Tropical Life Sciences Research, 24(2), 85–90, 2013

SHORT COMMUNICATION

Endophytic *Fusarium* spp. from Roots of Lawn Grass (*Axonopus compressus*)

Latiffah Zakaria* and Chua Harn Ning

School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

Abstrak: Kulat endofit ditemui di dalam tumbuhan perumah tetapi tidak menunjukkan sebarang tanda-tanda yang ketara. Dalam kajian ini, spesies *Fusarium* endofitik telah dipencilkan daripada akar rumput halaman (*Axonopus compressus*) dan sebanyak 51 pencilan telah diperolehi daripada 100 segmen akar. Berdasarkan ciri-ciri morfologi bentuk makrokonidia dan sel conidiogenus, dua spesies *Fusarium* telah dikenalpasti iaitu, *F. oxysporum* (53%) dan *F. solani* (47%). Kehadiran *F. oxysporum* dan *F. solani* endofitik di dalam akar rumput halaman menyumbang kepada pengetahuan mengenai taburan kedua-dua spesies *Fusarium* dan kepentingan akar sebagai nic endofitik spesies *Fusarium*.

Kata kunci: Endofit, *Fusarium*, Akar Rumput

Abstract: Fungal endophytes are found inside host plants but do not produce any noticeable disease symptoms in their host. In the present study, endophytic *Fusarium* species were isolated from roots of lawn grass (*Axonopus compressus*). A total of 51 isolates were recovered from 100 root segments. Two *Fusarium* species, *F. oxysporum* (53%) and *F. solani* (47%), were identified based on macroconidia and conidiogenous cell morphology. The detection of endophytic *F. oxysporum* and *F. solani* in the roots of lawn grass contributes to the knowledge of both the distribution of the two *Fusarium* species and the importance of roots as endophytic niches for *Fusarium* species.

Keywords: Endophyte, *Fusarium*, Grass Root

Endophytic fungi live inside plant tissues without causing any visible disease symptoms (Petrini 1991). Root endophytes are widespread among plants (Schulz *et al*. 2006). The interactions between endophytic fungi and host plants range from mutualism to parasitism (Schulz & Boyle 2005; Saikkonen *et al.* 1998). A “balanced antagonism”, the equilibrium between fungal virulence and plant defence, was postulated by Schulz *et al.* (1999) to define the endophyte-host association. However, this equilibrium may be affected by an imbalance in nutrient exchange (Kogel *et al.* 2006), environmental conditions (Moricca & Ragazzi 2008) and physiological stress, including senescence (Halmschlager *et al*. 1993). Therefore, Sieber (2002) redefined endophytic fungi as those found in apparently healthy, functional tissues at the time of sample collection.

From the beginning of fungal endophyte research, grasses have been the focus because fescue toxicosis in cattle is caused by *Sphacelia typhina,* a

*Corresponding author: Lfah@usm.my

fungal endophyte in tall fescue (*Festuca arundinacea*) (Bacon *et al*. 1977). Since then, fungal endophytes have been recorded from virtually all plant species (Saikkonen *et al*. 2006). Endophytes infect at least 80 grass genera. Among the well-known endophytic fungi associated with grasses and sedges are the Clavicipitaceae, which include *Balansia, Myriogenospora, Atkinsoniella, Balansiopsis, Epichloë* and *Neotyphodium* (Clay 1989), illustrating that fungal endophytes in grasses are ubiquitous.

Although symptomless infections by *Fusarium* species have been reported in many types of plants, including grass families (Sanchez Marquez *et al*. 2010; Walsh *et al*. 2010; Yuan *et al*. 2010; Marcia-Vicente *et al*. 2008a, b; Pamphile & Azevedo 2002; Katan 1971), the occurrence of endophytic *Fusarium* species in grasses in Malaysia has not been given much attention compared to that given to pathogenic *Fusarium* species. Therefore, the present study was conducted to isolate and identify *Fusarium* species from roots of lawn grass [*Axonopus compressus* (Sw.) P. Beauv.] from the Family Poaceae, which is widely planted in Malaysia and commonly used as ground cover.

