Short Communication

Naturally germinating seeds of the achlorophyllous orchid *Galeola septentrionalis* contained no fungal pelotons

UMATA Hidetaka[1], KUBOTA Katsuyoshi[2], INOUE Sachiko[2],
SHIIBA Yasuki[3], NAGASAWA Hisami[3]

1) University Forests, Faculty of Agriculture, Kagoshima University, Kōrimoto 1-21-24, Kagoshima 890-0065, JAPAN

2) Shiiba Research Forest, University Forests, Faculty of Agriculture, Kyushu University, Ōkawachi 949, Shiiba-son, Higashiusuki-gun, Miyazaki, 883-0402, JAPAN

3) Kasuya Research Forest, University Forests, Faculty of Agriculture, Kyushu University, Tsubakuro 394, Sasaguri-chō, Kasuya-gun, Fukuoka, 811-2415, JAPAN

Received Jun 30, 2006 / Accepted Oct 10, 2006

Abstract

Galeola septentrionalis is an achlorophyllous myco-heterotrophic orchid and has been known to establish a symbiotic association with several biological species of *Armillariella mellea* (Basidiomycetes). However, there have been no successful reports elucidating an association between a mycobiont and seeds of this orchid during germination. In this study, a field sowing experiment using seeds from this orchid was conducted for more than 14 months in the natural habitat of this species. Approximately 0.1% of the seeds germinated as the enlarging embryos ruptured the outer seed coat. Seeds that failed to germinate were collected and cultured *in vitro* on an agar medium containing mannitol, yeast extract, and other nutrients and incubated at 27.5℃. Approximately 4% of the seeds germinated six weeks after sowing. In this case, however, the two valves of the seed coat opened to release the enlarged embryo. The embryos of germinating seeds were devoid of fungal pelotons. Seed germination appears to take place in the absence of mycobiont infestation in this species.

Key Words: Achlorophyllous orchid, Field sowing, *Galeola septentrionalis,* Germination, Myco-heterotrophic plant, Peloton

Introduction

Because achlorophyllous orchids lack the ability to perform photosynthesis and so derive an independent source of organic nutrition, they are thought to be dependent throughout their lives on the mycobionts within their roots or tubercles. Furthermore, the seeds of achlorophyllous orchids are very small in size and contain only minimal nutritional reserves within their embryos, and it is therefore generally assumed that an external supply of carbon is required for germination. Mycobionts are capable of satisfying this requirement for germination. For example, the seeds of the achlorophyllous orchid *Erythrorchis ochobiensis* (Hayata) Garay (formerly *Galeola altissima* Reichb. f.) were found to germinate only in the presence of a mycobiont (Umata, 1995).

The achlorophyllous orchid *Galeola septentrionalis* Reich. f. has been shown to be symbiotic with the Basidiomycetous fungus *Armillariella mellea* (Vahl.: Fr.) Karsten (Hamada, 1939) and several biological species of *A. mellea* (Cha and Igarashi, 1996; Terashita, 1996). When protocorms of this orchid were co-cultured with *A. mellea*, they showed successive further growth to develop roots. Pelotons were formed within the roots and these pelotons exhibited the same microscopic characteristics as those observed within the roots of wild plants (Terashita, 1985). However, there have been no reports of an association between a mycobiont and seeds of *G. septentrionalis* during

Discussion

Though it was anticipated that a germination process similar to that of *G. elata* might occur also in *G. septentrionalis,* the present study showed that the germination processes were not identical in the two species. According to Xu & Mu, (1990) and Hong et al. (2002), *G. elata* requires mycobionts for germination of the seeds. And according to our unpublished data, a positive germination response was also observed in seeds of *G. elata* that had been buried *in situ* in a field sowing experiment similar to that conducted in the present study; seeds that germinated were found to contain fungal pelotons within their embryos (Fig. 7, 8). Thus the results suggest that whereas *G. elata* requires direct association with mycobionts for germination under natural conditions, *G. septentrionalis* does not. Given that seeds of *G. septentrionalis* germinated even on a simple agar medium *in vitro* (Nakamura, 1964), it appears that this orchid has the ability to germinate without an exterior nutrient supply in the wild. An association with mycobionts essential for subsequent development must be established after germination.

