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Necessity of particular seed-borne fungi for seed germination and seedling growth of *Xyris complanata*, a pioneer monocot in top soil-lost tropical peatland in Central Kalimantan, Indonesia

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Abstract Hawaii yellow-eyed grass (*Xyris complanata*: Xyridaceae) inhabits infertile, acidic peat soil in the rainy tropical zone in Southeast Asia, and this monocot plant produces a large number of dormant seeds to make a large deposit to seed bank in the soil. Under laboratory conditions, surface-sterilized *X. complanata* seeds were rarely able to germinate on sterilized peat moss bed, while they required inoculation with either seed epiphytic or soil fungi to facilitate active seed germination. In the present study, three different genera of seed epiphytic fungi were isolated, and two common fungal genera, *Fusarium* sp. (strain R-1) and *Penicillium* sp. (strain Y-1), were found to promote the seed germination of this plant. In the sterile peat moss beds, the germination-stimulating fungi also showed growth-promoting effects on *X. complanata* seedlings growing. These results suggested that the seed germination-promoting fungi likely function as genuine partners for *X. complanata* in tropical, open peat lands.

Keywords *Xyris complanata* – Seed germination – Tropical peat soil – *Fusarium* sp. – *Penicillium* sp. – Plant growth promoting fungi

Introduction

In some higher plants which produce tiny seeds, microorganisms are an essential partner during seed germination and/or seedling growth. One of the most famous examples of obligatory dependence during seed germination is that of orchids (family Orchidaceae). Orchids require the orchid mycorrhizal fungi (e.g. *Rhizoctonia*) as their obligate growth helper (Rasmussen 2002). Through the regeneration cycle, some mycorrhizal fungi (e.g. *Epulorhiza* for *Platanthera clavellata*) are associated to the seed germination (Zettler and Hofer 1998; McKendrick et al. 2002). The fragment of fungal mycelium in the albumen-less, tiny seeds travels with the seeds to their final destination and starts to develop the mycorrhizal fungi along the seed germination. Another obligatory relationship between plant seeds and microorganisms has been reported in non-photosynthetic, myco-heterotrophic plants, *Sarcodes sanguinea* and *Pterospora andromedea*, both of which belong to the family Ericaceae (Bruns and Read 2000). Seed germination of these plants is highly linked with *Rhizopogon* spp. (Basidiomycota) fungi. Under laboratory conditions, their seeds were unable to germinate without mycelial colony of the root-associating fungi. As a matter of fact, it was demonstrated by an experiment to separate seeds and mycelia physically with a cellophane film that certain diffusible chemical(s) served as signals for the promotion of seed germination (Bruns and Read 2000). Similar with these ericaceous, myco-heterotrophic plants, dormant seeds of remarkably small sizes often receive chemical stimulants from specific hosts or other biotic and/or abiotic chemical sources (Hauck

et al. 1992; Yoneyama et al. 1998; Flematti et al. 2004), while some plants that wait dormancy breakage for seed germination in seed bank in soil often required biological and/or physical decomposition of seed coat (Warrag and Eltigani 2005; Baskin and Baskin 2004).

Genus *Xyris* of family Xyridaceae contains over 250 species world-wide and this member of monocotyledonous plant is known to be tolerant to highly stressed, infertile soils (Sajo and Rudall 1999), including acidic soils (Hotta 1989; Guevara et al. 2005) and saline soils (Wilson et al. 1985). They are representative group of pioneer plants in wet, adverse open land, producing large numbers of very tiny seeds for their regeneration and survivals (Kraus et al. 1994). In *X. tennesseensis*, a typical aquatic emergent plant, it has been reported that they also produce tiny seeds to deposit to persistent seed bank in native soil. Nevertheless, the seed germination is highly difficult in non-native soil. Only in the native soil, *X. tennesseensis* can germinate effectively without any stimuli for dormancy breakage. This suggests that certain microorganism(s) in the native soil is associated with the seed germination of *X. tennesseensis* (Baskin and Baskin 2003).

