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Seed Germination Response to Storage Conditions of *Eriocaulon heleocharioides* (Eriocaulaceae), an Extinct Species in the Wild

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Abstract Seed germination experiments with *Eriocaulon heleocharioides* Satake (Eriocaulaceae) were performed to investigate the effect of air exposure on seeds during the storage period. Although the seeds germinated both above and under water, the response and durability of germination differed from each other. Moreover, the seeds that were dried for 1 month and sown in water showed the highest germination rate (84.4%) among all combinations of conditions. This result shows that treatment by drying prior to sowing promotes the germination of this species.

Key words: aquatic plant, *Eriocaulon heleocharioides*, *ex situ* conservation, extinct in the wild, re-introduction, seed germination.

Introduction

Eriocaulon heleocharioides Satake (Eriocaulaceae) is an annual aquatic herb endemic to Japan and an extinct species in the wild (Ministry of the Environment, 2012). The species was collected for the first time in Koshigaya, a city in central Japan, in 1938 and described in the following year (Satake, 1939). Thereafter, it was never found at this or other sites, and hence, was considered extinct (Satake, 1982). However, the species was rediscovered, collected as herbarium specimens and deposited in the Ibaraki Prefectural Museum, at Sanuma Lake, Shimotsuma, Ibaraki Prefecture, central Japan in 1975. The lake is a large water storage reservoir for paddy fields. Further, in 1994, this species also became extinct in Sanuma Lake. This species disappeared from its last habitat after a change in water management in 1994 and has been preserved only *ex situ* such as in botanical gardens.

Ex situ conservation is essential for extinct species in the wild. In a subsequent stage, reintroduction of extinct species to the wild is necessary, because an organism is a component of the ecosystem and can be maintained as a wild species with genetic diversity only in the wild (Maunder, 1992; Primack, 1995; Lawrence and Kaye, 2011).

It is necessary for a stable *ex situ* conservation to reveal seed storage and germination behavior, especially for annual plants, such as *E. heleocharioides*. Although seeds can generally be

preserved under low temperature and dry conditions (Roos and Davidson, 1992), information about seed storage and germination of this species and genus is insufficient. Thompson *et al.* (1997) showed that *E. aquaticum* formed a short-term persistent soil seed bank, with seeds surviving in the soil for at least 1 year but no more than 5 years. Miyamoto (1995, 1997) found that seed storage in water in the dark at 4℃ is suitable for *E. heleocharioides* and that after 35 days of this treatment, germination is promoted on dampened filter paper in the light at 25℃. In contrast, we observed that air exposure for several weeks may improve the seed germination rate. In papaya (*Carica papaya*, Caricaceae), drying treatment prior to sowing has also been reported to be promoted germination (Hore and Sen, 1993; Sippel and Claassens, 1993). In some plant species, seed germination after exposure to cycles of hydration and dehydration has been reported to be delayed (Allen *et al.*, 1993; Downs and Cavers, 2000; Ren and Tao, 2003) or reduced (Vincent and Cavers, 1978; Downs and Cavers, 2000).

This study aims to determine the effect of air exposure during the storage period on seed germination of *E. heleocharioides.*

Materials and Methods

The seeds for this study were collected in November 2009, 2010 and 2011 from multiple individuals of *E. heleocharioides* Satake (Eriocaulaceae) cultivated in the nursery of the Tsukuba Botanical Garden, National Museum of Nature and Science (TBG accession no. 137574). The plants were originally collected from Sanuma Lake in 1994 and have since been continuously subcultured every year by seed collection from propagated plants and seedings.

The experiment was performed from August 2012 to January 2013 and consisted of three different treatment methods after collection of seeds: Treatment 1, preservation in water; Treatment 2, air exposure; Treatment 3, preservation in water after air exposure. The germinated seeds were counted and removed daily until seven consecutive days without seed germination. The detailed methods are as follows, and combinations of experimental conditions are listed in Table 1. Two iterations of fifty seeds were used for each combination.

Treatment 1: Before the experiments, the seeds collected in November 2009, 2010 and 2011 were preserved in water in the dark at 4℃. Using these seeds, two sowing environments were prepared, and they were kept in the light at 25℃: "wet," placed on dampened filter paper in a 9-cm Petri dish, and "water", placed in water. Dormancy breaking was not applied in Treatment 1, because the storage period after collection functioned as dormancy breaking.

Treatment 2: The seeds exposed to air at room temperature since their collection in November 2011 were used for the germination test. The seeds were divided into two groups: with and without dormancy breaking (in water in the dark at 4℃ for 35 days) and then sown in either the "wet" or "water" environment, as described for Treatment 1.