The outer seed coat of *G. septentrionalis* is made up of two hard valve-shaped structures attached tightly to one another, as in a bivalve (see illustration in Nakamura (1964)). These protect the embryo. The results of the present study suggest that this seed-coat structure is intrinsically linked with the mode of germination, as either Type-Burst or Type-Open, depending on the manner in which the growing embryo is released from the coat. As to the nature of Type-Burst germination, this can be interpreted as a result of the enlarging embryo being unable to split open the two valves along their seal, because they are too tightly bound. Thus, only the few growing embryos strong enough to break through weak points in the seed coat were seen to germinate in this study (field sowing experiment and 31st March experiment). Because the culture methods for germination developed by Nakamura (1967, 1975) were not applied in the March and the April experiments here, the occurrence of Type-Burst germination may reflect either that some embryos had already enlarged within the seed coats, or that dormancy had been broken prior to 29th March. An interpretation of the manner in which the seed coat behaves may also be applicable to those seeds that exhibited Type-Open germination (24th April experiment). That is, following an event that loosens the seal between the two valves, the growing embryo is able to split open the seed coat. *E. ochobiensis,* which has a seed similar in structure to that of *G. septentrionalis,* also has a similar germination mode, Type-Open germination in the presence of mycobionts (Umata, 1998). In *G. septentrionalis,* given that the number of seeds that exhibited Type-Open germination far out-numbered those that exhibited Type-Burst and germination under asymbiotic culture is Type-Open as can be inferred from the illustration in Nakamura (1964), it is suggested here that majority of the seeds may go through Type-Open germination in nature.

It was reported that some myco-heterotrophic plants germinated without direct mycobiont-seed contact. That is, seeds of the two myco-heterotrophic plants belonging to Monotropaceae, *Pterospora andromedea* Nuttall and *Sarcodes sanguinea* Torrey can germinate if the seeds are placed at the edge of mycobiont colonies or on cellophane covering the colonies (Bruns and Read, 2000). It is suggested that a diffusible or volatile compound unique to the mycobiont stimulates germination. The embryos of germinating seeds of *G. septentrionalis* are also devoid of fungal pelotons. However, germination was accelerated when cultured on an agar medium containing nutrients and seeds exhibited Type-Open germination. In addition, various microorganisms were observed colonizing the medium. These suggest that nutrients or microorganisms probably accelerate the loosening of the seal between the two valves forming the seed coat.

In conclusion, the present investigation demonstrates that seeds of *G. septentrionalis* germinate independently from mycobiont invasion in nature. The germination process probably works as follows: firstly, the embryo swells within the seed coat and keeps swelling until the seed coat opens. Then, the seed coat opens as the seal between the two valves is loosened releasing the swelling embryo. However, the factors that bring about the enlargement of the embryo under natural conditions, whether atmospheric conditions as proposed by Nakamura (1975) or otherwise, have yet to be determined. Furthermore, the means by which the seal between the two valves of the outer seed coat are loosened to release the growing embryo and what kinds of mycobionts, *A. mellea* or other species as observed in *G. elata* invade first the germinating seeds, also remain unknown. Further work is required in order to resolve these issues.

Acknowledgments

We wish to thank Dr. Shin Inoue, the director of Shiiba Research Forest, for permitting us to carry out our experiments in the Research Forest and Mr. Stephan Gale (Kochi Prefectural Makino Botanical Garden) for proofreading the manuscript.

Literature cited

Bruns TD, Read DJ (2000) In vitro germination of nonphotosynthetic, myco-heterotrophic plants stimulated by fungi isolated from the adult plants. New Phytol. **148(2)**: 335-342.

Cha JY, Igarashi T (1996) *Armillaria jezoensis,* a new symbiont of *Galeola septentrionalis* (Orchidaceae) in Hokkaido. Mycoscience **37**: 21-24.

