RESEARCH ARTICLE

Germination of *Hemerocallis* Seeds as Influenced by Seed Development and Temperature Treatments

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Abstract

Germination of *Hemerocallis* seeds as influenced by cold stratification at 5°C (CS) and maturity of seeds evaluated using X-ray imaging has not been well investigated. Seeds of *H. lilioasphodelus*, *H. citrina*, *H. citrina* 'April Flower', and *H. minor* collected from China and *H. thunbergii* collected from Korea were germinated at 20°C without pre-temperature treatment, while *H. hongdoensis*, *H. dumortieri*, *H. minor*, and *H. vespertina* seeds were treated with CS. Harvesting 'Stella de Oro' capsules at 35–40 days after anthesis yielded mature seeds with well-developed embryo and cotyledons analyzed by X-ray images with a 92% germination in 17 days after sowing. Seeds of *H. thunbergii* and *H. citrina* germinated in less than 13 days without CS; two weeks of CS did not accelerate seed germination. Seeds of *H. hongdoensis* germinated in 24 days when seeds were stored at 25°C without CS and in less than 27 days when cold stratified. Therefore, 'Stella de Oro' capsules should be harvested at 35–40 days after anthesis to harvest mature seeds. Cold stratification is not required to accelerate seed germination in the *Hemerocallis* taxa evaluated in this study.

Additional key words: cold stratification, daylily, dormancy, embryo and cotyledon/endosperm ratio, maturity, seed development, X-ray imaging

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Introduction

The genus *Hemerocallis*, commonly known as daylily, is a popular perennial landscape plant that is native to China, Korea, and Japan. Flowers of most taxa open in the morning and last for about a day, thus giving rise to the common name, daylily. *Hemerocallis* thrives in full sun, although the plants can tolerate some shade during the day. Flowering starts in early to mid-spring with repeat blooming characters until frost. Flower buds develop continuously on each scape, and seeds of different maturity levels develop in capsules throughout the flowering season.

Hemerocallis 'Stella de Oro' is quite popular hybrid for landscape use due to its extended flowering season and can be propagated easily by division and seeds. Seeds formed early in the season will mature, while those formed later in the season sometimes fail to reach maturity. Hybrids are propagated

by division of root clumps (Gulia et al., 2009), although seeds can be used for propagation if the progeny from self-pollinated plants are uniform in terms of flower shape and color. 'Stella de Oro' seedlings obtained through open pollination did not show any genetic variation based on the sequence analysis of nuclear internal transcribed spacers 1,2 in a ribosomal RNA gene (nrITS) and a chloroplast interspacer region (cpIS), and morphological characteristics such as flower color and plant height (Gulia et al., 2009)

Seed germination percentage in *Hemerocallis* is affected by the number of empty seeds, by harvest dates, and by the geographic locations from which seeds were collected (Suzuki et al., 2003). In *Hemerocallis*, capsules are formed continuously from May to October, and thus the date of seed maturity varies over a period of months. On a branched scape, flowers and capsules often develop simultaneously. Therefore, on any particular seed harvest date, the seed development stage can vary widely. Furthermore, when capsules mature on a branched scape, their development is delayed as compared to that of single capsule developing on the scape (Fig. 1).

It is difficult to evaluate the maturity of seeds using methods other than germination testing. Magnetic resonance imaging revealed that *Styrax japonicus* Siebold & Zucc. seeds should be harvested 8 weeks from anthesis to improve the germination percentage (Horimoto et al., 2011). However, this technique requires the use of a sophisticated instrument, and it is difficult to screen many seeds at the same time. With *Corylopsis*, an immersion test in water was used to separate mature and viable seeds from immature and non-viable seeds. However, immersion of *Hemerocallis* seeds in water did not separate full and viable seeds from empty seeds. Therefore, X-ray imaging could be employed as an alternative method. Seeds of *Pinus nigra* ssp. *pallasiana* (Lamb.) Holmboe that sank after immersion in 96% ethanol had higher germination rates than those that floated. Similar results were found with *Casuarina equisetifolia* L. seeds dipped in petroleum, and X-ray imaging analysis

Fig. 1. Development of flower buds and seed capsules of *Hemerocallis* 'Stella de Oro' in branched scape (A) and non-branched scape (B). Based on anthesis day 0, flowers that were open 1, 3, and 5 days ago are indicated as -1, -3, and -5, respectively, and flower buds that will open in 2 and 4 days are indicated as +2 and +4, respectively. Capsules developing 7, 10, and 12 days after anthesis are indicated as -7, -10, and -12, respectively. The scape normally branches as it develops so that the buds are not all present at the top of the scape.

revealed that seeds that sank were fully developed (Sivakumar et al., 2007). In castor bean (*Ricinus communis* L.), seeds that produced translucent X-ray images or tissues with less than 50% of embryo reserves yielded low germination percentages (Carvalho et al., 2010).