Healthy lawn grasses were collected from Taman Mewah, Butterworth, Pulau Pinang; the field in front of the Vector Control Research Unit (VCRU), Universiti Sains Malaysia (USM); the convocation site, USM; and Tasik Harapan, USM. Young and healthy grasses were collected, placed in plastic bags and brought to the laboratory to be processed.

The grass samples were washed thoroughly to remove any soil and dirt adhering to the roots. The clean grass roots were then placed in a beaker and labelled. The beaker was covered with a piece of net cloth, and the grass roots were washed under running tap water for 24 hrs to remove soil particles and surface epiphytes.

Endophytic *Fusarium* samples were isolated from the roots using surface sterilisation techniques. The roots were sterilised by soaking in 75% ethanol for 1 min, 1% sodium hypochlorite for 3 min and 95% ethanol for 5 min. All samples were dried in a laminar flow hood using sterilised filter paper to remove excess water.

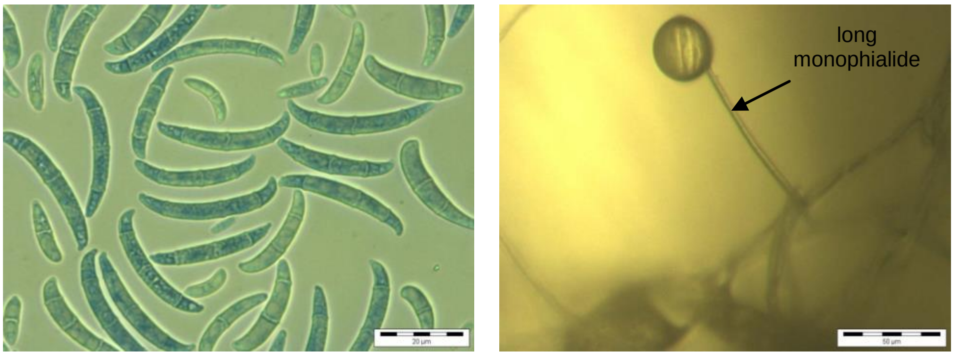
After the samples were completely dry, they were cut into 3 to 5 mm segments using a sterile scalpel. To check the efficacy of the surface sterilisation procedure and confirm that the isolates only originated from the internal tissues of the roots, the imprint method, i.e., pressing sterilised root segments gently onto potato dextrose agar (PDA), was used (Schulz *et al*. 1993).

Root segments were then transferred onto pentachloronitrobenzene (PCNB) media and incubated at 25±1°C until there was visible mycelial growth from the tissues. Mycelia were then subcultured onto fresh PDA plates.

To identify endophyte species, primary and secondary characteristics of the isolates were observed based on the methods described in The *Fusarium* Laboratory Manual (Leslie & Summerell 2006). The primary characteristics observed were the shapes of the macroconidia and microconidia, the formation of conidiogenous cells (i.e., monophialide or polyphialide) and the presence of chlamydospores on carnation leaf agar (CLA). The characteristics were observed using a light microscope (Olympus BX41, Tokyo). Secondary characteristics, such as the pigmentation and texture of the colony, were observed using PDA.

A total of 51 *Fusarium* isolates were isolated from 100 *A. compressus* root segments. The absence of any fungal growth on PDA after root imprint confirmed that the surface sterilisation procedure was effective in removing surface fungi. This result indicated that the epiphytic fungi could not grow after surface sterilisation and that the *Fusarium* isolates recovered were endophytic (Schulz *et al*. 1993).