X. complanata (Hawaii yellow-eyed grass) that was used in this study distributes throughout Southeast Asia, as relatively common species in this region, together with *X. indica*, *X. pauciflora*, and *X. capensis* (Yahiro 1997). *X. complanata* is a major Xyridaceae plant in Central Kalimantan and is well suited for growth in deforested open areas and wayside due to its relatively high adaptability to infertile, acidic soil. During the rainy season, *X. complanata* actively flowers and produces large numbers of tiny, football-shaped seeds that are approximately 0.2×0.3 mm in size (Yahiro 1997). At the end of the rainy season (March-April), it dispersed the seeds over the wet soil and effectively deposit to the seed bank in the soil. A portion of the seeds germinates irregularly on the surface of the moist, acidic soil to maintain its pure communities.

Open peat land where *X. complanata* dominated was originally peat swamp forest, but it has been destroyed by logging and further peat mining. As a result, large tracts of land have lost topsoil and exposed to open conditions. *X. complanata* tolerant to the severe nutrient-deficiency in the peat soil had migrated and has successfully regenerated in the destroyed peatland over the past 15 years (Salzar, unpublished data). In these areas, the pH of the flooding water is 3.3-3.7 throughout the rainy season, and the stagnant water in dry season (May-October) is generally pH 3.2 and it sometimes drops to level of pH 3.0 (Hashidoko et al. 2005). In a preliminary experiment under a laboratory condition, we sowed *X. complanata* seeds on vermiculite bed wet with 1/4 Hoagland's no. 2 medium (pH 3.5), and maintained at 23°C under a 16-h-photoperiod. However, these seeds failed immediate germination, similar to *X. tennesseensis* (Baskin and Baskin 2003). Almost 4-weeks later, at which point the emergence of whitish fungal mycelia was observed around some seeds on the bed surface,

the germination of *X. complanata* seeds occurred for the first time around the fungal mycelia. This preliminary observation encouraged us to investigate seed-borne, helper fungi that stimulate seed germination of *X. complanata*. Our final goal of this research is to understand ecological position of *X. complanata* in associating with soil microorganisms in the acidic peat soil ecosystem, we here report investigation of the functional fungi and evaluate their seed germination-promoting activity.

Materials and Methods

Fungal isolation and identification

When non-sterile *X. complanata* seeds were sown on some vermiculite bedded dishes, active germination that coincided with fungal emergence were reproducibly observed. A vermiculite particle in the actively seed-germinating area was placed on the center of 1/2 Hoagland's no. 2 agar plate with 0.5% mannitol (pH 5.0), and incubated at 27°C for 2-3 days. A small part of the emerging mycelia was then moved to 100 mg/L chloramphenicol-containing potato-dextrose agar plates, and this purification procedure was repeated several times. Isolated mycelia were distinguished from each other by their mycelial color and microscopic observations for produced conidia (Gilman, 1957).

Chromosomal DNA of each fungus was extracted from 5-day-liquid cultured mycelium by means of sonication with glass beads (0.6 mm i.d.) as reported by Van Burik *et al.* (1998). Partial sequences of 5.8S and 18S rRNA genes and the ITS regions between them were determined for tentative genetic identification purposes (Table 1). The resulting mixture was then subjected to DNA extraction with the Isoplant II DNA extraction kit (Wako Pure Chemical Industries Ltd., Osaka, Japan). After the chromosomal DNA was obtained, ITS regions were amplified with PCR using the ITS1 forward (5'-TCCCGTAGGTGAACCTGCGC-3') and ITS4 reverse (5'-TCCTCCGCTTATTGATATGC-3') primers for eukaryotic microorganisms and give rise to a 450 bp PCR product (Kretzer and Bruns 1999; Sugita and Nishikawa 2003). The amplified DNA fragments were directly sequenced using the BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with these primers according to its protocol and sequenced with the ABI PRISM[®] 310 Genetic Analyzer (Applied Biosystems). The resultant DNA sequences were assembled and then searched for homology on the BLASTN DNA searching system in DDBJ (Database of DNA Bank of Japan).

Seed germination promotion assay

Surface sterilization of the seeds.

Approximately 100~300 seeds of *X. complanata* were placed in a sterilized 0.5-ml

microcentrifuge tube and subjected to surface sterilization. Under aseptic conditions, 200 μ l of 70% EtOH was added to the seeds and the tube was shaken with tapping for 15 sec. The EtOH was removed by immediate rinsing with 400 μ l of sterilized water. The resulting seeds were next rinsed with 500 μ l of antiformalin solution (prepared as 50 ml of 5% active chlorine solution containing 2-3 drops of Tween 20), and the tube was slowly shaken for 15 min, and finally rinsed with sterilized water. The resulting wet seeds were spread over sterilized filter paper allowing to air-dry for approximately 15 min.

