Breaking dormancy by successive low and high temperature on the seed germination of *Glehnia littoralis*©

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Abstract

***Glehnia littoralis* Fr. Schmidt ex Miquel (*Apiaceae*, syn., *Umbelliferae*) is one of the typical seaside plants and is endemic to Japan, Korea, and China. The tap root has been used as a Kampo medicine and as a vegetable for the Japanese traditional dishes. In recent years, the extinction of *Glehnia littoralis* is worried about because of illegal harvesting from its natural habitats. For vegetation recovery, it is necessary to do seed propagation from seeds gathered in the same indigenous place to avoid disturbance of each ecosystem. Therefore we tried to clarify the effect of low temperature and successive low and high temperature treatments to seeds before sowing on the germination rate. Low temperature treatment promoted germination. However, germination rate of each fruit cluster was obviously different. The successive low (L), high (H) and low (L) temperature treatment remarkably accelerated seed germination compared with H and L treatments. Especially the L4H4L4 treatment caused the highest rate (58%) through these all experiments.**

INTRODUCTION

Japan has a long coastline in the country which consists of many islands. However, the natural coastal environment decreases remarkably by recent years' development activity and seashore constructions. Besides baggy cars for leisure enter into the remaining natural coast areas and have been destructed the beach ecosystem. The important vegetation of valuable seaside plants to maintain the beach ecosystem is being lost as a result. The plant which grows naturally in the location near the seashore strandline contributes to prevent blown sand and keep the ecosystem in stability. *Glehnia littoralis* Fr. Schmidt ex Miquel (*Apiaceae*, syn. *Umbelliferae*) is one of the typical seaside plants and is endemic to Japan, Korea and China. The tap root has been used as a Kampo medicine which eases the symptom of cold because of its alleviation of fever and an anodyne action (Ito et al., 2012). Moreover, in Japan, the leaf is used as a vegetable for the Japanese traditional dishes. In recent years, the extinction of *G. littoralis* is worried about because of illegal picking out from its natural habitats. For vegetation recovery, it is necessary to do seed propagation from seeds gathered in the same indigenous place to avoid disturbance of each ecosystem (Yahara and Kawakubo, 2002).

Besides it will be the result which stops the illegal harvesting when it is possible to supply the *G. littoralis* seedlings of the amount corresponding to the market demand. As a preliminarily, we have checked the effect of 24-h seed dipping in gibberellic acid (GA_3) solutions at the concentration of 0, 10, 100, 1000 ppm on the germination rate of *G. littoralis* seeds. But there was no effect of GA_3 on the promotion of seed germination. From this result, it seems that *G. littoralis* seeds have very deep physiological dormancy (Khan, 1996; Derkx, 2000; Baskin and Baskin, 2004).

In this reports, we examined the effects of low temperature and successive low and high temperature treatments on the germination rate of *G. littoralis* seeds before sowing. The successive low and high temperature treatments described as "hot and cold method" by Kamata (1987) through the series of seed germination experiments on wild *Lilium* species, especially on the so-called hypogeal germinating seed type. For example, seeds of *L. japonicum* and *L. rubellum* belong to the hypogeal type that require more than 1½ years for the start of true leaf emergence

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under natural condition (Kamata, 1987).

MATERIALS AND METHODS

We used seeds from wild plants (Experiment 1) collected from a natural habitat at the coast of Chigasaki, Kanagawa Pref., Japan and commercial ones (Experiments 2 and 3) purchased from a commercial company (Bio-plant Farm Ecoland Sansai-Kobo) located at Shibetsu, Hokkaido, Japan).

