato Dextrose Agar (PDA) with 0.01% (w/v) Penicillin at room temperature of 28-30°C. The isolates were observed daily and subcultured until pure colonies were achieved. The growth rate of the fungi was recorded, the morphology and colour of the colony was also observed.

Identification was done through morphological observation by staining the mycelium with lactophenol cotton-blue solution (Leck, 1999) and Acridine Orange. The isolates were grown under a sterile coverslip on a PDA plate (Mitchell & Britt, 1981). The coverslip together with the fungus was then taken out and stained. After 2 minutes of staining, the coverslip was washed gently with sterilized distilled water and then examined under the fluorescent microscope (Olympus).

In vitro Antagonistic Test against *Rhizoctonia solani*

Dual culture bioassay (Rahman *et al.,* 2009) was used to evaluate *in vitro* antagonistic activity of OMF against the pathogenic fungus, *R. solani* sourced from diseased rice given by Ms Allicia Jack of Malaysian Research and Development Institute (MARDI). Mycelia disk of 0.6 cm diameter of both pathogenic fungus and isolates were placed apart on a PDA plate (Figure 1) and considered as treatment plates while the control plates contained only *R. solani* in five replicates and measurements were done at day 3 and day 5. The percentage inhibition of radial growth (PIRG) was calculated based on the formula by Skidmore & Dickinson (1976):

$$\text{PIRG} = \frac{\text{R1} - \text{R2}}{\text{R1}} \times 100\%$$

Where,
R1 = radius of pathogenic fungus (*R. solani*) on control plate
R2 = radius of pathogenic fungus (*R. solani*) on treatment plate

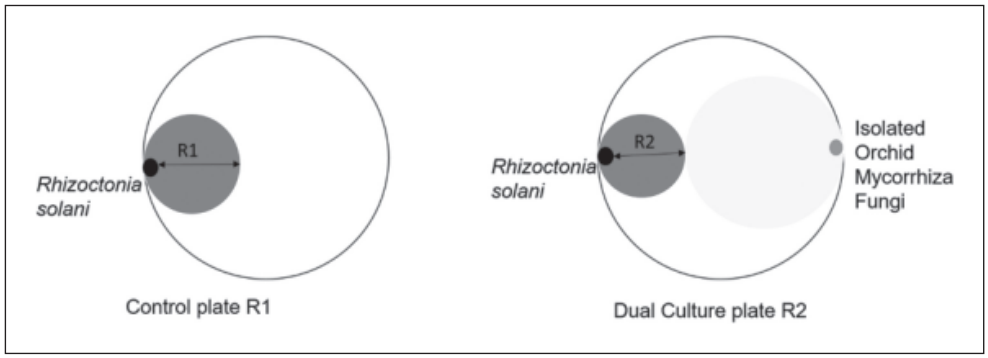

Fig. 1. Illustration of dual culture bioassay.

RESULTS AND DISCUSSION

Isolation and Identification of OMF

A total of four fungal pure cultures were isolated from the orchids; *P. pulcherrima*, *B. finlaysoniana* and *D. crumenatum*. Fungi identified as *Rhizoctonia* species C showed the fastest growth rate with 3.68 cm/day, followed by *Rhizoctonia* species B (1.08 cm/day), *Rhizoctonia* species A (0.86 cm/days) and *Daldinia* species slowest at 0.61 cm/day. The colony colour of all *Rhizoctonia* species A, B and C was white while *Daldinia* species turned greenish with age (Figure 2). The texture of *Rhizoctonia* species A, C and *Daldinia* species was fluffy while *Rhizoctonia* species B had a hyaline texture. All of the isolates did not have any spores and had septate to the neighbouring hyphae. The morphological characteristics of fungi are summarized in Table 1. However, further molecular identification is necessary as *Rhizoctonia* species are typically asexual, making it hard to identify at species level.