For the purpose of gnotobiotic seed germination assay, approximately 400 of the surface-sterilized seeds in one portion were sown on potato-dextrose agar medium (Difco, Sparks, MD, USA). As a means to assess fungal contamination, the plates were incubated at 27°C for 4 days. When fungal mycelia emerged from a seeds, the seed contaminated with fungus was immediately removed from the agar medium together with the fungal mycelia. In the sterilization procedure, the contamination rate of the seeds was approximately 2%. The sterilized seeds that had passed the contamination test were washed with a portion of sterilized water three times for the germination assay.

Preparation of X. complanata seedbeds.

The germination of *X. complanata* seeds was tested on approximately 30 ml of Kalimantan peat soil, vermiculite, and also peat moss seed beds. All of these seedbeds were previously sterilized in a deep Petri dish (60 mm i.d. \times 60 mm height), except Kalimantan peat soil used in preliminary germination test for *X. complanata* seeds. In the case of the vermiculite bed (1-2 mm, Shouwa Vermiculite Co., Isehara, Japan), 1/4 Hoagland's no. 2 solution (pH 3.0 with addition of H₂SO₄) was added just until the vermiculite became saturated with moisture. This mixture was then autoclaved (120°C for 15 min). The vermiculite bed was used as isolation sources of active fungi. In sphagnum peat moss (chopped, Mori Sangyo Co. Ltd., Shihoro, Hokkaido, Japan), it was moistened with half volume of ion-exchanged water and then autoclaved (120°C for 15 min) and then gently squeezed in order to remove excessive water. Twenty ml of the moisture-adjusted peat moss was placed in a deep Petri dish and was autoclaved an additional time (120°C for 15 min) for complete elimination of contaminants. The peat moss bed was always used in the seed germination assay through this study.

Germination and survival assays on peat moss.

Conidia suspension of strains R-1 and Y-1 was obtained from the conidia-producing 10-day-old mycelia of each grown on potato-dextrose broth agar. The resulting spore suspension was adjusted CFU (colony forming unit)/ml of approximately 5×10^3 , and 100 μ l was inoculated on each Petri dish. Fungus strain B-1, which does not produce any spores in 50 ml of the potato-dextrose broth

medium, was shaking-cultured at 27°C for several days, and the resulting mycelia were homogenized with a blender (Nippon Seiki, Nagaoka, Japan) after removal of the culture medium. The resultant hyphal fragments passed through a sterilized tea strainer had a greater turbidity than that of the spore suspensions, although its CFU was not measured. In the case of the hyphal suspension, 100 µl was also inoculated to the seedbeds per Petri dish.

The dishes sown with *X. complanata* seeds (exactly 100) and inoculated with spore/mycelial suspension were maintained at a constant temperature of 30°C under 16 h-photoperiodic conditions in a plant growth chamber (EYELA LTD-600SE, Tokyo, Japan) equipped with LED light panels (LED-B 470 nm and RED-R 660 nm photodiode light, mixed as 1:4, 45 × 45 cm², EYELA, Tokyo, Japan). The resulting peat moss bed was observed at an interval of 1-3 days in order to count the number of germinating cotyledons, up to 45 days. Because some seedlings died after germination, the denoted germination rate evaluation actually represents the germination and survival rate of *X. complanata* seedlings. The germinating seeds were usually counted with the naked eye, and if necessary, a mesoscope was used to facilitate the counting (× 5-70, SZ-H10, Olympus Co., Tokyo, Japan).

In the precise seed germination assay using gnotobiotic system, we used surface-sterilized *X. complanata* seeds that had passed contamination test. In this experiment, 100 of sterilized seeds were sown onto one peat moss bed, and then inoculated with isolated fungus. After the seeding and fungal inoculation, the peat moss beds set in triplication for one treatment were observed up to 23-days. Unlike the seedbeds in Fig. 1, all of the beds used in the gnotobiotic experiment possessed only mycelial flora originated from the inoculant.

