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Short Communication

Seed germination of *Galeola altissima*, an achlorophyllous orchid, with aphyllophorales fungi

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Seed germination test of *Galeola altissima* was carried out with five aphyllophorales fungi: *Erythromyces crocicreas, Ganoderma australe, Loweporus tephroporus, Microporus affinus* and *Phellinus* sp.. All five species were effective for seed germination of the orchid. *Erythromyces crocicreas,* which has hitherto been regarded as the only endomycorrhizal fungus of the orchid, was confirmed to be effective for further development of the orchid.

Key Words——aphyllophorales; germination of achlorophyllous orchid; *Galeola altissima.*

Little is known about the perfect stages of endomycorrhizal fungi of achlorophyllous orchids, and in most cases one or two fungal species were found specifically on one or a few orchid species. For example, *Marasmius coniatus* var. *didymoplexis* Berk. et Br. was the mycorrhizal fungus of *Didymoplexis pallens* Griff. and *D. minor* J. J. Smith (Burgeff, 1932); *Xerotus javanicus* Ade was that of *Gastrodia javanica* (Bl.) Lindl. (Burgeff, 1932); one or two groups of *Armillaria mellea* complex* were those of *Gastrodia elata* Bl. and *Galeola septentrionalis* Reichb. f. (Kusano, 1911; Hamada, 1939); *Armillaria tabescens* (Scop. ex Ff.) Emel. was that of *G. septentrionalis* (Terashita and Chyuman, 1987); and *Fomes* sp. was that of *Galeola hydra* Reichb. f. (Burgeff, 1936).

Erythromyces crocicreas (Berk. et Br.) Hjortst. et Ryv. (=*Hymenochaete crocicreas* Berk. et Br.), which is regarded as a mycorrhizal fungus of *Galeola altissima* Reichb. f. (Hamada and Nakamura, 1963), is the only fungal species hitherto found on the orchid.

The present article reports on the seed germination test of *G. altissima* with five species of aphyllophorales fungi, namely, *E. crocicreas, Ganoderma australe* (Fr.) Pat., *Loweporus tephroporus* (Mont.) Ryv., *Microporus affinus* (Fr.) Kunt. and *Phellinus* sp.

Materials and Methods

Fungi The fungal strains used in this test are shown in Table 1. All were obtained from trama or context of fruit bodies and maintained on potato-dextrose agar medium at room temperature.

Galeola altissima *Galeola altissima* (Bl.) Bl. (Fig. 1) is a climbing, liane-like, perennial achlorophyllous orchid which produces about 36 m long terrestrial stems (Makino and Nemoto, 1931), and is distributed from Tanegashima Island and Ryukyu Islands in Japan to Taiwan, the Philippines, Malaysia, India and Indonesia (Corner and Watanabe, 1969; Hatusima, 1971). The plant flowers from May to June and the fruit ripens from late October to November in Kutinoerabujima Isl., Kagoshima Prefecture. The ripe, but not yet dehiscing capsules, of *G. altissima* were collected at the island on 12 Nov., 1992 and the seeds from capsules were refrigerated at 5.5±1°C.

Cultures The medium for cultivation of the plant and test fungus consisted of $Ca(NO_3)_2 \cdot 4H_2O$, 170 mg; $MgSO_4 \cdot 7H_2O$, 240 mg; KCl, 80 mg; NH_4NO_3, 60 mg; KH_2PO_4, 40 mg; EDTA-Na-Fe Salt, 3,850 mg; $MnCl_2 \cdot H_2O$, 0.4 mg; $CuSO_4 \cdot 5H_2O$, 0.05 mg; $ZnSO_4 \cdot 7H_2O$, 0.05 mg; H_3BO_3, 0.6 mg; $H_2MO_4 \cdot H_2O$, 0.02 mg; D-mannitol, 20.0 g; yeast extract (Difco), 2.0 g; Bacto-agar (Difco), 10.0 g; and distilled water, 1,000 ml. The pH was adjusted to 5.7±0.1 with 0.5 M HCl or 0.5 M NaOH. Sawdust was prepared from the wood of broad-leaved trees (e. g., *Castanopsis sieboldii* Hatusima), which had been dried in an oven for 1 h at 150°C. Test tubes (18 mmϕ×180 mm) containing 10 ml of the above agar medium, and large test tubes (30 mmϕ×200 mm) containing 30 ml of the same liquid medium in which the agar was replaced by 5.0 g of sawdust, were autoclaved for 20 min at 121°C. The six strains of fungi were inoculated separately on these media and incubated at 23°C for 3-4 weeks in darkness.

The seeds of *G. altissima* were surface-sterilized by successively shaking for 1 min in 75% ethanol and 10 min in 10% solution of calcium hypochloride, and then rinsed twice or three times with sterilized distilled water. The sterilized seeds were dried in a sterilized

* *Armillaria mellea* Vahl. in Kusano (1911) and *A. mellea* (Vahl.) Quél. in Hamada (1940).

