New Mycorrhizal Fungi for Germinating *Goodyera schlechtendaliana*©

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A total of 24 mycorrhizal strains were obtained from wild *Goodyera* species (Orchidaceae) and their symbiotic activities for both seed germination and seedling growth of *G. schlechtendaliana* were evaluated. Isolates No. 9711 and No. 9735 were effective in promoting protocorm development.

INTRODUCTION

The symbiotic method of growing terrestrial orchids is well known as shown by the remarkable germination and seedling growth demonstrated by Clements (1986). In previous studies, the symbiotic culture technique was evaluated for orchid species in Japan (Tsutsui and Tomita, 1986; Tomita, 1995; Tomita and Konno, 1998; Tomita, 2001). *Goodyera schlechtendaliana* with binucleate *Rhizoctonia* grew markedly well compared with seeds on a nonsymbiotic culture (Tomita, 1995). The object of this study was to find an effective fungal isolate for *G. schlechtendaliana* in order to develop an effective propagation method for this orchid.

MATERIALS AND METHODS

Seed Material. Seeds of *G. schlechtendaliana*, from plants cultured in a greenhouse, were collected from fully ripened capsules and stored at 4°C in a small air-tight vial.

Collecting Fungal Isolates. Mycorrhizal roots of *Goodyera* species were collected from their habitats in Aomori prefecture and fungal isolates were obtained. The method for isolation of mycorrhizal fungi was identical to that used in a previous paper (Tsutsui and Tomita, 1986) and those cultures were maintained on potato dextrose agar (Clements et al., 1986).

Screening Test. Oat powdered agar (Tomita, 1995) was used. After surface sterilization, seeds were sown on the slope of a 25-mm × 150-mm test tube, with a sowing density of ca 300 seeds with embryos per test tube. At least five replicate test tubes were prepared. A 5-mm × 5-mm piece of agar block of a fungus subculture was added to the upper side of the slope. Cultures were maintained in a 16-h light and 8-h dark condition at 20°C. Isolate No.706, a good symbiont for *G. schlechtendaliana* (Tomita, 1995), was used for the control. Seeds were cultured for 16 weeks and then their germination rate and growth after germination were evaluated. The method of evaluating seedling growth were identical to that used in previous papers (Tomita, 1995;Tomita and Konno, 1998; Tomita, 2001).

RESULT AND DISCUSSION

Fungal isolates obtained from wild *Goodyera* are listed in Table 1. Forty plants of two *Goodyera* species were collected from six different habitats in Aomori prefec-

Table 1. Origin of orchid mycorrhizal fungi used in the experiment.

Isolate No.	Host orchid	Collection site
9711	*Goodyera schlechtendaliana*	Gosyogawara (A), Aomori
9712	"	Gosyogawara (A), Aomori
9713	"	Gosyogawara (A), Aomori
9714	"	Gosyogawara (A), Aomori
9715	"	Gosyogawara (A), Aomori
9716	"	Gosyogawara (A), Aomori
9717	"	Gosyogawara (A), Aomori
9718	"	Gosyogawara (B), Aomori
9719	"	Gosyogawara (B), Aomori
9720	"	Gosyogawara (B), Aomori
9721	"	Kanagi, Aomori
9722	"	Kanagi, Aomori
9723	"	Kanagi, Aomori
9726	*G. foliosa* var. *laevis*	Ajigasawa, Aomori
9727	"	Ajigasawa, Aomori
9728	"	Iwasaki (A), Aomori
9729	"	Iwasaki (A), Aomori
9730	"	Iwasaki (A), Aomori
9731	"	Iwasaki (A), Aomori
9732	"	Iwasaki (B), Aomori
9733	"	Iwasaki (B), Aomori
9734	"	Iwasaki (B), Aomori
9735	"	Iwasaki (B), Aomori
9736	"	Iwasaki (B), Aomori

ture. A total of 24 isolates were successfully obtained from those mycorrhizae (Table 1). All fungi were identified as belonging to *Rhizoctonia* (Sneh et al., 1991).

Screening test results are summarized in Table 2. Germination was observed on all orchid-fungus combinations; however, the germination percentages were significantly different among the isolate treatments. Among the fungal isolates, the effect of each isolate varied remarkably from non-effective to highly effective. Although germination percentages were not significantly different with Isolates No. 9711 and No. 9735, protocorms developed faster and were the same as for the control (Isolate No. 706). Isolate No. 706 is a good symbiont for not only *G. schlechtendaliana* (Tomita, 1995) but also other *Goodyera* species (Tomita and Konno, 1998). Further

Table 2. Effects of fungal endophytes on seed germination and seedling development of *Goodyera schlechtendaliana*.

Isolate No.	Germination (%)	Developmental index[z]
9711	52.7 a[x]	3.9 a[x]
9712	45.7 ab	2.2 cd
9713	55.8 a	3.2 b
9714	40.8 b	2.1 cd
9715	28.6 c	1.2 d
9716	42.2 ab	1.9 cd
9717	42.6 ab	1.6 d
9718	43.9 ab	1.5 d
9719	47.3 ab	2.4 c
9720	44.4 ab	2.3 c
9721	46.5 ab	2.2 c
9722	41.8 ab	2.6 c
9723	44.9 ab	2.9 bc
9726	34.2 bc	2.7 c
9727	22.8 c	1.0 d
9728	23.6 c	1.2 d
9729	22.9 c	1.0 d
9730	25.5 c	1.0 d
9731	27.6 c	1.0 d
9732	27.9 c	1.3 d
9733	22.9 c	1.1 d
9734	27.3 c	1.0 d
9735	52.9 a	3.8 a
9736	21.8 c	1.0 d
706 (Control)	55.9 a	3.7 a

[z] Tomita and Konno (1998).
[x] Mean separation within column by Duncan's multiple range test at 5% level.

investigation about the symbiotic activities of Isolates No. 9711 and No. 9735 on other *Goodyera* species is needed to improve symbiotic propagation systems for *Goodyera* species.

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