Growth promotion effect of the germination promoting fungi on *X. complanata* seedlings on peat moss

For seedling growth, 20 ml of autoclaved peat moss and 18 ml of 1/4 Hoagland's no. 2 solution (pH 2.5) were added to duplicated deep Petri dishes (60 mm in diameter). The peat moss beds were autoclaved again at 120°C for 15 min. The pH of the resulting liquid in the peat moss bed was elevated to pH 3.6 due to a buffering effect of the peat moss. Ten sterilized, 4-week-old seedlings pre-grown on the gellan gum gel bed were carefully transplanted under aseptic conditions. To treated seedling bed, the spore suspension (100 µl) of *Fusarium* sp. strain R-1 was inoculated, at which point these seedling beds were incubated for additional 8 weeks under the conditions as described above. Another dish was used for the control without any inoculation.

Dual culture experiment on the isolated fungi

A potato-dextrose agar medium allowing mycelial growth was cut off as a small piece, which was used as inoculant for the dual culture assay. Two different fungi were inoculated to the plate (\varnothing 9 cm) to keep 5 cm of distance between them, and then incubated for 3 days at 28°C. In three different combinations, resulting mycelial growth on the plates was observed.

Results

Identification of seed-epiphytic fungi

From the vermiculite beds which contained emerging seedlings, we isolated three distinct fungi. Morphologically, characters of these fungi (Table 1) are as follows: R-1; producing *Fusarium*-like conidia of reddish colored, B-1; without any conidia nor spore production, and Y-1; *Penicillium*-like conidia of a blue-green color. According to their DNA sequence homology in DDBJ database, R-1, B-1, and Y-1 were tentatively identified as *Fusarium* sp. (accession no. AB277209), *Curvularia* sp. (AB277211), and *Penicillium* sp. (AB277210), respectively.

Germination-promoting assay of seed epiphytic *Fusarium* sp. R-1 to surface-sterilized *X. complanata* seeds on peat moss

When 100 seeds of *X. complanata* were directly sown on a wet, autoclaved peat moss bed (pH 4.0~4.2), almost none of the seeds were capable of germination for 6 weeks. When 100 seeds of *X. complanata* were sterilized with 1% antiformin solution, the seeds did not germinate in the peat moss beds. Inoculation of *Fusarium* sp. R-1 to three replicate peat beds under the same conditions, the seeds started to germinate actively after 10 to 15-day-incubation period in 2 out of the 3 cases. In these cases, the germination and survival rates in the beds reached over 50% (Fig. 1).

Fungal microflora in all three beds (the two actively germinated and the less active bed) were re-investigated in an attempt to identify predominant microfloral components. In these seedbeds showing higher seedling germination and survival rates (R-1-A and R-1-B in Fig. 1), the predominant microfloral component was strain R-1. On the contrary, in the remaining bed that had relatively low seed germination despite the inoculation with *Fusarium* sp. R-1 (R-1-C), a blackish mycelial fungus identifiable as *Curvularia* sp. appeared as the most predominant microfloral component, along with *Fusarium* sp. R-1, a second-predominant fungus there due to contamination in the seeds.

Three isolates, *Fusarium* sp. R-1, *Curvularia* sp. B-1 and *Penicillium* sp. Y-1 were tested for their activity to promote seed germination toward sterilized *X. complanata* seeds selected on potato-dextrose agar plates. As shown in Fig. 2, both *Fusarium* sp. R-1 and *Penicillium* sp. Y-1 stimulated seed germination in the gnotobiotic assay. After 8 days, seeds in some dishes started to

germinate, and after 15 days, the germination and seedling survival ratio stabilized and was highly reproducible for Y-1 and R-1 inoculated seeds. When the survival ratio of the seedlings reached a stationary stage (2-3 weeks), *Penicillium* sp. Y-1-inoculated seedlings achieved statistically significant enhancement of germination and survival rates (78-86%) than *Fusarium* sp. R-1-inoculated seedlings (63-78%) (Fig. 2). In the *Curvularia* sp. B-1-inoculated seedbeds, the germination rate was nearly zero throughout the entire incubation period (for 3 weeks).

Growth promotion of the transplanted seedlings to peat moss.