Table 1. Species, families and collection sites of fungi effective for the seed germination of *Galeola altissima*.

Strain No.	Species	Family	Collection site
F209	*Loweporus tephroporus*[1)]	Polyporaceae	Kutinoerabujima, Kagoshima Pref.
F210	*Microporus affinus*[1)]	Polyporaceae	Kutinoerabujima, Kagoshima Pref.
WD592	*Erythromyces crocicreas*[2)]	Hymenochaetaceae	Amamiohshima, Kagoshima Pref.
F215	*E. crocicreas*[1)]	Hymenochaetaceae	Kutinoerabujima, Kagoshima Pref.
F216	*Phellinus* sp.[1)]	Hymenochaetaceae	Kutinoerabujima, Kagoshima Pref.
F217	*Ganoderma australe*[3)]	Ganodermataceae	Kutinoerabujima, Kagoshima Pref.

1) On the dead trunks of *C. sieboldii* to which *G. altissima* adhered.
2) In a natural forest containing *C. sieboldii*, kindly provided by Mr. T. Hattori, Forestry and Forest Products Research Institute, Ministry of Agriculture, Forestry and Fishery, Japan.
3) On a dead trunk of *C. sieboldii* in a forest inhabited by *G. altissima*.

desiccator for 2 or 3 days at room temperature.

The method of culture followed Tashima et al. (1978). Seeds were attached to bamboo needles (4 mmϕ × 50 mm), which were planted on the agar or sawdust surface in the test tubes where the fungi grew. Needles with attached seeds were also planted on the same agar medium without fungi.

These test tubes were incubated at 30°C in darkness for 8 months and the germination was observed under the binocular microscope.

Results and Discussion

Fugal species which were effective for seed germination The seeds successfully germinated with each of the five species of fungi: *E. crocicreas* (Fig. 2), *G. australe* (Fig. 3), *L. tephroporus* (Fig. 4), *M. affinus* (Fig. 5) and *Phellinus* sp. (Fig. 6). Germination did not occur in the absence of the fungi. Figs. 2-6 show the seed germination and the further growth of *G. altissima* with the fungi. In experiments by Nakamura (1962), orchid seeds were germinated sterilely on the Ebios-sucrose agar medium in sealed vessels after immersion in 1% KCl solution. In the present experiment, the seeds were not immersed in KCl solution and the test tubes were not sealed. The results suggested that the five fungal species had the ability to stimulate germination of the orchid seeds.

