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Seed Germination of *Gastrodia elata* Using Symbiotic Fungi, *Mycena osmundicola*

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The germination rate and longevity of seeds of *Gastrodia elata* Blume have been observed for 48 weeks using *Mycena osmundicola* strain H-21, one of fungi stimulating seed germination. Storage condition of post-harvest seeds was observed in the different temperature ranges of –30°C, –5°C, 5°C and 30°C for 48 weeks. After storage period of 48 weeks, the germination rate of *G. elata* was 65.7% at 5°C and 71.6% at –5°C, respectively. Although the germination rate of *G. elata* was 77.3% for 11 weeks at 25°C, the germination rate had been decreased gradually to 49.3% at 13 weeks, 0.3% at 23 weeks and then 0% at 25 weeks. The germination rate was reached to the level of 10% for 2 weeks at –30°C and then decreased to 0%.

KEYWARDS: ***Gastrodia elata* Blume, Germination rate, *Mycena osmundicola*, Storage condition**

Gastrodia elata Blume belongs to the Orchidaceae, and has been known to be distributed widely in Korea, China and Japan. The dried tubers of *G. elata* have been used as a traditional Chinese herb for curing human diseases such as vertigo, blackout, headache, gemiplegia and convulsions epilepsy under the name of "Cheonma" for several centuries in Asian countries (Huang, 1985; Chang and But, 1986).

G. elata is aphyllous and achlorophyllous orchid plant, and has been known to need a symbiont necessary to the growth of *G. elata* under the natural conditions. *Armillaria mellea*, one of these symbionts has been engaged in the growth of *G. elata* in the form of energy metabolism (Kusano, 1911; Zhang and Li, 1980; Choi and Lee, 1983; Hong *et al.*, 1990).

Zhang and Li (1980) observed the biological relationship between *G. elata* and *A. mellea*. They pointed out ontogenesis of *G. elata* has four stages: for example, four stages such as seedling formation, tuber formation, flowering and fruiting. They found that there were two modes of infection of *A. mellea* on *G. elata*. Under normal conditions, *A. mellea* has been known to infect the cortical layer of *G. elata*. On the contrary, the digestive cells posses both the functions of defense and infecting hyphae. Some researchers suggested a pathological infection often was caused under unfavorable conditions (Zhang and Li, 1980; Sung *et al.*, 1996).

To develop tubers of *G. elata* from its seedlings, the renewal of its vegetative organ has been known to depend on *A. mellea* (Zhang and Li, 1980; Lee, 1983; Sung *et al.*, 1995).

Although *G. elata* has been cultured widely in Korea and China, Korean farmers are faced with some troubles in tuber production of *G. elata*. The yields of *G. elata* have been recently decreased owing to the degeneration of spawn tuber arisen from a successive asexual reproduction and technical problem of managing fungal organisms caused by the mistake of farmers themselves (Guo and Xu, 1991; Hong *et al.*, 2002). One possible way to solve these problems had been suggested elaborately to use seeds instead of vegetative propagation (Clements *et al.*, 1986).

Since the seeds of *G. elata* are not only very small but do not possess an endosperm, the germination rate of its seed is poor or not at all in nature (Nakamura, 1982: Xu and Guo, 1990). Therefore, many researchers have done to elucidate some mechanism by applying the viewpoint of histology and enzymology to seed germination of *G. elata*.

Though *A. mellea* has an outstanding effect on tuber formation of *G. elata*, some strains of *A. mellea* have been known to inhibit the seed germination of *G. elata* in nature (Xu and Mu, 1990).