Experiment 1: Effect of low temperature on seed germination from wild plants

After collection from natural habitat, seeds were sown into five commercial garden planters and grown until maturity for 4 years under outside natural condition. Seeds from the mature plants were collected at three different fruit maturing stages: (G) green fruits stage, (GB) half the fruits had turned to brown, (B) color of almost fruits were brown. The viable seed (with embryo) numbers of respective fruit clusters were within the range between 200 and 300 in this experiment. The seeds were washed with tap water repeatedly until seeds absorbed water sufficiently, and then seeds of each fruit cluster were separately packed in a plastic bag to avoid drying and stored in a refrigerator at 4±2°C. After 4, 8, 12, 16 and 20 months from the start of low temperature treatment, germination rate of each fruit cluster was recorded. In this experiment, germination was defined as the seed with more than a 3-mm long root.

Experiment 2: Effect of low temperature on germination of purchased seeds

The method for Experiments 2 and 3 are those of Takahata et al. (2008, 2011). Purchased seeds were washed with tap water repeatedly until seeds absorbed water sufficiently, and then the seeds were sown in a 200-cell tray (Yanmar Co., Ltd., Osaka, Japan) cut in ¼ (50 seeds per treatment) filled with a commercial germinating soil mixture (Yosaku N-150; $N:P_2O_5:K_2O$ = 150:1000: 150 mg L^{-1}; Jcam Agri Co., Ltd., Tokyo, Japan). The trays watered and put in Uni-pack (J-4, 340 (long) × 240 (wide) × 0.04 (thick) mm, Seisan-nippon-sya Ltd., Tokyo, Japan). Then they were placed in a refrigerated chamber set at 5°C. The low temperature treatment was carried out for 2 to 20 weeks under continuous darkness. After the indicated period of low temperature treatment, each of the trays was moved to an germinating incubator (LH-200, Nippon Medical & Chemical Instruments Co., Ltd., Tokyo, Japan) at the set condition with 12 h-light (111 μmol m^{-2} s^{-1} PPFD)/12 h-dark and 17.5°C. The process of seed germination was recorded for 25 days. In this experiment, germination was defined as the visible observation of unfolded cotyledons.

Experiment 3: Effect of successive low and high temperature treatments on germination of purchased seeds

The seeded trays underwent the same procedure as in Experiment 2 and were treated as indicated in Table 1 in three temperature conditioned incubators as follows: low (L), middle (M) and high (H). The incubators were set at 5, 17.5 and 30°C respectively under continuous darkness. After the successive temperature treatment, each of the trays was moved to the germinating incubator mentioned above and the process of seed germination was recorded for 25 days. In this experiment, germination was defined as the visible observation of unfolded cotyledons.

Table 1. The experiment conditions of temperature and period.

Treatment[1]	Temperature (°C)			
	4 weeks	8 weeks	12 weeks	16 weeks
L4H8L4	5	30	30	5
L4H4M4L4	5	30	17.5	5
L4H4L4	5	30	5	
H8L4	30	30	5	
H4M4L4	30	17.5	5	
H4L4	30	5		
L4 (control)	5			

[1]L: Low (5°C), M: Middle (17.5°C), H: High (30°C).

RESULTS

Experiment 1: effect of low temperature on seed germination from wild plants

Low temperature treatment promoted germination of wild *G. littoralis* seed (Figure 1). However, germination rate of the fruit clusters from the different maturing stages was obviously different. The seeds of the GB stage germinated earlier, that is, half of seed germinated within 8 months. On the other hand, the G stage seeds required more than 1 year for half of them to germinate. Moreover, the germination progress was not linier, it changed step by step. This indicated that there were seeds in the same fruit cluster with varying chilling requirements for breaking dormancy.

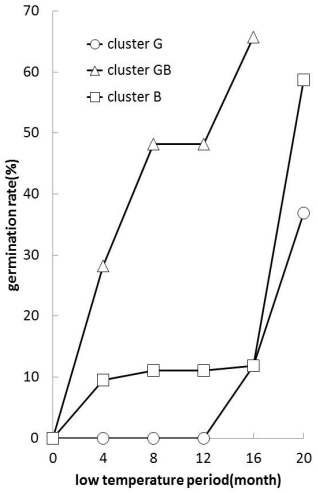

Figure 1. The germination rate of each cluster of *Glehnia littoralis* under 4°C.

