「森林総合研究所研究報告」(Bulletin of FFPRI) Vol.10 No.1 (No.418) 1 - 5 March 2011

研究資料（Research material）

Difference in germination response to cold stratification intervals between two dwarf bamboo species, *Sasa cernua* and *S. senanensis*

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Abstract
Dwarf bamboo species are monocarpic and seldom flower or bear fruit. We observed the small-scale flowering of two dwarf bamboo species, *Sasa cernua* and *S. senanensis*, at the Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo, Central Hokkaido and obtained their seeds. We investigated the germination response of these two species to repeated cold periods and found that the dormancy period of *S. senanensis* seeds was longer than that of *S. cernua* seeds. In the course of five repeats of cold treatments, most of the *S. cernua* seeds had germinated after the second cold period, while majority of *S. senanensis* seeds did not until after the fourth or fifth cold period. This difference between the two species was statistically significant.

Key words :acclimation, cold stratification, dwarf bamboo, *Sasa*, seed dormancy

Introduction

Dwarf bamboos have a vigorous vegetative regeneration system (Makita, 1992). Because of this, they often dominate the forest understory in temperate and subarctic forests in Japan. In Hokkaido, dwarf bamboo species rapidly colonize open sites created as a result of disturbances such as forest fires, wind damage, landslides, volcanic eruptions, artificial logging, and cultivation.

Bamboos are monocarpic (Kudoh & Ujiie, 1990; Janzen, 1976) and take several decades to flower, thus regeneration by seeds is rare. When dewarf bamboos bear fruit, they provide a large number of viable seeds, which are often consumed by insects (Kudoh et al., 1994; Nishiwaki & Makita, 1998) and mammals (Abe et al., 2001) as a source of food. Previous studies on dwarf bamboo species have revealed the existence of genetically variable genets in local populations (Suyama et al., 2000, Kitamura & Kawahara, 2009). This is a positive evidence of the contribution of seeds to the regeneration process in dwarf bamboo species. Furthermore, we observed seedlings of *S. cernua* at a study site 2 years after small-scale flowering and fruiting (K. Kitamura personal observation, 2005 and 2008). This observation suggests that dwarf bamboo seeds may be dormant for a minimum of 2 years.

In this study, we investigated the length of the cold acclimation period required to break the dormancy of seeds of two dwarf bamboo species, *S. cernua* and *S. senanensis*.

Materials and Methods

Seed collection

We observed small-scale flowering of *S. cernua* (Kitamura & Kawahara, 2007) and *S. senanensis* in 2006 in the experimental forest of Hokkaido Research Center, Forestry and Forest Products Research Institute, Sapporo, Hokkaido (42°58' 32" N, 141°23' 46" E, 130–260 m above sea level).

We selected 18 culms of *S. cernua* and 10 of *S. senanensis*, that flowered and bore fruits. We covered all caryopses of every culm with a coarse fiber cloth bag in June for *S. cernua* and in early July for *S. senanensis*. In late July, after all the seeds had matured and been shed from the culms, we collected the bags containing the seeds.

Compound stratification

The collected seeds were rinsed and soaked in water overnight. To prevent mold growth on the seed surface, seeds were soaked in 0.05% benomyl for 3 h. Seeds from each culm were placed separately in Petri dishes and kept moistened with water-soaked cellulose sponges throughout the experimental period. The compound stratification

原稿受付：平成 22 年 7 月 21 日 Received 21 July 2010 原稿受理：平成 23 年 2 月 9 日 Accepted 9 February 2011
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method (Asakawa 1957) was employed to germinate the seeds. Five cycles of incubation under cold (3℃) and warm (25℃) temperatures were conducted. Due to the restriction in using the equipment, we were not able to manage the constant intervals of cold and warm temperatures. The cycles were as follows: 54 days cold and 49 days warmth, 64 days cold and 51 days warmth, 38 days cold and 30 days warmth, 31 days cold and 53 days warmth, and 231 days cold and 34 days warmth.

Significant differences in germination between the two species were investigated after each cold period using the χ^2 test.

Results and Discussion

We observed the germination of *S. cernua* and *S. senanensis* seeds after each cold period except for the third (Table 1). The germination response of *S. cernua* was the highest after the second cold period, at which 79.3% of seeds had germinated. Most of the *S. cernua* seeds (99.6%) had germinated after the fourth cold period.

On the other hand, majority of the *S. senanensis* seeds, 69.9%, germinated after the fifth cold period.