Guo and Xu (1991) overcame an obstacle of degeneration of *G. elata* by using seed propagation method. Also, they pointed out some enzyme promoted seed germination in orchidaceae plant. Especially, they demonstrated that estrase isozyme of six fungal strains could promote the seed germination of *G. elata* by comparing the enzyme patterns of five fungal strains with those of *Mycena osmundicola* (Guo and Xu, 1991; Li *et al.*, 1999). To

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develop protocorm from seeds, Xu and Mu (1990) observed the symbiotic relationship of both *M. osmundicola* and *G. elata*. They reported the cytological observation that hyphae of *M. osmundicola* invaded seed coat in the process of seed germination of *G. elata*. To obtain the nutrient sources necessary to sprout seeds of *G. elata*, the seeds should be cultured with some fungi helpful to formation of protocorm, because the seed is small and does not contain an endosperm in itself (Xu and Guo, 1990; Li *et al.*, 2000).

Hong *et al.* (2002; 2004) have observed optimal factors of seed germination such as an optimal medium in the storage of seeds, protocorm formation, optimum range of temperatures and substrates suitable for fungal species promoting seed germination (Hong *et al.*, 2002, 2004).

They concluded that favorable seed germination of *G. elata* depended on optimal temperatures during the storage of its seeds.

In this experiment, we tried to check the optimal storage period and temperature for the storage of post-harvest seeds.

Materials and Methods

Microbial cultures and inocula. *Mycena osmundicola* strain H-21, one of fungal strains inducing seed germination of *G. elata* was received from Guo (Institute of Medicinal Plant Development, China, Xu and Guo, 1989) and then maintained on PDA for the culture (Hong *et al.*, 2002). For a germination assay of *G. elata*, the culture media which *M. osmundicola* H-21 was cultured were prepared by mixing fallen leaves of *Quercus acutissima* with rice bran at the ratio of 8 : 2 (V/V). The culture media were inoculated with *M. osmundicola*, and then cultured for 4 weeks at 25°C (Hong *et al.*, 2004). After 4 weeks of incubation, the culture media were fully colonized with *M. osmundicola*. Several pieces of leaves which were infested with *M. osmundicola* were placed on water agar. Several hundred seeds were spread on the surface of leaves infested with *M. osmundicola*, and then cultured for 48 weeks at different temperatures. The optimal conditions of seeds were checked in the range of 5°C, 25°C, −5°C and −30°C, respectively.

Observation of seed germination. After seeds were spread on leaves, the germination rate was evaluated periodically Germination rate was observed every week for 2 months, and then two or four weeks. The degree and evaluation of seed germination were performed by using light microscope (40 ×). The seed germination was evaluated by swollen form of seeds and then converted into the percentage. The experiment was done with three replications.

Results and Discussions

The storage of post-harvest seeds has been carried out in 4 different temperatures for 48 weeks. After 48 weeks of the storage, the germination rate of *G. elata* was 65.7% at 5°C and 71.6% at −5°C, respectively. Although the germination rate was 77.3% for 11 weeks at 25°C. The germination rate was decreased rapidly to 49.3% at 13 weeks and stopped nearly at 23 weeks. The germination rate was

Table 1. Germination rate of *G. elata* on the leaves of *Quercus acutissima* inoculated with *Mycena osmundicola*

Temperature / Date (week)	Seed storage (°C)			
	25	+5	−5	−30
1	100.0 ± 0.0	98.2 ± 7.6	98.0 ± 7.7	4.0 ± 5.2
2	73.7 ± 7.1	97.0 ± 6.1	90.0 ± 6.9	10.3 ± 7.1
3	79.3 ± 6.0	88.6 ± 4.2	94.0 ± 5.6	0
4	84.7 ± 10.3	90.0 ± 5.3	93.0 ± 5.0	0
7	76.5 ± 9.7	87.0 ± 4.1	93.0 ± 4.2	0
11	77.3 ± 7.0	83.3 ± 4.2	90.0 ± 2.6	0
13	49.3 ± 5.8	82.6 ± 9.3	90.0 ± 5.5	0
15	48.3 ± 9.9	80.3 ± 11.2	90.0 ± 5.7	0
17	26.7 ± 9.1	79.0 ± 2.6	88.3 ± 4.1	0
19	24.3 ± 3.5	83.3 ± 8.9	80.5 ± 10.6	0
21	26.3 ± 10.7	78.3 ± 6.7	85.0 ± 12.5	0
23	0.3 ± 0.6	80.0 ± 7.2	80.0 ± 9.0	0
25	0	79.6 ± 2.1	87.2 ± 4.6	0
32	0	80.3 ± 9.5	79.7 ± 9.7	0
35	0	77.7 ± 9.9	71.5 ± 7.2	0
39	0	73.0 ± 4.6	64.3 ± 5.7	0
41	0	67.7 ± 4.8	64.7 ± 4.7	0
43	0	64.5 ± 4.9	66.2 ± 10.7	0
48	0	65.7 ± 9.6	71.6 ± 6.6	0

