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NOTE

Teleomorph formation of *Setosphaeria monoceras*, a perfect state of *Exserohilum monoceras*, by Japanese isolates

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Abstract *Setosphaeria monoceras*, a teleomorph of *Exserohilum monoceras*, has been produced in culture through the crossing of compatible mating-type isolates from Japan. In this study, Sachs' agar medium + maize leaf were placed at a constant temperature of 25°C with 16 h light and 8 h dark cycles for 25–30 days. When assessed, the morphological characteristics were almost identical to those of original description by Alcorn, using Australian isolates. This note presents the second report investigating the development of the perfect stage of *E. monoceras.*

Keywords Ascoma · Ascospores · Bioherbicide · Mating type · Sexual reproduction

The fungal pathogen *Exserohilum monoceras* (Drechsler) K.J. Leonard & Suggs is a causal agent of severe leaf blight disease in the *Echinochloa* weed species and a promising bioherbicide agent (Tsukamoto et al. 1997; Zhang and Watson 1997). The perfect stage *Setosphaeria monoceras* Alcorn was observed in 1978 through in vitro crossing in Australia (Alcorn 1978). However, no observations of the sexual state have been reported in environment and laboratory crossing experiments in other locations. In our previous study, we developed multiplex polymerase chain reaction (PCR) primers to determine mating type of this fungus (Morita et al. 2011). The method successfully identified the mating type of *E. monoceras* Japanese field isolates in a single reaction, indicating that both mating types are naturally distributed in the field and that potential sexual encounters are common. An absence of either mating type is therefore not the reason for the sparse number of reports regarding the sexual state of *E. monoceras*. The isolates appear capable of sexual reproduction in laboratory conditions, which may allow us to improve the fungus to act as a more effective mycoherbicide using conventional sexual hybridization breeding strategies.

In this study, we examined crosses between eight selected *MAT1-1* field isolates (D.delta-1, D.mukai-2, YM-1, YM-2, nishi1-1, -2, -3, and C.ex-1; q.v. Table 1 in Morita et al. 2011) and the laboratory strain 9.29 (*MAT1-2*), which was isolated from diseased *Echinochloa* weed and keeps high virulence under laboratory maintenance for years (Morita et al. 2011). A pair of the strains was inoculated on opposite sides of a 3- to 4-cm piece of sterilized maize leaf in Sachs' agar medium (1.0 g KNO_3, 0.5 g NaCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g Ca $(NO_3)_2$, 0.5 g $Ca_3(PO_4)_2$, trace of $FeCl_3$, 15.0 g agar per 1,000 ml distilled water) and incubated at 25°C for 16-h light and 8-h dark cycles, as described previously (Ueyama and Tsuda 1975). Protothecia were evident 10 days after inoculation and developed to pseudothecia within 2 weeks in some paired cultures. Under our conditions, ascospores matured in 3–4 weeks. Similar results were obtained using basal salt agar [1 × basal salts solution for complete medium (CM; Tanaka et al. 1991) was solidified with 1.2%(w/v) agar], of which salts can be stocked as a 100 × solution and are routinely used for preparation of CM in our laboratory (data not shown).

The majority of ascomata were formed on the exposed edges of the corn leaves, whereas some were formed while immersed in the agar. Matured ascomata appeared black, were ellipsoidal to globose, and measured 338–483 μm in height and 310–447 μm in diameter (Fig. 1a). The outer

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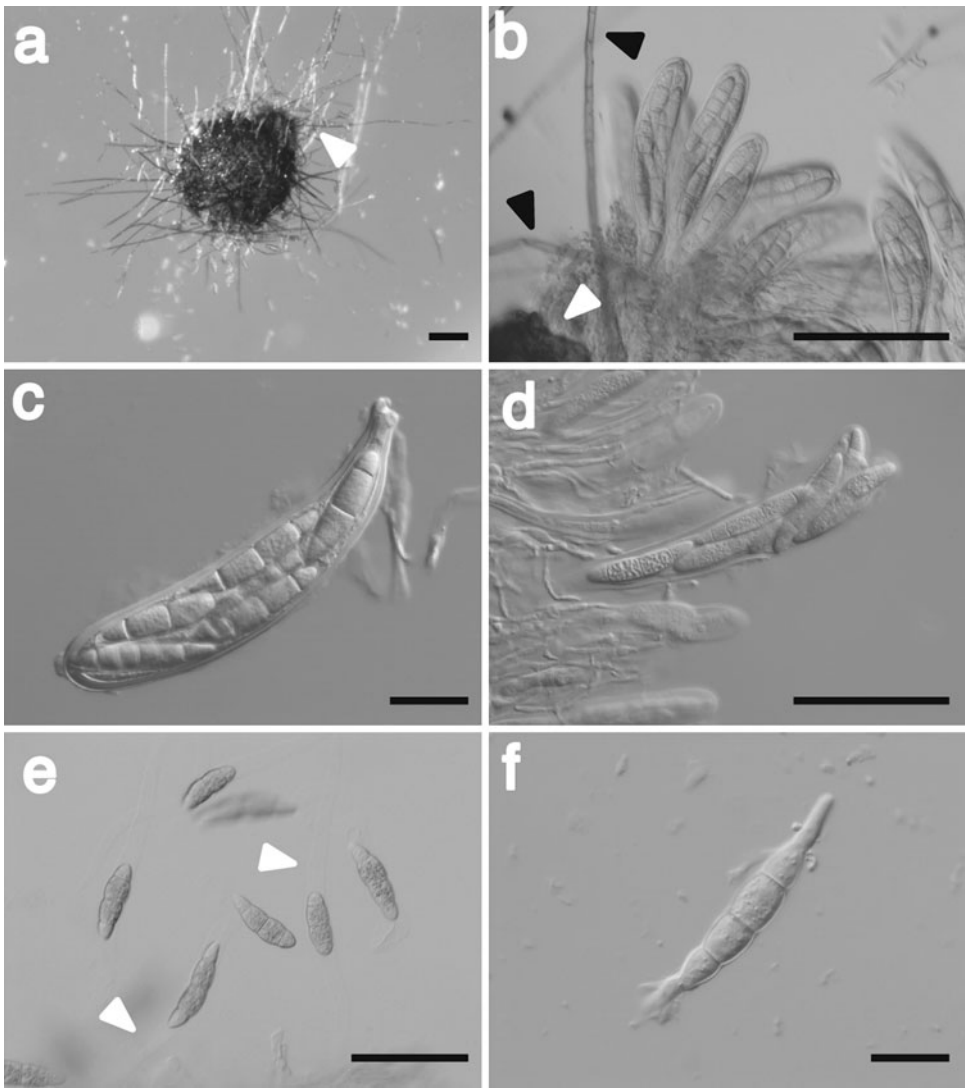