The gnotobiotic assay for 10 seedlings of *X. complanata* seeds with or without *Fusarium* sp. R-1 inoculation showed different growth performance of the test seedlings. The surviving seedlings grown for 2 months were qualitatively compared for the aboveground portions. The R-1-inoculated seedlings were approximately 20-40 mm long with 5-6 leaves, while those from non-inoculated controls were only 10 mm long although it contained 4-5 leaves (Fig. 3). This preliminary growth test indicated that *Fusarium* sp. R-1 assisted the growth of *X. complanata* seedlings under moistened peat moss bed containing excessive humic substances. Similarly, *Penicillium* sp. Y-1 inoculated to *X. complanata* seedlings also showed growth promoting activity to *X. complanata* seedlings in the gnotobiotic system on a moistened peat moss bed (data not shown).

Competitions between isolated fungi in dual culture assay

As shown in Fig. 4, dual culture of R-1 with B-1 did not show any significant interference between them. In the boundary area between two of the mycelia, slight growth suppression of both B-1 and R-1 was observed. Approaching hyphae of B-1 and Y-1 reached to the other, suggesting their noninterference each other on their mycelial growth. In contrast, boundary area between B-1 and Y-1 showed a clear boundary as several millimeters of interference area, leading to obvious growth inhibition of B-1 by Y-1. The third dual culture between Y-1 and R-1 showed suppression of Y-1 mycelium but not in R-1. Altogether, it was apparent that R-1 is the toughest fungus among them, whilst Y-1 severely suppressed emergence of B-1, identical as phytopathogenic *Curvularia* sp.

Discussion

Properties of *X. complanata* seeds

A primary strategy of *Xyris complanata* for acquiring their niche is to produce thousands of tiny seeds and to deposit them to seed bank in the soil. Multiple factors in their natural environment, such as moisture levels, light conditions, ground temperature and the water table affect their chance for

germination, and among the successfully germinated seeds, only seedlings which possess enough space for leaf development can survive and maintain their pure community. In our experiments, germination of the seeds was inhibited in microbe-free, humic substances-rich soils, no matter how surface-sterilized or unsterilized (Figs. 1 and 2). In such adverse soil in microorganisms-free conditions, however, inoculation of two seed-attaching fungi, *Fusarium* sp. R-1 and *Penicillium* sp. Y-1, led to active germination of the dormant *X. complanata* seeds. In preliminary observation, these fungi also assisted growth of the germinated seedlings in the nursery bed soils. The presence of such helper fungi is likely an important component for the life cycle of *X. complanata* in natural habitats.

The partnerships between *X. complanata* and the seed germination-promoting fungi are thought to be a modest relationship unlike typical mutualistic symbiosis. This contrasts the tight relationship between orchids and orchid mycorrhizal fungi (Rasmussen 2002). Unlike orchid seeds which lack albumen to be dependent on symbiotic fungi, *X. complanata* seeds possess a tiny but genuine albumen (Rudall and Sajo 1999).

Ecological roles of the seed-borne, seed germination promoting fungi

Neither in *Fusarium* sp. strain R-1 nor in *Penicillium* sp. strain Y-1, dense colonization was rarely observed on the rhizoplane and/or in the root tissues of *X. complanata* seedlings. Our microfloral investigation of bulky soil in the acidic peat land in this research site indicated that *Trichoderma* spp. and some unidentified basidiomyceteous fungi are the predominant composers of the soil, while *Penicillium* spp. and *Fusarium* spp. were rarely found as predominate composers (data not shown). Both *Fusarium* sp. R-1 and *Penicillium* sp. Y-1 are thus found as seed epiphytes of *X. complanata*, and these seed-borne fungi are likely to be more specific to *X. complanata*.

Seed-borne fungi in many cereal crops are generally known to be phytopathogens (Richardson 1996), including many of *Fusarium* spp. known as common seed germination-suppressors world-wide (Ahmed et al. 2001; Klironomos 2002). *Curvularia* sp. strain B-1 is also categorized into a phytopathogenic seed-borne fungus. On the other hand, some seed-borne fungi are known to function as growth helpers for host plant (Barrow et al. 1997; Yamaji et al. 2001). A strain of *Penicillium damascenum* which had been isolated from *Picea glehnii* seeds was reported as a defensive partner of the host to be associated with the seedling roots (Yamaji et al. 2001). Some *Fusarium* spp. are also known to be defensive partners of the seedlings of family Brassicaceae (Ishimoto et al. 2004) and function as seed germination promoting fungi for an orchid (Vujanovic et al. 2000).

