論 文 (Original article)

Germination characteristics of a pioneer shrub, *Stachyurus praecox* (Stachyuraceae)

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Abstract

To clarify the effect of bird ingestion for germination and the effect of moist stratification for dormancy release, germination experiments for a common pioneer shrub, *Stachyurus praecox*, were examined using current and two-years stored seeds (dry- and moist-storage). Germination percentage of moist-storage seeds immediately reached 100% regardless of stratification treatments. Dry-storage seeds without stratification did not germinate while other treatments promoted germination slowly. Germination of all moist-storage seeds suggests that physiological longevity was at least two years. In current seeds, acid stratification was the most effective, which suggest that bird ingestion is a trigger of germination. These characteristics can be advantageous to germinate suitable timing in the safe site of pioneer *S. praecox*.

Key words : acid stratification, dormancy release, germination percentage, moist stratification, *Stachyurus praecox*

Introduction

Bird ingestion and trigger of dormancy release are important for germination in many plants (Baskin & Baskin, 1998; Jordano, 2000; Probert, 2000). These characteristics may affect growth advantage of following life stage in association with the spatio-temporal fluctuation of suitable conditions. Stachyurus praecox is a pioneer shrub endemic to Japan (Ohba, 1999), and is common in forest gaps and edges of temperate forests. Fleshy fruits of S. praecox ripen in summer and are dispersed by birds. The seedlings are sometimes found in forest gaps or samples of soil seed banks (Kobayashi & Kamitani, 2000; Goto, 2004; Sakai et al., 2005). Germination characteristics of S. praecox would adapt to detect the suitable growth condition for pioneer plants. However, germination characteristics such as dormancy and the effective stratifications of this shrub have not been reported even in encyclopedias about seeds (International Seed Testing Association, 1993; Katsuta et al., 1998).

In this study, we examined the effect of bird ingestion for germination by acid scarification that assumes passage through bird alimentary canal. In addition, to clarify the mechanism to start germination when the unsuitable environmental conditions improve, we examined the effect of moist stratification for dormancy release on *S. praecox* by comparing the germination percentages among the treatments on stored and current seeds.

Methods

Seed collection and storage

Seeds were collected from 5 females (two populations) in October 2003 and 2005 in Ibaraki Prefecture, Japan. Until use for the germination tests, seeds collected in 2003 were subjected to removal of the pulp and stored at 3°C in the dark for two years. Two storage methods were examined: seeds stored in polyethylene bags with wet sphagnum (moist) and in bottles (dry). Seeds in 2005 were sown soon after collection.

Seed stratification and germination

To eliminate empty seeds, seeds sunk in water were used for the germination tests.

For 2003 seeds, we set up 5 treatments prior to the germination test: two acid scarifications (soaking in 97% sulfuric acid for 3 min and 5 min), two moist stratifications (14 d and 21 d at 3°C in Petri dishes of 0.9% agar substrate), and no treatment (control). Acid scarifications are assumed the effect of bird ingestion that promotes seed germination by a mechanical or chemical scarification of the seed coat (Barnea *et al.*, 1990; Izhaki & Safriel, 1990). For germination test, 100 seeds were used per treatment with 50 seeds being placed in each two Petri dishes matted a moist filter paper. Seeds were incubated in 8 h light (30 °C, 6000 lx) and in 16 h darkness (20°C) for 112-133 d using a Shimazu FLI-301NH incubator. The duration of incubation

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was finished when the germination rate became zero or nearly zero. Germination was defined as the emergence of the radicle from the seed coat and was checked mostly every one-week and germinated seeds were removed from the Petri dish. After the germination test, ungerminated seeds were categorized as fresh, dead or embryoless by cutting. Germination percentage was calculated based on the number of fresh and germinated seeds. Seed weights per 100 grains were 0.081 ± 0.003 g (n = 5, mean \pm SD) in dry storage and 0.085 ± 0.004 g (n = 5) in moist storage.