Antagonistic Potential of Isolates against *R. solani* *In vitro*

Dual culture bioassay is an establish method used to screen the potential of isolated endophytic fungi with antagonistic characteristics in demand to create a biological control agent (BCA). Percentage Inhibition of Radial Growth (PIRG) values served as a benchmark in determining the efficacy of an isolate as BCA. In this study, the PIRG value was recorded on the day 3 and day 5. On the day 3, *Rhizoctonia* species B displayed the highest values of PIRG with 51.5% and followed by *Daldinia* species (50.0%), *Rhizoctonia* species A (40.0%) and *Rhizoctonia* species C (23.5%). The control plate which contained only *R. solani* had reached its maximum growth on the day 5 with 8.5 cm of radial

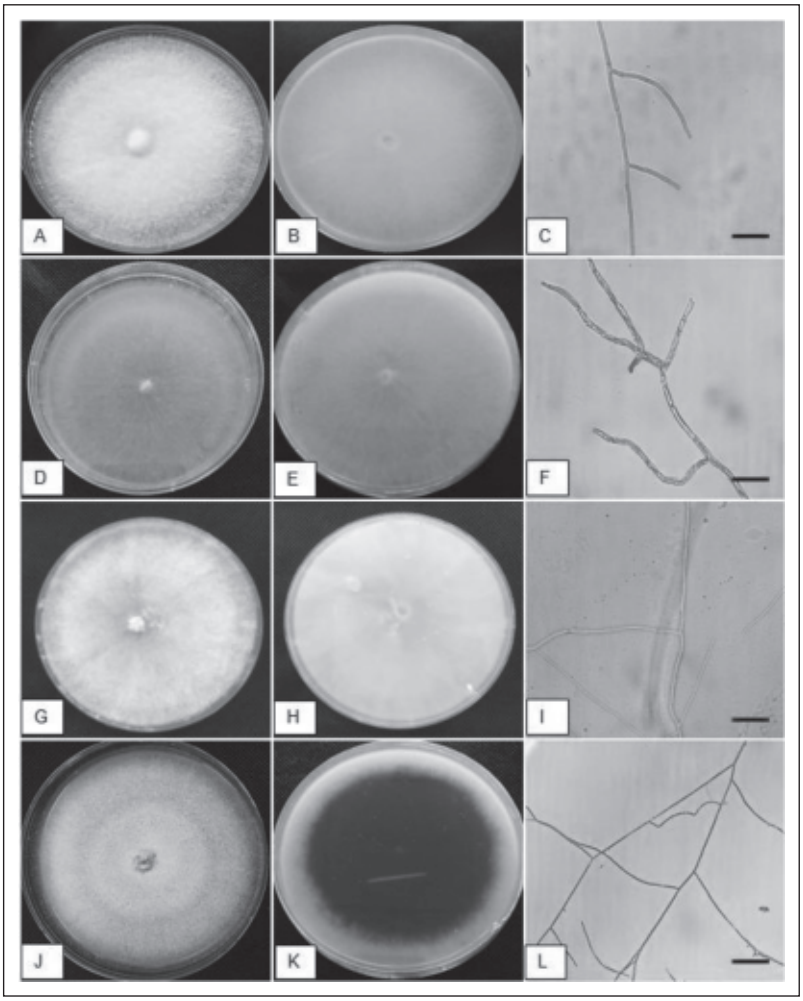

Fig. 2. Morphological characteristics of *Rhizoctonia* species A (A,B,C), *Rhizoctonia* species B. (D,E,F), *Rhizoctonia* species C (G,H,I) and *Daldinia* species (J,K,L). Surface of 7-day-old culture on PDA (A,D,G,L); Colony on reverse plate (B,E,H,K); Fungal hyphae under bright field fluorescence microscope (C,F,I, L); Bars = 20 μm.

Table 1. Morphological observation of the isolated fungi

	Isolated From	Growth rate (cm/day)	Colony colour	Texture	Spore	Septa
Rhizoctonia sp. (A)	*Phalaenopsis pulcherrima*	0.86	White	Fluffy	No	Yes
Rhizoctonia sp. (B)	*Phalaenopsis pulcherrima*	1.08	White	Hyaline	No	Yes
Rhizoctonia sp. (C)	*Dendrobium crumenatum*	3.68	White	Fluffy	No	Yes
Daldinia sp.	*Bromheadia finlaysoniana*	0.61	Greenish	Fluffy	No	Yes