* Germination rate has been observed for 12 months (48 weeks).

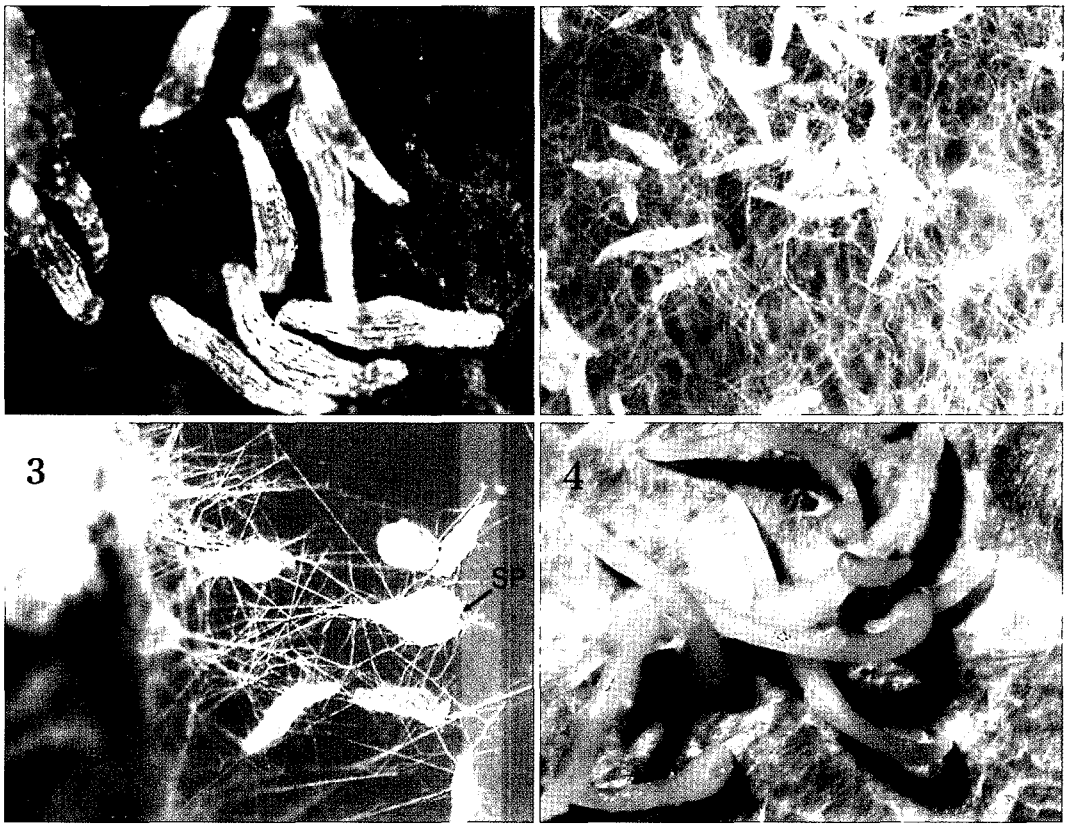

Fig. 1. The seed germination of *Gastrodia elata*.
1. The seed of *G. elata*. 2. The seeds of *G. elata* spread on the mycelial culture of *Mycena osmundicola*, one of fungi inducing seed germination. 3. The seeds of *G. elata* undergoing seed germination. The swollen portion (SP) of seed was considered as seed germination. 4. The small protocorm of *G. elata* formed on the surface of oak leaf infested with *M. osmundicola*.

reached to the level of 10% for 2 weeks at −30°C and decreased to 0% (Table 1 and Fig. 1).