For seeds collected in 2005, we set up 5 treatments (soak in running water for 2 d, soak in sulfuric acid for 20 min, cold stratification for 14 d and 90 d at 3°C, and no treatment). In each treatment, 400 seeds (8 replications of 50 seeds per Petri dish) were incubated for 126 d. The other procedures of the germination tests there after were same as for the 2003 seeds. Current seed weight per 100 grains was 0.149 ± 0.005 g (n = 20).

The effects of treatments on final germination percentage were examined using logistic regression. As the monitoring periods differed between treatments for 2003 seeds, final germination was defined as that on 112 d, which was the last day of counting germination in all treatments. Germination for 2003 seeds was evaluated by two two-way interactions: one was for the effects of the storage methods and the periods of acid stratification, and the other was for the effects of the storage methods and the periods of cold stratification. Germination for 2005 seeds was evaluated by two one-way analyses: the softening treatment of seed coat (water, acid, and control) and cold stratification (14 d, 90 d, and control). The storage methods were regarded as a categorical variable, and the period of acid scarification and moist stratification were included as continuous variables for 2003 seeds. Softening treatment was included as a categorical variable, and the periods of cold stratification were regarded as a continuous variable for 2005 seeds. All statistical analyses were performed using JMP software (Sall et al., 2004).

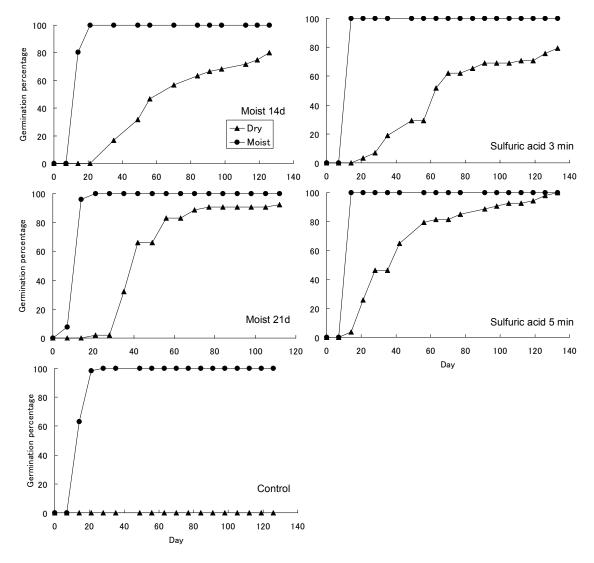


Fig. 1. Comparison of germination rates between dry and moist storage for 2 years. Mean germination percentages are shown for two replications (100 seeds).

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Effects*	G	df	Р
Storage	104.4	1	> 0.0001
Moist	0.0	1	0.9925
Storage \times moist	0.0	1	0.9918
Storage	94.6	1	> 0.0001
Acid	0.0	1	0.9923
Storage \times acid	0.0	1	0.9917
* "Storage" Dry va Ma	iat "Maiat"	. 114.	.a. 01.d

Table 1. Effects of storage methods and treatments for germination of 2003 seeds.

* "Storage": Dry vs. Moist, "Moist": 14d vs. 21d

Results

Two-years stored seeds

Germinability of *S. praecox* was maintained for at least two years. The germination rate of moist-storage seeds was faster than that of dry-storage seeds (Fig. 1). In moist-storage seeds, germination percentage reached 100% within one month irrespective of pretreatments to promote germination. On the other hand, dry-storage seeds without stratification did not germinate while other treatments promoted germination. Even in other treatments, dry-storage seeds germinated slowly, and some of the treatments yielded a lower germination percentage than moist-storage seeds on the final observation day. Final germination percentages significantly differed between dry- and moist-storage rather than treatments and stratification × storage interactions (Table 1).