Two *Fusarium* species, *F. oxysporum* (Schlechtendahl emend. Snyder & Hansen) and *F. solani* [(Martius) Appel & Wollenber emend. Snyder & Hansen] were recovered from symptomless root segments. *F. oxysporum* (53%) was isolated more frequently than *F. solani* (47%). The species descriptions corresponded with the descriptions of *F. oxysporum* and *F. solani* from The *Fusarium* Laboratory Manual (Leslie & Summerell 2006). On PDA, isolates of *F. oxysporum* produced abundant floccose mycelia with violet pigmentation, and isolates of *F. solani* produced sparse mycelia with cream pigmentation. The two species were identified primarily based on the shapes of macroconidia and conidiogenous cells. Isolates of *F. solani* produced long monophialides and stout and robust macroconidia (Fig. 1), whereas isolates of *F. oxysporum* produced short monophialides and straight or slightly curved macroconidia (Fig. 2).



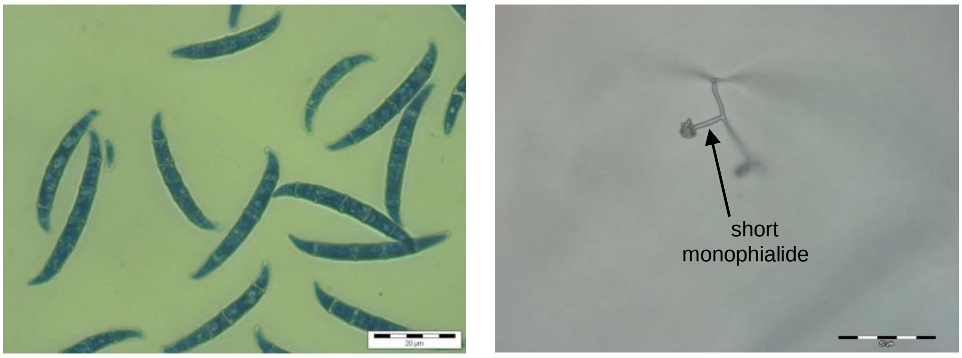
(a) (b)

Figure 1: Stout and robust macroconidia (a) and long monophialide (b) of *F. solani.*

Endophytic *Fusarium* species were among the fungal isolates recovered from the plant roots of the grass family Poaceae (formerly known as Gramineae) (Sieber 2002). In the present study, only two species, *F. oxysporum* and *F. solani,* were isolated from *A. compressus* roots. Both *Fusarium* species are cosmopolitan, occur on a wide range of hosts and are common soil saprophytes. Endophytic *F. oxysporum* and *F. solani* have been isolated from roots of different grasses, such as *Holtus lanatus,* which grows in humid water logged soils (Sanchez Marquez *et al*. 2010), *Oryza granulata* (Naik *et al*. 2009) and *Oryza sativa* (Yuan *et al.* 2010). In addition to grasses, endophytic *F. oxysporum* and *F. solani* have been recovered from roots of several plants from the family Leguminosae (Marcia-Vicente *et al*. 2008a), and isolates of endophytic *F. oxysporum* have been reported from roots of several vegetables (Kim *et al*. 2007). Isolates of endophytic *F. solani* have been found in roots of

Rhizophoraceae mangrove trees (Xing & Guo 2011). These studies suggest that endophytic *F. oxysporum* and *F. solani* are common fungal endophytes occupying the roots of grasses and other plants.

Endophytic *Fusarium* species from grasses are hemibiotrophs, i.e., they infect healthy tissues as biotrophs, but the fungus may infect the host after a latency period or become a saprophyte when then host plant dies (Bacon & Yates 2006). The association between the endophytic fungus and the plant host may also switch from mutualistic to pathogenic depending on several factors (Moricca & Ragazzi 2008; Kogel *et al*. 2006; Johnson *et al*. 1997; Halmschlager *et al*. 1993). Therefore, the association between the fungal endophyte and the host plant is likely transitory (Bacon & Yates 2006). Although the association is transitory, the fungal endophyte may help the plant host adapt to its habitat, promote plant growth and protect plants from biotic and abiotic stress (Rodriguez & Redman 2008; Schulz & Boyle 2005; Sieber 2002).