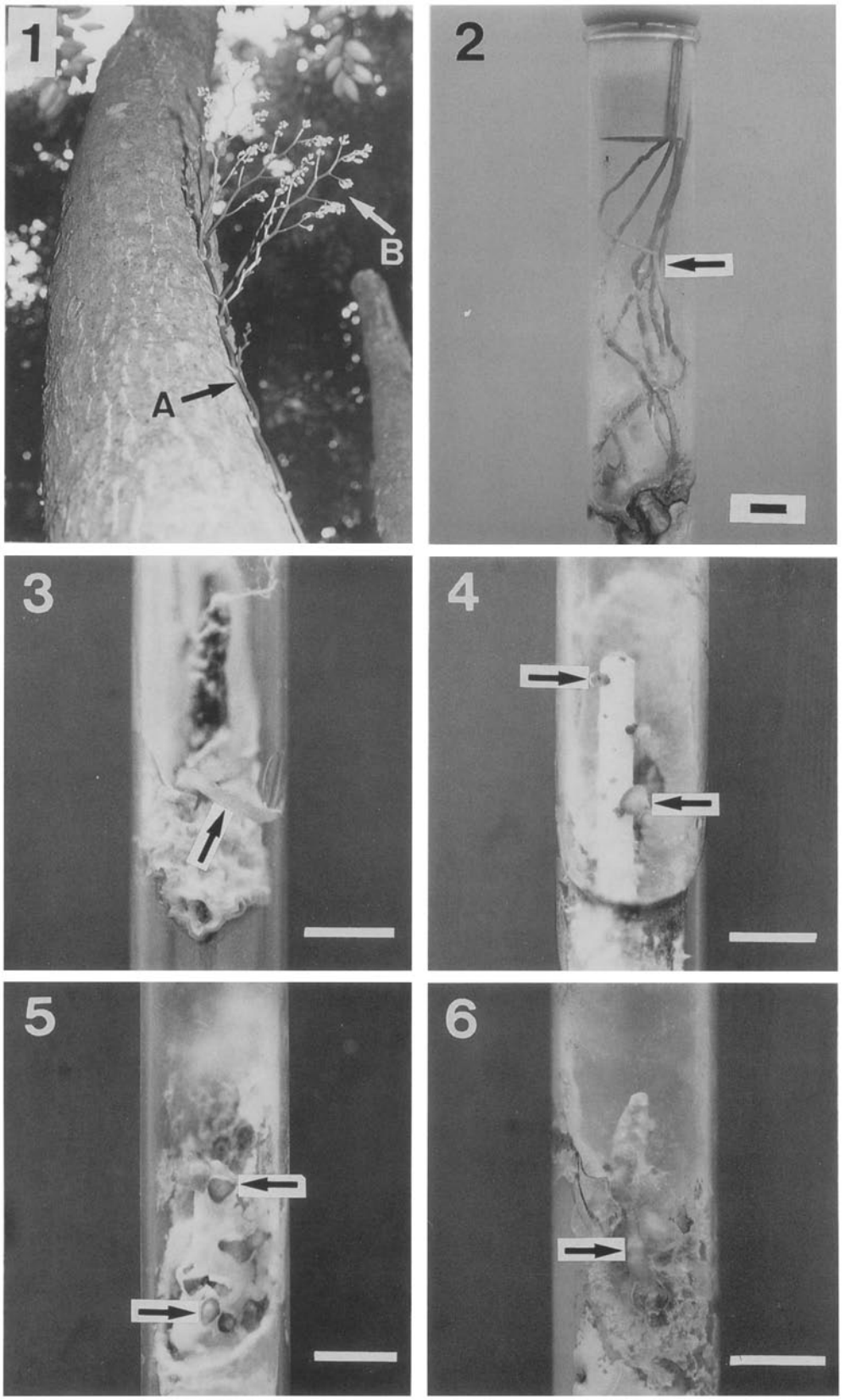
According to Tashima et al. (1978), in preliminary experiments on the culture of the achlorophyllous orchid *Gastrodia verrucosa* Bl., 18 of about 100 fungal strains were effective for its seed germination. These about 100 fungal strains were isolated from the roots of *G. verrucosa* and *G. nipponica* Tuyama and isolated from fruit bodies of some basidiomycetes which were collected around the two *Gastrodia* orchids. And two of them, one isolated from the roots of *G. verrucosa* and the other from *G. nipponica*, were effective for further development of *G. verrucosa*. Masuhara and Katsuya (1991) showed that the seedlings of *G. septentrionalis* associated with 8 of 12 strains of *Rhizoctonia repens* Bernard which were obtained from roots and protocorms of several species of green orchid.

Table 1 shows the species and family names of the five fungi effective for the seed germination of *G. altissima*. These five species are diverse in their taxonomy. Whether *G. australe, L. tephloporus, M. affinus* and *Phellinus* sp. are mycorrhizal fungi of *G. altissima* will only be confirmed by observation of these four fungi in association with the roots of the plant under natural and in vitro conditions. It is possible that the five fungi produce similar secretions effective for the seed germination of *G. altissima*.

Erythromyces crocicreas *Erythromyces crocicreas* is distributed from Cape Sata in Japan (Aoshima and Ogimi, 1974) to the Philippines, Indonesia, Cambodia, Malaysia, Fiji, Australia and Africa (Gabon) (Hjortstam and Tellería, 1990). Its distribution in Asia is similar to that of *G. altissima* in many localities. In Japan, this fungus was first reported as the mycorrhizal fungus of *G. altissima* by Hamada and Nakamura (1963), and is the only mycorrhizal fungus of the orchid hitherto found. The WD1459** strain isolated by Hamada and Nakamura was effective for the seed germination of *G. altissima* (Umata, 1992). In the present study, two fungal isolates from fruit bodies were also effective for the seed ger-

Fig. 1. *Galeola altissima* climbing a trunk of *Castanopsis sieboldii*. A: terrestrial stem. B: inflorescence.
Fig. 2. Roots of *Galeola altissima* developing 15 months after culture with *Erythromyces crocicreas* (WD592). Note that roots show negative geotropism. Bar = 1 cm.
Figs. 3-6. Germination and growth of *Galeola altissima* 8 months after culture with fungi. Arrows show germinated seeds (4, 5) and roots (3, 6).
3. *Ganoderma australe*. 4. *Loweporus tephroporus*. 5. *Microporus affinus*. 6. *Phellinus* sp. Bars = 1 cm.

** This is preserved in the Forestry and Forest Products Research Institute, Ministry of Agriculture, Forestry and Fishery, Japan, and is the same as Ga1002 in the previous paper (Umata, 1992).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mination. These two fruit bodies had been collected at different localities: Kutinoerabujma Isl. and Amamiohshima Isl. Furthermore, the germinated seeds with those fungi developed to form a very short stem and long roots which showed negative geotropism (Fig. 2).

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