Table 2. Antagonistic activities of endophytic fungi against *R. solani*

Days of inoculation	Radial growth (cm)	Isolate	Percentage Inhibition of Radial Growth (%)
3	4.0	*Rhizotonia* sp. A	40.0
	4.0	*Rhizotonia* sp. B	51.5
	4.0	*Rhizotonia* sp. C	23.5
	4.0	*Daldinia* sp.	50.0
5	8.5	*Rhizotonia* sp. A	52.9
	8.5	*Rhizotonia* sp. B	61.9
	8.5	*Rhizotonia* sp. C	40.4
	8.5	*Daldinia* sp.	50.2

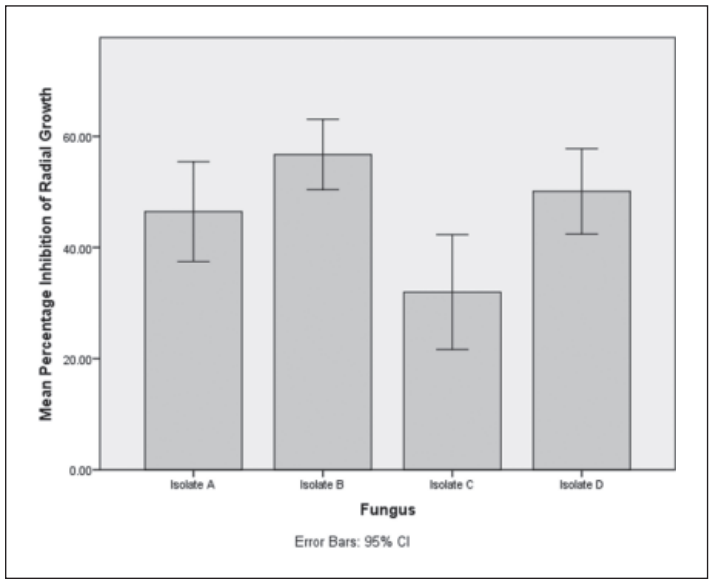

Fig. 3. Graph Percentage Inhibition of Radial Growth (PIRG) of isolated fungi against *Rhizoctonia solani*.

growth while for PIRG value, *Rhizoctonia* species B showed the dominancy of inhibition among the other isolates with 61.9% followed by *Rhizoctonia* species A (52.9%), *Daldinia* species (50.2%) and *Rhizoctonia* species C (40.4%). The PIRG value of the tested fungi was recorded as mentioned in Table 2. The fungi's percentage inhibition of radial growth (PIRG) was compared using one-way ANOVA and Tukey Post Hoc Test. The mean with one letter (*a and *b) in each column is significantly different at $p \leq 0.05$.

The graph depicted in Figure 3 shows the mean percentage inhibition of radial growth of the isolated endophytic fungi against pathogenic *R. solani*. The mean PIRG values were; *Rhizoctonia* species A (46.45%), *Rhizoctonia* species B (56.73%), *Rhizoctonia* species C (31.97%) and *Daldinia* species (50.11%). Overall from the graph, it was revealed that *Rhizotonia* species A, *Rhizotonia* species B and *Daldinia* species are not significantly different from one another while has significant difference from *Rhizotonia* species C. *Rhizotonia*

species A, *Rhizoctonia* species C and *Daldinia* species generated a 'barrage' (arrow, Figure 4) at the meeting point with the *R. solani*. This indicates the capability of the isolates to defend itself against the pathogenic *R. solani*. Barrage reaction happens when there is contact of cytoplasm via hyphal fusions where the tips of the interacting hyphae may branch profusely but a line of contact is clearly visible as the isolates mature. Even though fusion of hyphae occur, no nuclear exchange takes place and the two different mycelia form abnormal and often lethal hyphal fusions (Ikeda *et al*., 2011).

On the other hand, *Rhizoctonia* species B shows mycelial fusion (Figure 5) with *R. solani* after inoculated for 5 days. This result is interesting indeed as both of the isolates might be the same species within different host as it was capable to fuse their mycelium together. This fusion is characterized by a shared intermingling at the contact zone and fusions of mycelia via anastomosis formation (Esser & Meinhardt, 1984). In *Rhizoctonia*, hyphal anastomosis is used to taxonomically group isolates of *Rhizoctonia* anastomosis groups (AGs). Hyphal fusion lead to either self-pairings (acceptance) or somatic incompatibility (rejection) between isolates belonging to the same AG (Eken & Demirci, 2004). Thus, it is suspected that isolate B may be the same species and AG group of *R. solani* as the pathogenic *R. solani* from diseased rice.