Current seeds

For all treatments, germination occurred within one month for current seeds (Fig. 2). The differences in germination percentage both among softening treatment and among cold stratification were significant (softening: G = 168.9, df = 2, P < 0.0001, cold: G = 68.9, df = 1, P < 0.0001). Seeds with acid scarification exhibited the highest germination rate among the treatments (Fig. 2). On the other hand, germination rate was relatively low with no treatment and sinking in water. Among the softening treatments, acid scarification significantly differed from the control (G = 122.5, df = 1, P < 0.0001) and from water treatment (G = 150.6, df = 1, P < 0.0001). Germination percentage was higher with longer duration of cold stratification (G = 8.0, df = 1, P = 0.0046 in control vs 14 d cold; G = 69.6, df = 1, P < 0.0001 in control vs. 90 d cold; G = 32.1, df = 1, P < 0.0001 in 14 d cold vs. 90 d cold). Final germination percentage was also highest in acid scarification, but did not reach 100% in any treatment.

Discussion

Moist stratification and acid scarification were effective to promote germination of *S. praecox* seeds and either one of the two could promote germination. Acid scarification was the most effective for current seeds. This suggests that germination of *S. praecox* seeds is promoted by bird ingestion. Bird dispersal enhances the chance of seeds being transported to a safe site (Howe & Smallwood, 1982; Hoppes, 1988; Murray, 1988; Wenny & Levey, 1998). Higher germination percentage of current seeds with cold stratification is reasonable to lie as dormant seeds during winter, and then, dormancy breakdown occur when temperature become increase in next spring. For *S. praecox*, combination of seed dormancy during winter and quick germinability in next spring would be optimum germination strategy as a pioneer shrub.

In stored seeds, moist stratification seems to be the most effective means to break inhibition of germination of stored seeds. Seed dormancy is a strategy to avoid unsuitable

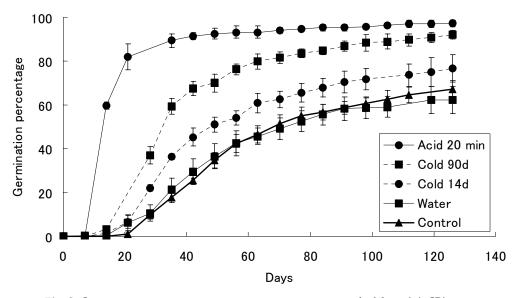


Fig. 2. Germination rates among treatments in current seeds. Mean (\pm SD) germination percentages are shown for 8 replications (400 seeds).

conditions for germination and initial growth (Harper, 1977; Baskin & Baskin, 1998). If fruits that are dispersed unsuitable site germinate immediately, it would be difficult for small seedlings to survive following winter. So, moist chilling as a trigger of dormancy breakdown is likely to favor selecting the suitable germinate timing for *S. praecox*. The ease of germination and almost 100% germinability of moist-storage seeds suggest that the physiological longevity of *S. praecox* seeds is at least two years, and probably, *S. praecox* seeds can persist for longer duration as a viable seed bank when the environment conditions are unsuitable for germination.

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キブシ(キブシ科)の種子発芽特性

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要旨

低木性先駆種であるキブシの発芽特性に関して,鳥による摂食の効果と休眠打破のための低温湿層処理 の効果を明らかにするため,当年生種子と2年間保存した種子(乾燥保存と保湿保存)を用いて発芽実験 をおこなった。保湿保存した種子では処理条件に関わらず全ての種子が速やかに発芽した。乾燥保存した 種子は無処理では全く発芽せず,それ以外の処理では緩やかに発芽した。保湿保存種子が全て発芽したこ とから,キブシの種子の生理的寿命は少なくとも2年以上あるものと思われる。当年生種子では硫酸処理 が発芽促進に最も有効であり、鳥の消化管を通過することで発芽が促進される可能性が示唆された。これ らの諸特性は先駆種であるキブシの生育適地において適切なタイミングで発芽するための機構であると考 えられた。

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