The difference in germination responses between the two species was significant ($p<0.01$), which indicated that the dormancy period of *S. senanensis* seeds was significantly longer than that of *S. cernua* seeds.

The genus *Sasa* generally shows seed dormancy; some species such as *Sasa tsuboiana* (Makita et al. 1993), *S. kurilensis* (Makita, 1992) have been reported to show short period of dormancy, however, *Sasa kurilensis* var. *jotanii* (Nishiwaki & Makita, 1998) showed no dormancy. The present study revealed that the two species, *S. cernua* and *S. senanensis*, show longer seed dormancy than previously studied species.

To accelerate germination of seeds having longer dormancy periods, the “compound stratification” or “warm followed by cold stratification” method is effective (Asakawa 1957). By employing this method, we obtained higher germination rates for *S. senanensis* (69.9%) than those found in previous studies (Matumura & Nakajima, 1981; Nishiwaki, 1988; Koyama, 2000). Although the interval between each cold and warm period was not kept the same throughout the experiment, repeated treatments with cold–warm periods may be a major factor in increasing the germination rate as well as the cumulative length of the cold period.

The germination response of *S. cernua* was the highest after the second cold period, i. e., after 118 days of cold period. This result coincided with that of a previous observation in which germination of seedlings in the natural population of *S. cernua* was observed 2 years after fruiting (K. Kitamura personal observation, 2005 and 2008). These results may indicate that seed dormancy of *S. cernua* can be broken by either two cold periods or 2 years after fruiting in natural populations.

On the other hand, most *S. senanensis* seeds germinated after the fifth cold period, i. e., after 418 days of cold period. This is significantly longer than that obtained in previous reports on *S. senanensis* (Matsumura & Nakajima 1981; Nishiwaki, 1988) and *S. cernua* in this study, which indicates longer dormancy period of seeds of this species. Matsumura & Nakajima (1981) have previously shown that *S. cernua* requires a significant prechilling period. The present study proved that the significant length of cold period was necessary to enhance germination of the two dwarf bamboo species.

Acknowledgements

We thank Akiko Takazawa, and Yukiko Sakamoto for their support. This study was supported by Grants-in-Aid for Scientific Researches (18580153 and 20580167) from the Japan Society for the Promotion of Science.

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Table 1. The number of germinated seeds from each culm of *S. cernua* and *S. senanensis* after each cold period. Germination was not observed after the third cold period.

Species	Culm	Cold period 1	2	4	5	Total
S. cernua	1	10	46	6	0	62
	2	18	62	15	1	96
	3	10	78	4	1	93
	4	13	24	11	1	49
	5	6	51	1	0	58
	6	9	62	8	0	79
	7	5	48	13	1	67
	8	2	121	12	0	135
	9	2	50	5	0	57
	10	1	28	6	0	35
	11	4	36	11	0	51
	12	3	98	5	1	107
	13	3	94	10	0	107
	14	1	50	0	0	51
	15	2	31	7	0	40
	16	3	63	7	0	73
	17	5	39	14	0	58
	18	6	5	15	0	26
	Total	103	986	150	5	1244
S. senanensis	1	0	0	2	0	2
	2	0	0	2	12	14
	3	0	2	11	9	22
	4	0	0	8	0	8
	5	0	0	3	0	3
	6	0	0	0	15	15
	7	1	0	1	11	13
	8	0	0	3	0	3
	9	0	2	6	0	8
	10	0	1	1	53	55
	Total	1	5	37	100	143

χ^2=950.03 (p<0.01, df=3)

オクヤマザサおよびクマイザサ種子における休眠打破に関わる低温期間の違い

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要 旨

一回結実性植物として知られているササは、数十年に一度一斉開花し枯死する。ササの開花は稀であり、種子に関する研究はほとんど行われていない。本研究では北海道の林床に広く分布するオクヤマザサ（*Sasa cernua*）およびクマイザサ (*S. senanensis*) の小面積開花集団から種子を採取し、２種における休眠打破に関わる低温期間の違いを調べた。低温期は５回繰り返し、３回目を除く全ての低温期間後に発芽が観察された。オクヤマザサの発芽は低温期２回目、クマイザサでは５回目に最大数が発芽した。オクヤマザサが低温２回目で発芽がピークを示したことは、野外において開花結実２年後に実生が観察されたことと一致する。カイ二乗検定の結果、低温回数と発芽数の関係に両種間で有意な差が認められ、クマイザサの種子はオクヤマザサのものより休眠が深いことが示唆された。

キーワード：一回結実性植物、ササ属、休眠、部分開花、低温湿層処理

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