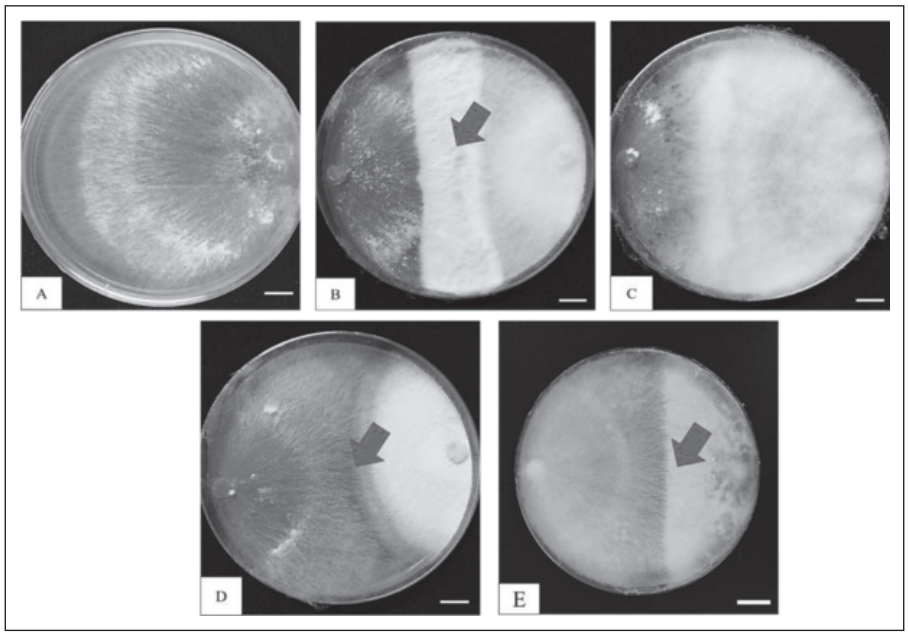

Fig. 4. Antagonistic screening of isolates against *R. solani*. 'Barrages' (indicated as red arrow) of *Rhizoctonia* species A (B), *Rhizoctonia* species C (D) and *Daldinia* species (E); (A) Control plate with *R. solani* only; (B-E) Isolates were inoculated on the right side while pathogen, *R. solani* on the left side of the PDA plate. Bars: A = 8.5 cm, B = 4.2 cm, C = 3.4 cm, D = 5.1 cm, E = 2.5 cm radial growth of *R. solani*.

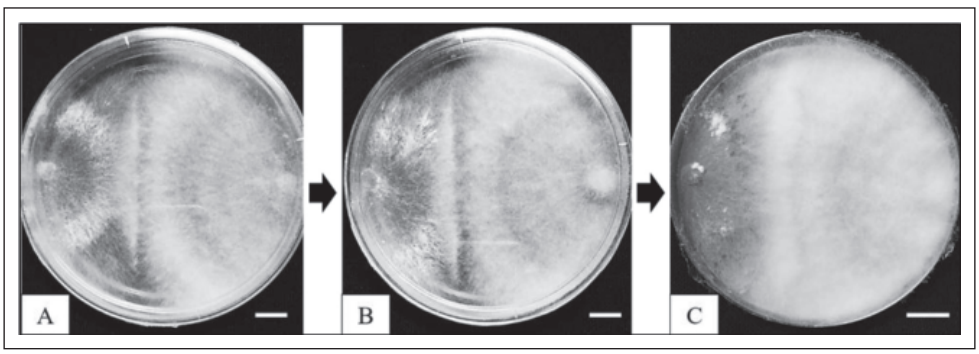

Fig. 5. Mycelial interaction in PDA plate between *Rhizoctonia* sp. B and *R. solani* at 5 days of inoculation (PDA diameter: 85 mm). Left is the inoculation of *R. solani* while right is *Rhizoctonia* sp. B; (A) 2 days inoculation with *R. solani*; (B) 3 days inoculation with *R. solani*; (C) 5 days inoculation with *R. solani*. Bars: A = 2.0 cm, B = 2.5 cm, C = 3.2 cm radial growth of *R. solani.*

CONCLUSION

In conclusion, isolates from orchids in BRIS is found to typically consist of *Rhizoctonia* species, which is a ubiquitous orchid mycorrhizal fungi. The isolates show high inhibition activities against the pathogenic *R. solani* even though they may be of the same species. This preliminary investigation shows the potential of orchid mycorrhizal fungi to be studied in order to further understand the mechanism of plant pathogen interactions.

