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Seed storage longevity of *Hosta sieboldiana* (Asparagaceae)

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ABSTRACT

Seed storage longevity of *Hosta sieboldiana* (Lodd.) Engler was studied. Mature and immature seeds of *H. sieboldiana* were stored immediately after collection at −20 °C and 5 °C with a moisture content of about 65%. In addition, the seeds were dried to either about 10% or 5% moisture content (MC) and then stored at either −20 °C or 5 °C. Seeds of *H. sieboldiana* were desiccation-tolerant but short-lived, with the P50 (the time required to reduce germination to half of the maximum germination) under storage conditions ranging from 0.5 to 221.9 months and 2.3 to 56.7 months for mature and immature seeds, respectively. Seed longevity of *H. sieboldiana* was increased by decreasing moisture content, but the responses of the seeds to temperatures were different from typical orthodox seeds. The dry seeds of *H. sieboldiana* (~5% to 10% MC) lost viability more quickly at −20 °C than at 5 °C. Storage allowed immature seeds to continue some maturation, but the longevity of these seeds was generally shorter than that of the mature seeds stored under the same conditions.

1. Introduction

Hosta sieboldiana (Lodd.) Engler (Asparagaceae) is a popular ornamental plant in Japan. This beautiful perennial herbaceous plant is endemic to southwestern and central Japan, where it is widely distributed. Because it is such an important ornamental, there is interest in developing seed production technologies to facilitate the commercial availability of this plant. Seed biology information is available for 264 species in the Asparagaceae, and all are reported to have orthodox seeds (Liu et al., 2008). But desiccation sensitivity has been shown in the Asparagales, e.g. *Ophiopogon japonicas* (Suzuki et al., 2007). *H. sieboldiana* seeds were reported to be orthodox as they survived liquid nitrogen storage at moisture contents of 10% to 12% (Stanwood, 1984).

H. sieboldiana usually grows along streams or rivers in valleys but also can be found in mesic open meadows and forests (Fujita, 1976). Like *Salix* species (Salicaceae), which also occur in similar habitats and have short-lived seeds (Zasada and Densmore, 1977; Maroder et al., 2000), seeds of *H. sieboldiana* may also be predicted to have a short life span (Walters et al., 2005; Probert et al., 2009). Indeed, in our pre-experiment, dry seeds of this species died after 15 months at room temperatures. Optimal conditions are crucial to prolong the seed longevity of *H. sieboldiana* during storage. However, seed storage longevity of this species has not been investigated.

In this study, we carried out a four-year seed storage study of *H. sieboldiana* seeds from two locations in the central district of Japan to investigate the effects of seed maturity status, seed storage moisture content and temperature on the seed storage longevity of the species.

2. Materials and methods

2.1. Seed lot details

Seeds of *H. sieboldiana* were collected from two locations on 18 September 2005 from central Japan: Asagiri Height, Fujinomiya, Shizuoka (N 35°24′ 04″, E 138°33′ 33.6″, 830 m) and Ashikawa, Yamanashi (N 35°33′ 33.19″, E 138°44′ 11.33″, 1350 m). At the time of collection, seeds from seed lot Ashikawa (YA) were at the point of natural dispersal. However, seeds from seed lot Asagiri Height (AS) were immature. They were collected two weeks prior to the time of natural seed dispersal.

2.2. Seed storage experiments

Three MC (moisture content) levels were targeted for storage: ~65% (the initial MC of seeds on collection); ~10% (dry) and ~5% (ultra dry). For each seed lot, seeds were separated into three groups. One group of seeds was sealed in polyethylene bags immediately after seed

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collection and then stored at 5 °C and −20 °C. These seeds had a moisture content of around 64% to 66%. Seeds of the other two groups were dried for nine to 18 days using silica gel to adjust their moisture content. After drying, seed moisture contents of seed lot YA and AS reached 9.6% and 4.5%; and 7.7% and 5%, respectively. These seeds were then sealed in polyethylene bags and stored at both 5 °C and −20 °C.

Seed storage experiments were conducted for more than four years. The preliminary seed germination experiments indicated that, although germination occurred over a wide temperature range (10 °C to 30 °C), germination was most rapid at 25 °C and 30 °C; therefore, 25 °C was used in all the experiments. To test the initial seed viability of the seed lots, on the commencement of the storage experiments, three replicates of 50 fresh seeds each of each seed lot were placed on top of two sheets of filter paper soaked in water in a 9-cm diameter petri dish and incubated at 25 °C. During the roughly four-year storage, germination tests were conducted every one to three months, and up to 50 germination tests for each treatment were done. For each germination test, three replicates of 50 seeds were used.

In addition, seed moisture content was assessed gravimetrically on 180 seeds in total (three replicates) per target moisture content by drying seeds at 105 °C for 16 h. MCs are expressed on a fresh-weight basis (% fr.wt.).