Xu and Guo (1989) reported that some fungus was associated symbiotically with seed germination of *G. elata*. They isolated *Mycena osmundicola* as a symbiotic fungus inducing seed germination of *G. elata*, and also demonstrated the symbiotic relationship of both *G. elata* and *M. osmundicola*.

Guo and Xu (1990) reported that the seed germination of *G. elata* depended entirely on *M. osmundicola* in the embryonic cells of the plant. However, it is reasonable that further development of the protocorm needs an invasion of *A. mellea*.

Xu and Mu (1990) reported the cytological observation on an invading hyphae of *M. osmundicola* in the process of seed germination of *Gastrodia elata*. It was proved from *M. osmundicola* that *G. elata* obtained the nutrient sources necessary to sprout seeds of *G. elata*. Therefore, it is reasonable that the seeds of *G. elata* should be cultured with some fungi such as *M. osmundicola* capable of inducing seed germination of *G. elata* (Xu and Mu, 1990).

The hyphal mass of *M. osmundicola* inducing seed germination is enclosed and digested by embryonic cytoplasm after hyphae of *M. osmundicola* penetrates the embryo through suspensor cells, and then meristem cells begin to be divided. The digested hyphae penetrates into large cells to be further digested. Consequentially, the embryonic part enlarges to form tissue, and continues to grow under the system of meristem cells using the nutrition thus obtained (Xu and Mu, 1990). On nutrient sources necessary to seed germination of *G. elata*, Xu and Mu (1990) proved that seeds of *Gastrodia elata* lack an endosperm and other stored nutrition, and thus do not easily germinate. The nutrition necessary for seed germination is derived from *M. osmundicola* invading cells of embryo. It has been known that the leaves of *Q. acutissima*, one of coniferous trees are not only considered as a culture medium for the favorable growth of *M. osmundicola* but also offer an indirect nutrient source for seed germination of *G. elata* (Xu *et al.*, 1990).

Hong *et al.* (2004) pointed out that optimum temperature for seed germination was 25°C for the storage of 1 month, and the substrate suitable for a favorable growth of *M. osmundicola* was *Q. acutissima*. Also, Xu and Mu (1990) clarified seed germination of *G. elata* was optimal within the range of 3~4 weeks after seed harvest. In this

experiments, the mycelial growth of *M. osmundicola* was favorable on the leaves of *Quercus acutissima* (Hong *et al.*, 2004). After seed harvest, seed germination of *G. elata* was continued at 5°C and −5°C during storage period of 48 weeks. Although the germination rate of *G. elata* was kept to the level of about 70% for 11 weeks at 25°C, the germination rate was decreased rapidly after 11 weeks. On the other hand, the seed germination of *G. elata* was stopped nearly within 2 weeks at −30°C (Table 1).

Our result was different excessively with that of Guo and Xu (1991). They have tested seed germination of *G. elata* for storage period of 1 month. With that result they recommended that storage period of 1 month was optimal for seed germination of *G. elata*, because the germination rate was decreased rapidly below 50% after 1 month (Unpublished data, personal communication). However, it was observed from our results that germination rate of *G. elata* was more than 60% at 5°C or −5°C during storage period of 48 weeks. The reason that germination rate of *G. elata* was more than 60% at 5°C or −5°C seems to be attributable to the influence of an optimal temperature and *M. osmundicola*. Hong *et al.* (2004) tested storage conditions and optimum temperature of *G. elata*. They concluded that the culture condition of *G. elata* was optimal at 25°C. Also, they pointed out that storage of seed pod was good in the range of −5°C or 5°C for storage period of 1 month. This was the same as our result. Therefore, it could be proved in the case of *G. elata* that the desirable storage of seeds could be kept for 1 year (or 48 weeks) at −5°C or 5°C and the propagation of seeds could be probable for 1 year after their harvest.

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