Hamada M (1939) Studien uber die Mykorrhiza von *Galeola septentrionalis* Reich. f. – Ein neuer Fall der Mykorrhiza-Bildung durch intraradicale Rhizomorpha. Jpn J Bot. **10**: 151-212

Hong IP, Kim HK, Park JS, Kim GP, Lee MW, Guo SX (2002) Physiological characteristics of symbiotic fungi associated with the seed germination of *Gastrodia elata.* Mycobiology **30 (1)**: 22-26.

Kusano S (1911) *Gastrodia elata* and its symbiotic association with *Armillaria mellea.* Jour Agri Col Tokyo Imp Univ. **4(1)**: 1-65, pl. I - V, Tokyo

Mori K, Hamaya E, Shimomura T, Ikegami Y (1969) Production of virus-free plants by means of meristem culture. J Cent Agr Exp Sta **13**: 45-110

Nakamura SI (1962) Zur Samenkeimung einer chlorophyllfreien Erdorchidee *Galeola septentrionalis* Reichb. f. Z Bot **50**: 487-497

Nakamura SI (1964) Einige Experimente zur Zamenkeimung einer cholorophyllfreien Erdorchidee *Galeola septentrionalis* Reichb. f. Memoirs of the College of Agriculture, Kyoto University **86** (Botanical Series No.4): 1-48

Nakamura SI, Uchida T, Hamada M (1975) Atmospheric condition controlling the seed germination of an achlorophyllous orchid, *Galeola septentrionalis.* Bot Mag Tokyo **88**: 103-109.

Terashita T (1985) Fungi inhabiting wild orchids in Japan (III). A symbiotic experiment with *Armillaria mellea* and *Galeola septentrionalis.* Trans Mycol Soc Japan **26**: 47-53.

Terashita T (1996) Biological species of *Armillaria* symbiotic with *Galeola septentrionalis.* Nippon Kingakukai Kaihho **37**: 45-49. (In Japanese)

Umata H (1995) Seed germination of *Galeola altissima,* an achlorophyllous orchid, with aphyllophorales fungi. Mycoscience **36**: 369-372.

Umata H (1998) In vitro symbiotic association of an achlorophyllous orchid, *Erythrorchis ochobiensis,* with orchid and non-orchid fungi. Mem Fac Agr Kagoishima Univ **34**: 97-107

Xu JT, Ran K, Guo S (1989) Studies on the life cycle of *Gastrodia elata.* Acta Academiae Medicinae Sinicae **11(4)**: 237-241

Xu JT, Mu C (1990) The relation between growth of *Gastrodia elata* protocorms and fungi. Acta Botanica Sinica **32**: 26-31

埋設処理により発芽した無葉緑ラン科植物ツチアケビの胚には菌球がなかった

馬田　英隆[1)]・久保田勝義[2)]・井上　幸子[2)]・椎葉　康喜[3)]・長澤　久視[3)]

1）鹿児島大学農学部附属演習林　〒890-0065　鹿児島市郡元一丁目21-24

2）九州大学農学部附属演習林宮崎演習林　〒883-0402　宮崎県東臼杵郡椎葉村大河内949

3）九州大学農学部附属演習林福岡演習林　〒811-2415　福岡県糟屋郡篠栗町津波黒394

和文要約

ツチアケビは無葉緑の菌寄生性のラン科植物で，ナラタケ菌の生物学的種の数種と共生することが知られている。しかしながら，これまで本種の種子がその発芽の過程の中でナラタケ菌と共生したという報告はない。本研究では，ツチアケビ種子を母植物の発生地に14ヶ月間以上埋設する試験を行った。その結果，約0.1％の種子が外種皮を破って発芽していた。また，その際に回収した未発芽種子をマンニット，精製酵母などを含む培地で27.5℃で培養すると，約４％の種子が外種皮の殻を開いて発芽した。発芽した種子の胚の内部に菌球は見あたらなかった。ツチアケビの種子においては共生菌の侵入がなくても発芽するものと思われる。

キーワード：無葉緑ラン，野外播種，ツチアケビ，発芽，菌寄生植物，菌球