2.3. Data analysis

Logistic regression (Scott et al., 1984) was used to model the relationship between seed germination percentage and three predictors as follows: storage time (in months, denoted by x), storage temperature and seed moisture content. The three predictors and all their interactions were included in the full model; then the contribution of individual predictor/interaction was assessed by the likelihood ratio test (Hosmer and Lemeshow, 2000). The selected model consisted of predictors and interactions that significantly (at $P < 0.05$) decreased the deviance. The goodness-of-fit of the selected model was evaluated by the likelihood ratio test, which compared the deviance of the selected model to the deviance of the null model. For the mature seeds (seed lot YA), the seed germination measurements of seeds stored at −20 °C and 4.5% MC were taken much more frequently during the first two years than the third and fourth years so that each measurement was weighted to adjust this imbalance. The selected model successfully captured the linear relationship between the log-odds of seed germination and storage time at various storage conditions. It achieved a significance of <0.0001 in the goodness-of-fit test. For immature seed (seed lot AS), the relationship between the germination percentage and the storage time was not monotonic, so the linear model was not appropriate to fit the relationship between the log-odds of germination and the storage time. Hence, a transformed term $x^{1/2}$ was added into the model to achieve a better fit. The selected model then achieved a significance of <0.0001. The effects of the predictors were represented by the regression coefficients. The mean seed germination percentages at various storage times and conditions were estimated using the selected models. The time for germination to decrease by 50% (P50) was also estimated. All the analyses mentioned above were performed using the R statistical package (R Development Core Team, 2013, version 2.15.3).

3. Results

Mature seeds from YA had an initial germination of 82%; the immature seeds from AS had an initial germination of only 18%. During storage, the viability of seeds from YA decreased significantly in all the treatments ($P \leq 0.001$, Table 1, Fig. 1), except at 4.5% MC and −20 °C ($P > 0.05$). Seeds with a high MC of 65.7% died rapidly at both 5 °C and −20 °C ($\beta_1 = -3.2$, Table 1, Fig. 1). Seeds at 9.6% MC stored at −20 °C lost viability much faster ($\beta_1 = -0.034$, Table 1, Fig. 1) than seeds with 9.6% MC and 4.5% MC stored at 5 °C.

Because of the immaturity of the seeds, the selected model for AS seeds was not a monotonic function of the storage duration (Fig. 1, Table 1). During storage, the viability of seeds appeared to increase in the first two to 19 months, depending on temperatures and MCs, and then started to decrease. This relationship was modeled by function $y = \beta_0 + \beta_1 x + \beta_2 x^{1/2}$ where y was the logit transformed germination percentages and x was the number of storage months. Following the same interpretation of a slope in a linear function, the first order derivative $\beta_1 + \beta_2/(2x^{1/2})$ (Table 1, Fig. 1) described the rates of the viability change during seed storage.

At both 5 °C and −20 °C, the viability of seeds stored with 7.7% MC increased and decreased faster than that of seeds stored with 5% MC (Table 1, Fig. 1). At the same MC level of 5% or 7.7%, the viability increased at similar rates between the two temperatures but declined much faster at −20 °C than at 5 °C (Fig. 1). Seeds with 63.8% MC stored at 5 °C quickly reached a germination percentage of 56% and then lost all the viability quickly (Fig. 1, Table 1). Seeds with 63.8% MC stored at −20 °C lost all viability before the second germination test.

The time required to reduce germination to half of the maximum germination (P50) ranged from 0.5 (MC = 65.7% and stored at −20 °C) to 221.9 (MC = 4.5% and stored at −20 °C) months for YA and ranged from 2.3 (MC = 63.8% and stored at 5 °C) to 56.7 (MC = 5% and stored at −20 °C) months for AS (Table 1). Under the same

Table 1
Selected models for the effects of storage months (x), moisture contents and storage temperatures on seed germination percentages and the calculated time for germination to decrease from the maximum germination to half of the maximum germination (P50) of seeds from YA and AS. β_1 and β_2 indicate the coefficients of x and $x^{1/2}$; t_1, P_1 and t_2, P_2 indicate the t and P values of β_1 and β_2, respectively.