Fig. 1 Perfect state of *Exserohilum monoceras* (*Setosphaeria monoceras*). **a** Ascoma; *white arrowhead* indicates a beak. **b** Asci; *black arrowheads* indicate setae, *white arrowhead* shows pseudoparenchyma cells of the pseudothecium. **c**, **d** Ascospores break out from the bitunicate asci. **e** Ascospores; hyaline long tubular mucous sheaths visible in the water mounts (*white arrowheads*). **f** Ascospore germinates bipolarly. *Scale bars* 100 μm in **a**, **b**, **d**, **e** and 25 μm in **c**, **f**

layers were composed of a pseudoparenchyma with thick, dark-brown walls. On occasions, a short, broad beak was observed at the apex of the ascoma, with hyaline cells protruding from the ostiole of a matured ascoma. Over the upper half of the ascomata, short, stiff, brown, spin-like hairs (setae) were densely produced around the ostiole. The asci were cylindrical, clavate or ellipsoidal, bitunicated, and narrow at the base (Fig. 1b–d). The asci contained (1–)4–8 spores and measured 117–229 × 23–38 μm. Ascospores were fusoid, straight or slightly curved, measured 43–78 × 11–22 μm, (1–)3(–4)-septate, and were constricted at the septa (Fig. 1e, f). The cells were hyaline to brown, whereas the median doliform cells were darker. In water mounts, hyaline long tubular mucous sheaths were visible along each side of the ascospores. These characteristics and sizes were consistent with the original description by Alcorn (1978) using Australian isolates. The ascospores were germinated bipolarly in water or liquid CM for 2 h (Fig. 1f).

The progenies of successfully mated pairs were randomly isolated from an ascoma, and their mating types were determined by multiplex PCR (Morita et al. 2011). Of the 40 progenies, 18 isolates were *MAT1-1* and 22 were *MAT1-2*. Segregation of the two mating types was estimated to be in equal proportions ($\chi^2 = 0.23$ with Yates's correction for continuity, $P > 0.05$). The production of ascomata was sparse and varied among isolates in *E. monoceras*. In the crossing examination, 6–37 ascomata per plate were found on four mating cultures (D.mukai-2 × 9.29, YM-1 × 9.29, YM-2 × 9.29, and nishi1-1 × 9.29) from eight combinations. Another four mating cultures (D.delta-1 × 9.29, nishi1-2 × 9.29, nishi1-3 × 9.29, and C.ex-1 × 9.29) were unsuccessful in crossing. The paring cultures with the same mating type combination of the field isolates or single cultures of the field isolates were prepared as negative controls and also failed to form ascomata. In the cultures of these unsuccessfully mated pairs, black protothecia or scolecite like

structures were often observed, whereas these were not found in the cultures of 9.29 alone. This suggested that the Japanese field isolates used in this study display maternal factors and that laboratory strain 9.29 lost its female characteristics as a result of successive cultivation in artificial laboratory media. Similar results were reported in the heterothallic species, including *Cochliobolus heterostrophus*, *S. turcica*, *C. miyabeanus*, and *Magnaporthe oryzae* (Lutterell 1958; Tsuda and Ueyama 1975; Ueyama and Tsuda 1976; Chang and Fan 1986; Zeigler 1998). A large number of genes are involved in complex multisteps of fungal sexual reproduction (Kronstad and Staben 1997; Turgeon 1998; Paoletti et al. 2007; Izumitsu et al. 2009). In the basidiomycetes fungi *Filobasidiella neoformans* (anamorph: *Cryptococcus neoformans*), mutational accumulation more easily occurs during successive cultivations for usual laboratory maintenance than in vegetative growth or in asexual reproduction (Xu 2002). Subtle effects may lead to disorders of the complex of maternal factor-related genes of the ascomycete fungus for the stored period, as authors in previous works on other ascomycetes have suggested (Kwon-Chung et al. 1974; Ueyama and Tsuda 1976).

Improving the sexual reproduction of this fungus will help facilitate research into its use and genetic-based herbicidal properties. In general, many fungal species are composed of isolates with varying degrees of sexual ability (Dyer et al. 1992; Notteghem and Silué 1992). The four field strains (D.delta-1, nishi1-2, nishi1-3, and C.ex-1) have mating types that are compatible with the 9.29 strain and appear to possess maternal potential but were unsuccessful in sexual hybridization with the strain. The progenies obtained by successful crossing in this study showed various degrees of fertility with the father strain 9.29 or sister strains with compatible mating types (data not shown). It is therefore necessary to examine the fertility of additional isolates from field populations or laboratory progenies. Exploration of more suitable *E. monoceras* crossing conditions is also required to further investigate the sexual reproduction of this fungus.

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