Similar to these seed-borne helper fungi, *Fusarium* sp. R-1 and *Penicillium* sp. Y-1 surely assisted not only seed germination (Fig. 2) but also seedling growth as reliable partner fungi of *X.*

complanata in peat soil (Fig. 3). Interaction between *X. complanata* and its helper fungi, thus, spotlighted adapting and surviving strategies of *Xyris* species in the open, acidic peatland. Germination-promoting effect of the native soil on *X. tennesseensis* seeds, as reported by Baskin and Baskin (2003), is probably explainable by certain soil fungi that are playing the similar role to R-1 and Y-1 in *X. complanata*. Seed germination promoting effect of R-1 on *X. complanata* seeds was, however, severely diminished by coexistence with *Curvularia* sp. B-1 (Fig. 1). This was reasonable because R-1 was unable to eliminate B-1 in the dual culture as shown in Fig. 4. In other words, it is more acceptable that *Penicillium* sp. Y-1 is characterized as the most active fungus to assist *X. complanata*, since Y-1 was able to defeat *Curvularia* sp. B-1 in competition *in vitro* (Fig. 4) and also showed the best effect on the gnotobiotic seed germination assay in Fig. 2. In either case, we will report elsewhere the effects of these fungi in sterilized and non-sterilized local land soil sampled from Central Kalimantan.

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Figure Title and Figure Legends

Fig. 1 Long-term seed germination and seedling surviving rate of *X. complanata* seeds in fungus-inoculated seedbeds of peat moss.

Effect of fungal inoculation on the germination of *X. complanata* seeds was investigated using seeds that had been swept seed surface with 0.25% antiformalin only. **(A)** Plots for germination rate of seedbeds sown surface-sterilized seeds. **(B)** Germination rate of seedbeds inoculated with *Fusarium* sp. strain R-1. Both **A** and **B** were in triplication. Seedling germination and survival rate (%) reflects the ratio of germinating seeds that survived on the beds, because some seedlings are killed soon after the germination by mycelia emerged from the peat moss beds. From the seedbeds in **B**, R-1-A, in which the most active seed-germination was observed, possessed only *Fusarium* sp. identical with inoculated R-1. In R-1-B and R-1-C, both of which showed relatively lower seed germination rates than R-1-A, *Curvularia*-like mycelium along with inoculated R-1 emerged. Particularly, emerging frequency of *Curvularia*-like mycelium in R-1-C was higher than R-1-B, reflecting the lowest germination rate of R-1-C. Solid medium used for microfloral investigation of the seedbeds was potato-dextrose agar. The agar plates were kept at 27°C for 2 days, after the inoculation with a small portion of the vermiculite from the seedbeds.

Fig. 2 Effect of isolated fungi on growth of completely surface-sterilized *X. complanata* seeds on the seedbeds of peat moss.

Each treatment was done in triplicate. (□) *Penicillium* sp. strain Y-1-inoculated dish, (■) *Fusarium* sp. strain R-1-inoculated, (○) *Curvularia* sp. B-1-inoculated, and (▲) control. Note that (○) and (▲) did not show any significant seed germination or survival of the seedling. Bars on the plots indicate standard deviation (\pm SD).

Fig. 3 Effect of *Fusarium* sp. R-1 on growth of *X. complanata* seedlings on the peat moss beds.

Four-week-old aseptically grown seedlings were transferred to a sterilized peat moss bed and were treated inoculation of *Fusarium* sp. R-1 (left) or a non-inoculated control (right). Two months subsequent to the transfer of the seedlings. *X. complanata* seedlings in the R-1-inoculated bed were thickly covered with whitish mycelia (arrow) and exhibited healthy growth without any indication of damage nor nutrient deficiencies (left). Also note that the tops of seedling leaves in the control (right) turned yellow to brown colors, indicative of nutrient-deficient symptoms (arrows).

Fig. 4 Competition between isolated fungi in dual culture assay.

PDA was used as the solid medium. The plates were incubated for 3 days at 27°C in the dark.