Seed lot	MC (%)	Temp (°C)	β_1	β_2	Intercept	t_1	P_1	t_2	P_2	P50 (months)
YA	4.5	−20	-0.009 ± 0.005^a	–	1.68 ± 0.15	−1.676	0.096	–	–	221.9
	4.5	5	-0.028 ± 0.004^b	–	2.07 ± 0.15	−6.220	<0.0001	–	–	82.0
	9.6	−20	-0.034 ± 0.004^c	–	1.16 ± 0.12	−8.660	<0.0001	–	–	48.4
	9.6	5	-0.011 ± 0.003^d	–	0.57 ± 0.11	−3.304	0.001	–	–	120.6
	65.7	−20	-3.224 ± 0.488^e	–	0.95 ± 0.39	−6.609	<0.0001	–	–	0.5
	65.7	5	-3.201 ± 0.488^f	–	3.56 ± 0.63	−0.563	<0.0001	–	–	1.1
AS	5	−20	-0.106 ± 0.021^a	0.885 ± 0.109^a	−1.59 ± 0.18	−5.077	<0.0001	8.155	<0.0001	56.7
	5	5	-0.078 ± 0.020^b	0.885 ± 0.109^a	−1.77 ± 0.19	−3.834	0.0003	8.155	<0.0001	NA
	7.7	−20	-0.150 ± 0.019^c	0.885 ± 0.109^a	−0.78 ± 0.18	−7.719	<0.0001	8.155	<0.0001	34.8
	7.7	5	-0.122 ± 0.017^d	0.885 ± 0.109^a	−0.96 ± 0.18	−7.019	<0.0001	8.155	<0.0001	48.6
	63.8	5	-2.966 ± 0.643^e	4.642 ± 1.101^b	−0.83 ± 0.33	−4.611	<0.0001	4.217	<0.0001	2.3

For β_1 and β_2, values with the same letters within each column and for the same seed lot were not significantly different at $P < 0.05$.
Data for seeds from AS stored at 63.8% MC and −20 °C were not analyzed using the model because too few effective data were available (see Fig. 1).
P50 for seeds from AS describes the time for germination to decrease from the maximum germination to half of the maximum germination. The time for the initial germination increase was not included.
NA: P50 values for these treatments couldn't be estimated because of insufficient data observed for viability loss (see Fig. 1).

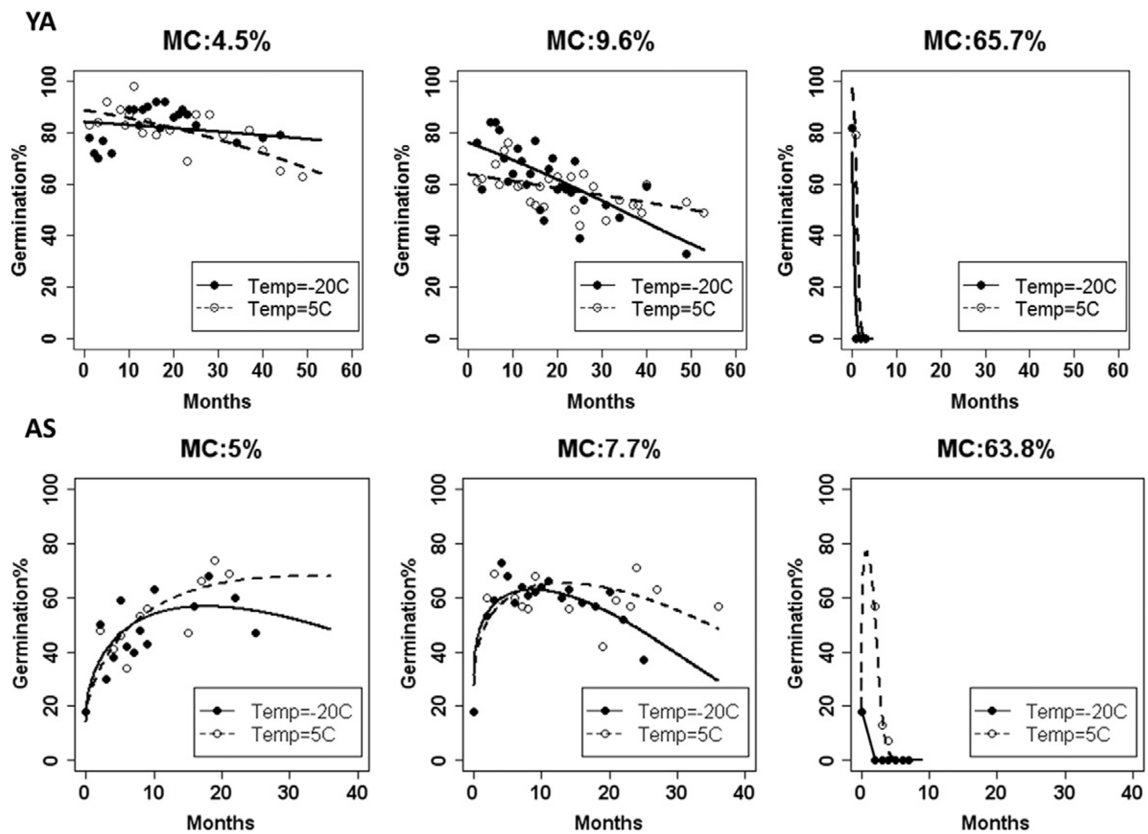

Fig. 1. Germination (%) of *Hosta sieboldiana* seeds after storage at ~5%, ~10% and ~65% moisture content and at 5 °C (open circle, dotted line) and −20 °C (closed circle, solid line). Values are means of three replicates of 50 seeds for the germination tests. Solid and dotted lines represent the selected models of storage time versus germination at 5 °C and −20 °C. Details of the models are given in Table 1.

storage conditions, P50 for seeds from AS was lower than that for YA seeds (Table 1).