Treatment 3: The seeds collected in November 2009, 2010 and 2011 were preserved in water in the dark at 4℃. They were then exposed to air for 1–3 months before dormancy breaking and sown in either of the two environments, as described for Treatment 1. The air exposure was applied for each combination of temperature (4℃ or 35℃) and illumination (light or dark). For air exposure, the moisture content of seeds was adjusted to 12% in a desiccator.

For Treatment 3, a classification tree model (CTM) (Breiman *et al.*, 1984) was used to detect the important conditions for germination rate among the multiple combinations of experimental conditions. The germination rate was used as the response variable. Duration, temperature and illumination in seed drying, dormancy breaking, and sowing environment were used as predictor variables. The most appropriate tree size in the CTM was obtained by cross-validation. Package "mvpart" of R 2.14.2 (R Development Core Team, 2012) was used for the analysis.

To confirm the viability of non-germinated

Table 1. Germination rate of *Eriocaulon heleocharioides* seeds in each treatment conditions

Conditions of drying seeds			Dormancy breaking (with or without)	Sowing environment (water or wet)	Germination rate (%)
Duration (month)	Temperature (°C)	Illumination (light or dark)			
Treatment 1: preservation in water					
0	—	—	with	water	37.0
0	—	—	with	wet	44.0
Treatment 3: preservation in water after air exposure					
1	4	light	with	water	72.0
1	4	light	with	wet	50.0
1	4	dark	with	water	85.3
1	4	dark	with	wet	58.0
1	25	light	with	water	96.7
1	25	light	with	wet	82.0
1	25	dark	with	water	96.7
1	25	dark	with	wet	86.7
2	4	light	with	water	54.7
2	4	light	with	wet	35.3
2	4	dark	with	water	80.7
2	4	dark	with	wet	49.3
2	25	light	with	water	33.3
2	25	light	with	wet	22.0
2	25	dark	with	water	68.7
2	25	dark	with	wet	57.3
3	4	light	with	water	64.0
3	4	light	with	wet	42.7
3	4	dark	with	water	85.3
3	4	dark	with	wet	75.3
3	25	light	with	water	0.7
3	25	light	with	wet	0.0
3	25	dark	with	water	48.0
3	25	dark	with	wet	44.7
1	4	light	without	water	67.0
1	4	light	without	wet	18.0
1	4	dark	without	water	82.0
1	4	dark	without	wet	30.0
1	25	light	without	water	100.0
1	25	light	without	wet	43.0
1	25	dark	without	water	69.0
1	25	dark	without	wet	51.0
2	4	light	without	water	47.0
2	4	light	without	wet	10.0
2	4	dark	without	water	52.0
2	4	dark	without	wet	33.0
2	25	light	without	water	19.0
2	25	light	without	wet	12.0
2	25	dark	without	water	58.0
2	25	dark	without	wet	33.0
3	4	light	without	water	45.0
3	4	light	without	wet	19.0
3	4	dark	without	water	71.0
3	4	dark	without	wet	8.0
3	25	light	without	water	0.0
3	25	light	without	wet	0.0
3	25	dark	without	water	14.0
3	25	dark	without	wet	3.0

Note: Data for Treatment 2 and for 2009 and 2010 seeds were not shown, because none of their seeds germinated as described in Results.

seeds after the germination test, both fluorescein diacetate (FDA) staining and a germination test using gibberellic acid (GA) were performed. For FDA staining, washed seeds were placed on 1% SeaKem agarose in a 9-cm Petri dish and incubated at room temperature for 1 day. Further, the FDA solution was poured in the Petri dish. The dish was then exposed to 470 nm illumination from a 470-nm EX Flash Light (Bio Tools Co. Ltd.) and observed through a yellow filter. Moreover, for GA test, seeds were placed on a filter paper dampened with 200 mg/l GA solution in a 9-cm Petri dish, and the dish was placed in the light at 25℃.

Results

In the present study, none of the seeds collected in 2009 and 2010 germinated under any of the treatments. Therefore, only the results for 2011 seeds are presented in Table 1.

In Treatment 1, preservation in water, the seeds germinated in both "wet" and "water" environments (Table 1). The cumulative number of germinated seeds is shown in Fig. 1. In the seeds placed in "water," the first germination was observed on day 3 after the start of the experiment and it continued until day 7 (final germination rate, 37.0%). In the "wet" treatment, germination began on day 5 and continued until day 20 (final germination rate, 44.0%).

In Treatment 2, air exposure, no seeds germinated under any condition (data not shown in Table 1).

In Treatment 3, preservation in water after air exposure, germination was observed under most experimental conditions (Table 1). The most appropriate tree is shown in Fig. 2. The highest germination rate (84.4%) among all combinations of conditions was obtained by drying for 1 month and sowing in "water". Moreover this rate is highest also compared with Treatment 1. In each node, sowing with dormancy breaking and in "water" resulted in higher rates than that without dormancy breaking and in "wet", respectively.