Experiment 2: effect of low temperature on germination of purchased seeds

Low temperature treatment also promoted germination of purchased seeds (Figure 2). However, there was no significant tendency in the relationship between low temperature treatment period and accelerated effect of germination. Germination rate was less than 10% when the period of low temperature was less than 6 weeks and 14 weeks. In this experiment, seed treated at 12-week low temperature shown the earlier germination and the highest rate of germination (32%).

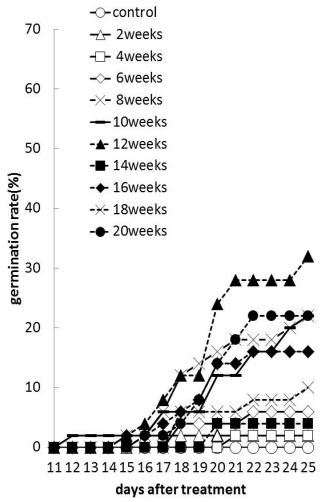

Figure 2. The germination rate of each cluster of *Glehnia littoralis* under 4°C.

Experiment 3: effect of successive low and high temperature treatments on germination of purchased seeds

The successive low (L), high (H) and low (L) temperature treatment remarkably accelerated seed germination compared with H and L treatments (Figure 3). Especially the L4H4L4 treatment caused the highest rate (58%) through these whole experiments. However, the accelerating effect declined when the H/M period between L and L was long (l4H8L4, L4H4M4L4).

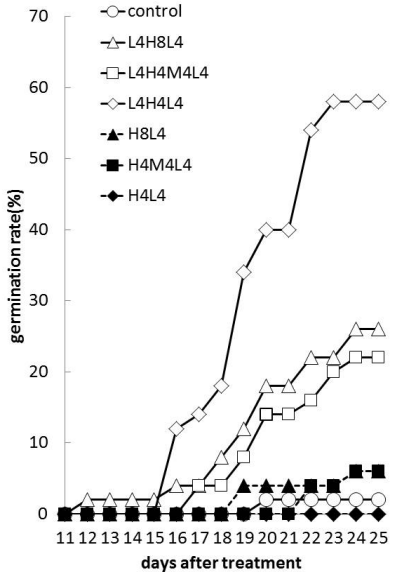

Figure 3. Effects of successive temperature treatments on seed germination in *Glehnia littoralis*. L: 5°C, M: 17.5°C, H: 30°C.

DISCUSSION

From the result of Experiment 1, *G. littoralis* wild seed has considerably deep dormancy and the level of dormancy deepness was remarkably different depending on the developmental stage of fruit and/or each individual plant. At least, the seed dormancy at the BG fruit stage was relatively light compared with the younger (G) and the older (B) stage (Figure 1). If *G. littoralis* wild seeds are sown under the natural condition without pretreatment, it is necessary for more than 2 years from seed sowing to get more than 50% of seed germination. The diversity of dormant depth seems to be functioning as one of necessary safety devices to leave next generation in the natural environment. On the other hand, the diversity is troublesome in cultivation. The diversity was also shown from purchased seeds. The low temperature treatment was effective in stimulation of germination in purchased seed, but the effect was variable (Figure 2). It seems that the variable effect caused by the coexistence of different dormancy depths in the seeds. However, it was shown that even with seeds having a deep dormant it was possible to break dormancy in a short time by the low-high-low temperature treatment (Figure 3).

From the preliminary observation of embryo development in seeds just after harvest from the natural habitat, the embryo developmental stage of the seeds was not uniform (data not shown). Thus we guess that the first low temperature accelerate the after ripening of immature embryos, the second high temperature raise the cold-sensitivity of developed embryos, and the final low temperature broke the dormancy of the embryo. There was a possibility that a longer high temperature period between low and low temperatures caused secondary embryo dormancy. In this experiment, the highest value of germination was 58% after the L4H4L4 treatment. This value will make it possible to obtain enough seedlings within a year after seed sowing to make seed propagation possible in practice.

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