4. Discussion

Seeds of *H. sieboldiana* were able to survive low moisture contents of about 5% and low temperature of −20 °C, suggesting that these seeds are not recalcitrant. However, these seeds are not typical orthodox. Reductions in seed moisture content and storage temperature within broad limits are known to enhance longevity in orthodox seeds (Roberts and Ellis, 1989). For successful long-term storage, orthodox seeds require low (e.g., 5%) moisture content and low temperature (e.g., −20 °C). This study showed that, for both the mature and immature seeds, decreasing moisture content increased seed longevity of *H. sieboldiana*, but the responses of the seeds to temperatures did not occur in the same predictable way as for orthodox seeds (Roberts and Ellis, 1989), and seeds of *H. sieboldiana* lost viability quickly under all the storage conditions. With a high MC of ~65%, seeds of *H. sieboldiana* lost all viabiliy within five months. It is not surprising because these seeds would have been killed by ice crystal formation at subzero temperatures or rapid viability loss/aging at temperatures above zero. At the MC levels below the unfrozen water contents (~5% to ~10%), however, the calculated P50s of the mature seeds stored at 5 °C and −20 °C were only 48.4–221.9 months (4.0 to 18.5 years). Long-term storage studies provide direct evidence of changes in seed viability with storage time. Because these types of studies are time-consuming, relative data are usually rare. Lack of data means that we are not able to compare our results with storage data for congener species or species from the same family. However, in a survey of 276 species across 18 families, under storage conditions of 4% to 8% MC and −18 °C, only 25% of the species had predicted half-lives of less than 50 years, and the median P50 was 54 years (Walters et al., 2005), suggesting that seeds of *H. sieboldiana* are comparatively short-lived.

Unlike typical orthodox seeds, the dry seeds of *H. sieboldiana* (~5% to ~10% MCs) lost viability more quickly at −20 °C than at 5 °C. Similar responses of seeds to subzero (−20 °C) and cool (0 °C) temperatures were also reported for the intermediate seeds of coffee (*Coffea arabica* L.) (Ellis et al., 1990). However, coffee seeds from many seed lots were more sensitive to desiccation, and seed viability was lost or declined largely after the seeds were desiccated to 5% MC (Ellis et al., 1990). Bonner (1990) defined seeds that can be stored under the same conditions as typical orthodox seeds, but for shorter period to be suborthodox. This type includes *Populus* and *Salix*, both of which could survive 6% to 10% MCs but lost viability rapidly at temperatures below −20 °C or −10 °C (Bonner, 1990). Therefore, seed storage behavior of *H. sieboldiana* is similar to that of *Populus* and *Salix*, and may be classified as suborthodox.

During storage, after-ripening occurred in the immature seeds (AS) of *H. sieboldiana*. Generally, the increase in germination was faster at higher MCs; at the same MC, the rates were not much different at 5 °C and −20 °C. When ripening ended, the germination percentages decreased faster at higher MCs and lower temperature. The lower P50 of the immature seeds compared with the mature seeds suggests that maturity status had significant effects on the seed storage longevity of this species. Though storage (at the MC range between 5% and 65% and at 5 °C and −20 °C) allowed some continuation of maturation, the maturation process of these immature seeds may not be completed during storage (resulting in lower maximum germination percentages), and their longevity was shorter than that of the initially mature seeds stored under the same storage conditions.

It has been suggested that seed storage longevity is related to the climate of species' origin, and that seeds from cool and wet regions are more likely to be short-lived (Walters et al., 2005; Probert et al., 2009). Consistent with this prediction, our data showed a short seed lifespan for this wet-climate-originated species. Compared with species from similar habitats, *H. sieboldiana* seeds lost viability faster than short-lived Liliaceae species (Walters et al., 2005) but more slowly than *Salix* species (Zasada and Densmore, 1977; Maroder et al., 2000) when stored in the same conditions.

Standard seed banking protocols recommend that orthodox seeds should be dried to 3% to 7% moisture content and then stored at −18 °C (FAO/IPGRI, 1994). Our data indicate that while short-term storage of *H. sieboldiana* seeds under standard seed bank conditions may be feasible, cryogenic storage might be a more efficient method for long-term conservation for these comparatively short-lived seeds. Indeed Stanwood (1984) has shown that *H. sieboldiana* seeds survive in liquid nitrogen.

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