FDA staining was invalid for *E. heleocharioides* seeds, because the embryo is too small for the staining to be visually recognizable. In addition, the staining of an esterase-like enzyme, exuded from living seeds was not observed on the agar medium. The non-germinated seeds in the experiments did not germinate with GA treatment, although 100% of the control seeds germinated.

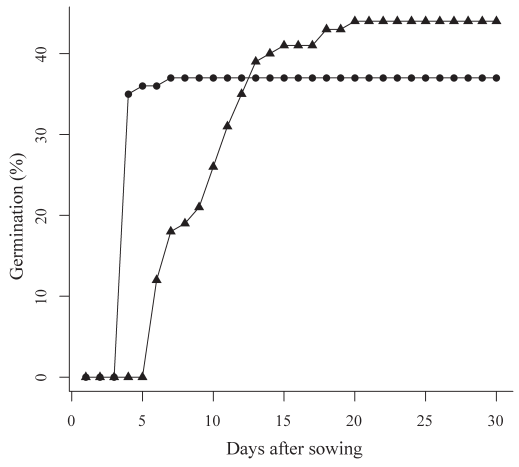

Fig. 1. Germination rate of *Eriocaulon heleocharioides* in Treatment 1. Cumulative percentages for "wet" (▲) and "water"(●) environments are shown.

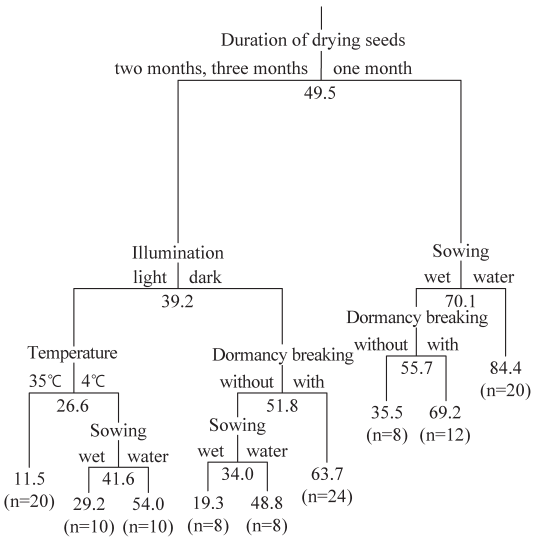

Fig. 2. The classification tree diagram for germination rate in Treatment 3. The number beneath node represents the germination rate of the node.

Discussion

In the present study, none of the seeds collected in 2009 and 2010 germinated under any test condition, indicating that *E. heleocharioides* seeds cannot be preserved for more than two years under the storage conditions of this study. This short-term survival behavior is consistent with that of other *Eriocaulon* species (Thompson *et al.*, 1997).

In this study, drying the seeds for 1 month prior to sowing promoted their germination. However, in some plants, exposure to cycles of hydration and dehydration is known to delay and/or reduce seed germination (Allen *et al.*, 1993; Downs and Cavers, 2000; Ren and Tao, 2003; Vincent and Cavers, 1978). Promoting germination by dehydration of seeds, as in the present study, has also been reported for papaya. In papaya, drying treatment prior to sowing promoted germination (Hore and Sen, 1993; Sippel and Claassens, 1993). The only two natural habitats of *E. heleocharioides*, in which the species has ever been recorded, become marshy from late September to early April due to lowering of water level. Therefore, most of the seeds are never submerged for several months after seed maturation. Moreover, some of the flower stalks remain in an upright position for several weeks, supporting the seeds that are exposed to air. This environmental condition is similar to that for dehydration prior to sowing. Therefore the seed germination behavior of *E. heleocharioides* revealed in this study is believed to relate to these habitat environments.

The seeds in "water" germinated faster than those in "wet" and quickly reached the final germination rate. In contrast, seed germination in "wet" was slow but sporadic. Kagaya *et al.* (2005) reported that hydration and dehydration cycles of seeds render the germination of *Aster kantoensis* (Asteraceae) sporadic. They considered this behavior to be associated with the plant's habitat, where the seeds are exposed to several cycles of hydration and dehydration. Moreover, in some aquatic plants, seed germination is markedly promoted by immersion in water (*Monochoria korsakowii* Regel and Maack.; Godo, 1997) and by sowing in deoxygenated water (*Zostera marina* L.; Moore *et al.*, 1993). In the case of *M. korsakowii*, exposing the seeds above water strongly reduces their germination. In contrast, germination of *E. heleocharioides* seeds seems to be controlled by some factors. This behavior may be associated with a habitat in which the moisture content of environment changes in a complex with the difference of relative elevation, soil texture, and weather.

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