(a) (b)

Figure 2: Straight and slightly curved macroconidia (a) and short monophialide (b) of *F. oxysporum.*

New plant pathogenic fungi, including pathogenic *Fusarium,* may evolve. These pathogenic isolates can colonise other plants without causing disease symptoms (Leslie & Summerell 2004). Symptomless root infections in plants have been reported from several temperate *Fusarium* species, such as *F. graminearum, F. nivale* and *F. culmoru*m (Sieber *et al*. 1988), and *F. crookswellense* (Boshoff *et al.* 1996). Both *F. oxysporum* and *F. solani* are well-known plant pathogens, and both species have special forms (*forma speciales*) that infect specific crop plants. The pathogenic isolates may arise from endophytes when new plants or crops are planted in the same field (Summerell & Leslie 2004).

Fusarium usually dominates root endophytic communities specialised for the colonisation of herbaceous plants (Sieber 2002). Therefore, the association of endophytic *F. oxysporum* and *F. solani* with roots of lawn grass (*A. compressus*) may suggest the importance of the roots as an endophytic niche for *Fusarium* species.

ACKNOWLEDGEMENT

This work was supported, in part, by USM Short Term Grant 304/PBIOLOGY/639067.

REFERENCES

Bacon C W, Porter J K, Robbins J D and Luttrell E S. (1977). *Epichloe typhina* from toxic tallfescue grasses. *Applied and Environmental Microbiology* 34(5): 576–581.

Bacon C W and Yates I E. (2006). Endophytic root colonization by *Fusarium* species: Histology, plant interactions and toxicity. In B J E Schultz, C J C Boyle and T N Sieber (eds.). *Microbial root endophytes*. Berlin: Springer-Verlag.

Boshoff W H P, Pretorius Z A, Swart W J and Jacobs A S. (1996). A comparison of scab development in wheat infected by *Fusarium graminearum* and *Fusarium crockswellense*. *Abstracts of APS/MSA Joint Annual Meeting*. Indianapolis, USA, 27–31 July 1996, 86(11): S58 .

Clay K. (1989). Clavicipitaceaous endophytes of grasses: Their potential as biocontrol agents. *Mycological Research* 92(1): 1–12.

Halmschlager E, Butin H and Donabauer E. (1993). Endophytic fungi in leaves and twig of *Quercus petrea*. *European Journal of Forest Pathology* 23(1): 51–63.

Johnson N C, Graham J H and Smith F A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continumm. *New Phytologist* 135(4): 575–585.

Katan J. (1971). Symptomless carriers of the tomato wilt pathogen. *Phytopathology* 61(10): 1213–1217.

Kim H-Y, Choi G J, Lee S-W, Lim H K, Jang K S, Son S W, Lee S O, Cho K Y, Sung N D and Kim J-C. (2007). Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. *Letters in Applied Biology* 44(3): 332–337.

Kogel K-H, Franken P and Hückelhoven R. (2006). Endophyte or parasite – what decides? *Current Opinion on Plant Biology* 9(4): 358–363.

Leslie J F and Summerell B A. (2006). *The* Fusarium *Laboratory Manual.* Iowa, USA: Blackwell Publishing Ltd.

Marcia-Vicente J G, Jansson H-B, Abdullah S K, Descols E, Salinas J and Lopez-Llorca L V. (2008a). Fungal endophytes from natural vegetation in Mediterranean environments with special reference to *Fusarium* species. *FEMS Microbiology Ecology* 64(1): 90–105.

Marcia-Vicente J G, Rosso L C, Ciancio A, Jansson H B and Lopez Llorca L V. (2008b). Colonisation of barley roots by endophytic *Fusarium equiseti* and *Pochonia chlamydosp*oria: Effects on plant growth and disease. *Annals of Applied Biology* 155(6): 391–401.