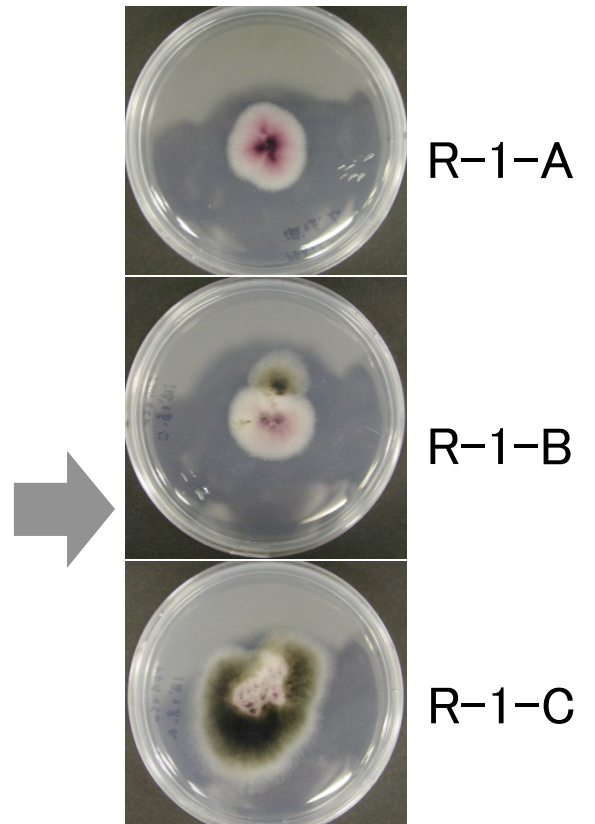
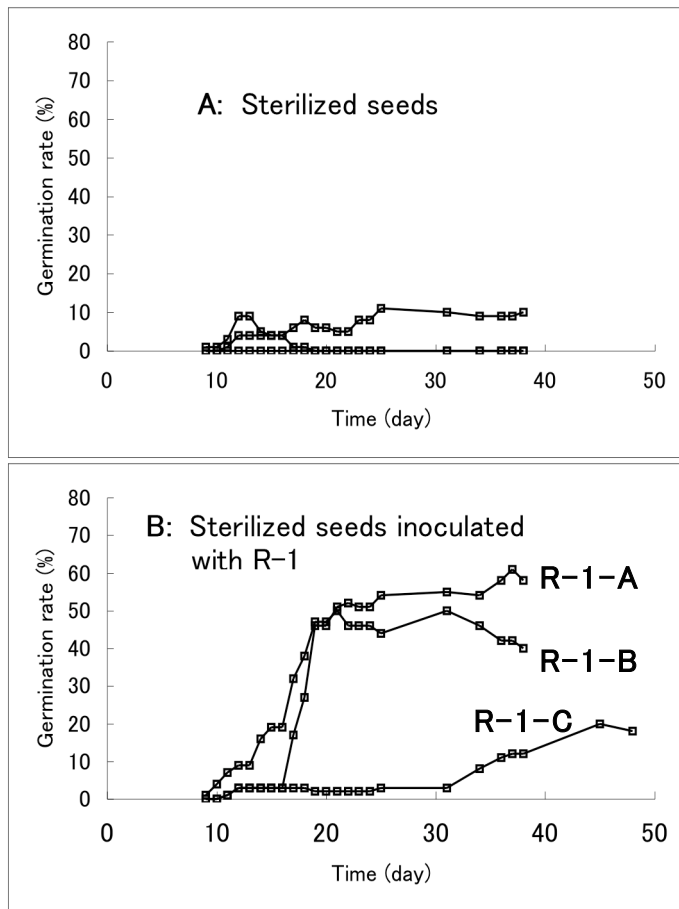