ACKNOWLEDGEMENTS

This research was funded by Universiti Malaysia Terengganu. We would like to acknowledge all the parties involved especially from the School of Fundamental Science.

REFERENCES

Mosquera-Espinosa, A.T., Bayman, P., Prado, G.A., Gómez-Carabalí, A. & Otero, J.T. (2013) The double life of *Ceratobasidium*: orchid mycorrhizal fungi and their potential for biocontrol of *Rhizoctonia* solani sheath blight of rice*, Mycologia,* **105(1)**: 141-150.

Bougoure, J., Ludwig, M., Brundrett, M. & Grierson, P. 2009. Identity and specificity of the fungi forming mycorrhizas with the rare myco-heterotrophic orchid *Rhizanthella gardneri. Mycological Research,* **113(10)**: 1097-1106.

Brundrett, M. 2004. Diversity and classification of mycorrhizal associations. *Biological Reviews*, **79**: 473-495.

Eken, C. & Demirci, E. 2004. Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates from bean in Eruzum, Turkey. *Journal of Plant Pathology*, **86(1)**: 49-52.

Esser, K. & Meinhardt, F. 1984. Barrage formation in fungi. In: Linskens H.F., Heslop-Harrison J. (eds) Cellular interactions. Encyclopedia of plant physiology (New series), vol 17. Springer, Berlin, Heidelberg.

Ikeda, K., Inoue, K., Nakamura, H., Hamanaka, T., Ohta, T., Kitazawa, H. & Park, P. 2011. Genetic analysis of barrage line formation during mycelial incompatibility in *Rosellinia necatrix*. *Fungal Biology*, **115(1)**: 80-86.

Jamilah, M.S., Faridah, M. & Rohani, S. 2015. Setiu: More than a wetlands. In Setiu Wetland, In *Setiu Wetland: Species, Ecosystem and Livelihoods*, Faridah, M., Jamilah, M.S., Jarina, M.J. & Rohani, S. (eds.). Universiti Malaysia Terengganu. 87-100 pp.

Lah, M.K.B.C., Nordin, M.N. Bin, Isa, M.B.M., Khanif, Y.M. & Jahan, M.S. 2011. Composting increases BRIS soil health and sustains rice production. *Science Asia*, **37(4)**: 291-295.

Leck, A. (1999). Preparation of lactophenol cotton blue slide mounts. *Journal of Community Eye Health*, **12(30)**: 24.

Mitchell, T.L. & Britt, E.M. 1981. A coverslip technique for preparing permanent fungus mounts. *Mycopathologia*, **76**: 23-25.

Rahman, M.A., Begum, M.F. & Alam, M.F. 2009. Screening of *Trichoderma* isolates as a biological control agen t against *Ceratocytis paradoxa* causing pineapple disease of sugarcane. *Mycobiology,* **37(4)**: 277-285.

Rasmussen, H.N. & Rasmussen, F.N. 2009. Orchid mycorrhiza: implications of a mycophagous life style. *Oikos*, **118**: 334-345.

Rice Knowledge Bank. 2017. Retrieved November 25, 2017, from http://www.knowledgebank.irri.org/training/fact-sheets/pest-management/diseases/item/sheath-blight

Skidmore, A.M. & Dickinson, C.H. 1976. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Transactions of the British Mycological Society*, **66**: 57-64.

Taheri, P. & Tarighi, S. 2011. Cytomolecular aspects of rice sheath blight caused by *Rhizoctonia solani*. *European Journal of Plant Pathology*, **129**: 511-28.

Warcup, J.H. & Talbot, P.H.B. 1967. Perfect states of *Rhizoctonia*s associated with orchids. *New Phytologist*, **66**: 631-641.