Moricca S and Ragazzi A. (2008). Fungal endophytes in Mediterranean oak forests: A lesson from *Discula quercina*. *Phytopathology* 98(4): 380–386.

Naik B S, Sashikala J and Krishnamurthy Y L. (2009). Study on the diversity of endophytic communities from rice (*Oryza sativa* L) and their anatagonistic activities in vitro. *Microbiological Research* 164(3): 290–296.

Pamphile J A and Azevedo J L. (2002). Molecular characterization of endophytic strains of *Fusarium verticillioides* (*F. moniliforme*) from maize (*Zea mays* L). *World Journal of Microbiology and Biotechnology* 18(5): 391–396.

Petrini O. (1991). Fungal endophytes of tree leaves. In J H Andrews and S S Hirano (eds.). *Microbial ecology of leaves*. New York: Springer-Verlag.

Rodriguez R and Redman R. (2008). More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. *Journal of Experimental Botany* 59(5): 1109–1114.

Sanchez Marquez S, Bills G F, Dominquez Acuna L and Zabalgogeazcoa I. (2010). Endophytic mycobiota of leaves and roots of the grass *Holcus lanatus*. *Fungal Diversity* 41(1): 115–123.

Saikkonen K, Fath S H, Helander M and Sullivan T J. (1998). Fungal endophytes: A continumm of interactions with host plants. *Annual Review of Ecology and Systematics* 29: 319–343.

Saikkonen K, Lehtonen P, Helander M, Koricheva J and Faeth S H. (2006). Model systems in ecology: Dissecting the endophyte-grass literature. *Trends in Plant Science* 11(9): 428–433.

Schulz B and Boyle C. (2005). The endophytic continuum. *Mycological Research* 109(6): 661–686.

Schulz B, Boyle S and Sieber T. (2006). *Microbial root endophytes*. Dordrecht, Netherlands: Springer.

Schulz B, Roemmert A K, Dammann U, Aust H J and Strack D. (1999). The endophyte-host interaction: A balanced antagonism? *Mycological Research* 103(10): 1275–1383.

Schulz B, Wanke U, Draeger S and Aust H-J. (1993). Endophytes from herbaceous plants and shrubs: Effectiveness of surface sterilization methods. *Mycological Research* 97(120): 1447–1450.

Sieber T N. (2002). Fungal root endophytes. In Y Waisel, A Eshel and U Kafkafi (eds.). *Plant roots: The hidden half*. New York/Basel: Marcel Dekker, 887–917.

Sieber T, Risen T K, Muller E, Fried P M. (1988). Endophytic fungi in four winter wheat cultivars (*Triticum aestivum*) differing in resistance against *Stagonaspora nodorum* (Berk.) Cast Germ = Septorianodorum (Ber.) Berk. *Journal of Phytopathology* 122(4): 289–306.

Summerell B A and Leslie J F. (2004). Genetic diversity and population structure of plant-pathogenic species in the genus *Fusarium*. In M Gillings and A Holmes (eds.). *Plant microbiology*. Oxford: Garland Science/BIOS Scientific Publishers, 207–223.

Xing X K and Guo S X. (2011). Fungal endophyte communities in four Rhizophoraceae mangrove species on the south coast of China. *Ecological Research* 26(2): 403–409.

Yuan Z-L, Zhang C-L, Lin F-C and Kubaik C P. (2010). Identity, diversity and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Oryza granulata*) from nature reserve in Yunnan, China. *Applied and Environmental Microbiology* 76(5): 1452–1652.

Walsh J L, Laurence M H, Lieu E C Y, Sangalang A E, Burgess L W, Summerell B A and Petrovic T. (2010). *Fusarium*: Two endophytic novel species from tropical grasses of northern Australia. *Fungal Diversity* 44(1): 149–159.