Fig. 1

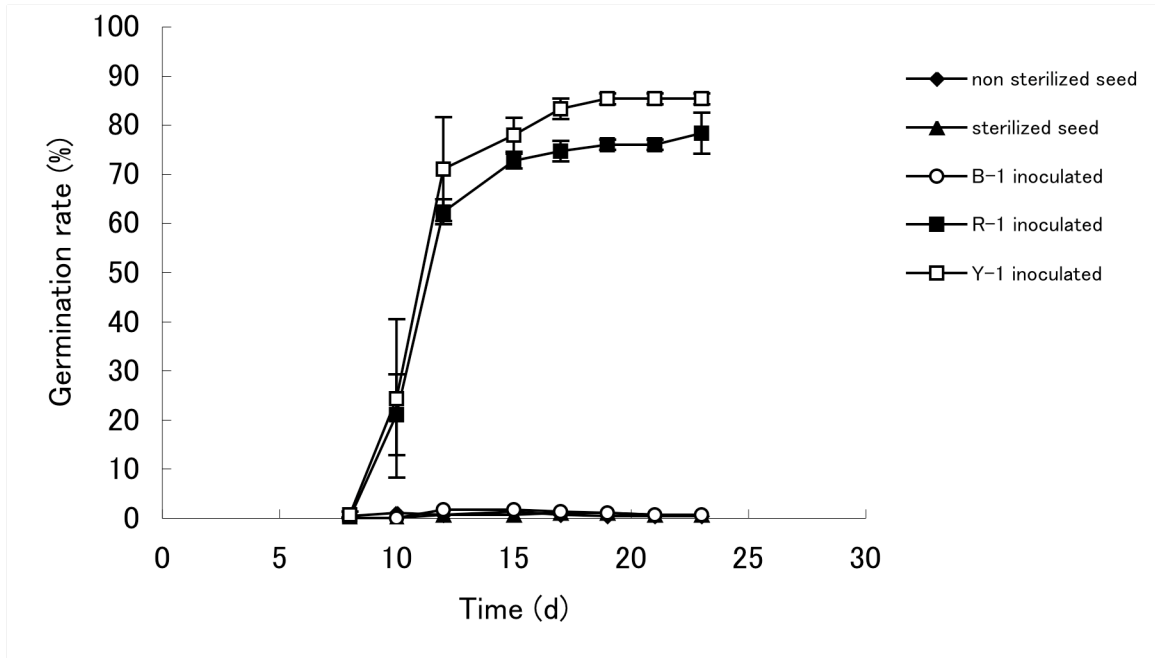


Fig. 2



R-1-inoculated seedlings



Non-inoculated seedlings

Fig. 3

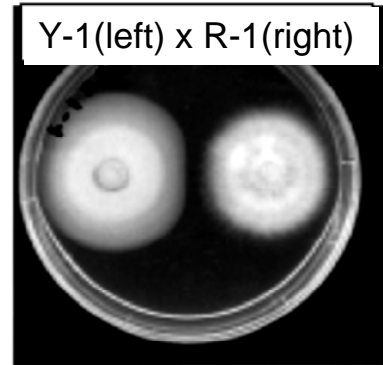
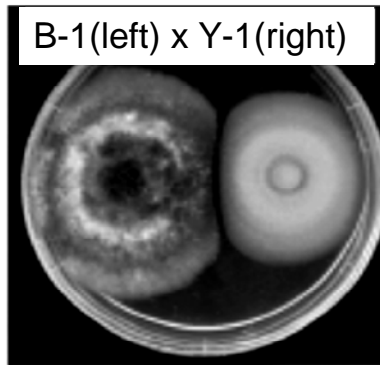
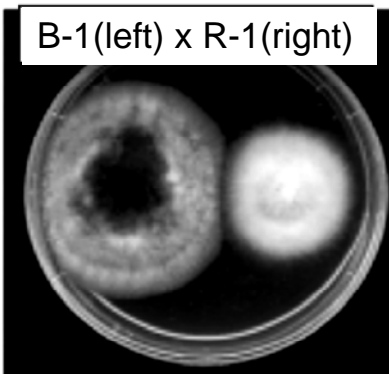


Fig. 4

Table 1 Fungal isolates from *X. complanata* seed-incubated vermiculite bed and their characteristics

Isolated fungus	Mycelial color	Conidia	Accession no.
<i>Fusarium</i> sp. R-1	reddish	+	AB277209
<i>Curvularia</i> sp. B-1	blackish	-	AB277211
<i>Penicillium</i> sp. Y-1	yellowish	+	AB277210

The fungi isolated from vermiculite sown unsterilized *X. complanata* seeds. For purification, potato-dextrose agar plates with 100 mg/l chloramphenicol were used.