The optimum temperature range for seed germination in *H. minor* and hybrids is 22–25°C for freshly harvested seeds without cold stratification (CS). Seeds stratified at 3.5°C germinated at supraoptimal temperatures and CS at 1°C for 32 days promoted germination (Griesbach and Voth, 1957). Germination of *H. dumortieri* var. *esculenta* and *H. fulva* var. *littorea* seeds was also promoted by CS. However, some seeds germinated immediately when sown (17%), although CS increased the germination percentage to 43% at 4 weeks, suggesting that seed dormancy was broken or may not be present (Suzuki et al., 2003).

Germination behavior, particularly the requirement for CS, may vary among taxa and the level of maturity of seeds if dormancy is induced after harvest. Therefore, this study was initiated to investigate germination of 'Stella de Oro' seeds with various levels of maturity and to examine the relationship between seed germination and the development of cotyledons and endosperm as revealed by X-ray analysis. Seeds of *H. lilioasphodelus* L., *H. citrina* Baroni, *H. citrina* 'April Flower', and *H. minor* Mill. collected from China, and seeds of *H. thunbergii* Baker, *H. hongdoensis* M.G. Chung & S.S. Kang, *H. dumortieri* C. Morren, *H. minor*, and *H. vespertina* Engl. collected from Korea, were used to investigate whether CS treatment is required for seed germination.

Materials and Methods

Seed development and X-ray imaging

Capsules of *Hemerocallis* 'Stella de Oro' were collected from plants that reached anthesis during different flowering times, as well as immature and mature seed capsules in Ann Arbor, MI, USA on August 7, 2013 (Fig. 1). Capsules were assigned to categories from 1 through 6 based on size and age, where 1 represents a mature capsule, harvested at 35–40 days after anthesis (DAA) and 3.5 cm in length, and 6 represents an immature capsule, 15–20 DAA and 2.3 cm in length (Table 1). The capsules were split open and the seeds were separated from the placenta (Fig. 2), dried at room temperature (17±2°C) for 7 days, subjected to X-ray imaging, and delivered in 10 days to Dankook University for germination testing.

Table 1. The number of seeds of *Hemerocallis* 'Stella de Oro' of various colors as affected by the size of seed capsules at harvest.

Capsule category[z]	DAA at which capsules were harvested[y]	Capsule size		No. of seeds by color per capsule			Total	Percentage of black seeds
		Length (cm)	Width (cm)	Black	Brown	White		
1	35-40 (mature)	3.5 a[x]	2.8 a	13.5 a	0.5 a	0.0 c	14.0 a	96.8 a
2	30-35	3.2 b	2.4 b	11.3 a	1.5 a	0.8 bc	13.6 a	83.8 ab
3	25-30	2.7 c	2.1 c	6.5 b	2.0 a	0.3 c	8.8 b	72.5 ab
4	20-25	2.7 c	1.8 d	6.5 b	3.8 a	1.8 abc	12.6 a	70.3 ab
5	15-20	2.4 d	1.9 d	3.3 b	3.3 a	5.0 a	11.6 a	39.5 b
6	15-20 (immature)	2.3 d	1.8 d	2.5 b	1.8 a	4.3 ab	8.6 b	36.3 b

[z]Capsules of various sizes were harvested on Aug. 7. A - capsules prior to splitting, and B - capsules after splitting. Capsules range from category 1, which is easily split open with a gentle squeeze to category 6, which is difficult to split open. Refer to Fig. 2 for more details.
[y]DAA: days after anthesis.
[x]Mean comparison by DMR at $p \leq 0.01$, F-test. Means compared within a column followed by the same letter are not significantly different.

Fig. 2. Various sizes of capsules and seed development of *Hemerocallis* 'Stella de Oro'. Capsules were harvested on Aug. 7 (A and B) and on Aug. 27 (C). Capsules were assigned to categories 1 through 6 based on age, where 1 through 6 represent 35-40 days after anthesis (DAA), 30- 35 DAA, 25-30 DAA, 20-25 DAA, and15-20 DAA (two different sizes), respectively. Capsules prior to splitting (A) capsules split open, showing seeds along the placenta (B), and capsules harvested on Aug. 27 (C) that were split; a = seeds were dispersed and capsules are empty, b = seeds separated from the placenta and are easily removed from open or split capsules, c = capsules beginning to split, showing seeds inside the capsule. Bars = 2 cm.

Seeds from mature and immature capsules (Fig. 2) were glued (50 seeds) onto a 8 × 15 cm card using a white multi-purpose glue and imaged at the Ornamental Plant Germplasm Center (Columbus, OH, USA) using a Faxitron MX-20 (Faxitron Corporation, Wheeling, IL, USA). Seeds were exposed to 20kV for 15 seconds and X-ray digital images were collected. Seeds showing various stages of development of embryo and endosperm were selected and classified in categories A through E based on the X-ray images (Fig. 3), where seeds in category A exhibited no endosperm or embryo formation (empty) and seeds in category E exhibited fully developed endosperm and embryo. After imaging, the seeds were mailed to Dankook University and sown for germination tests as described in the germination testing section.

Taxa and temperature treatments

Seeds of *H. lilioasphodelus*, *H. citrina*, *H. citrina* 'April Flower', and *H. minor* collected from China and *H. thunbergii* collected from Korea were stored dry at 5°C or otherwise specified and germinated at 20°C without any pre-temperature treatment. Seeds of *H. hongdoensis*, *H. dumortieri*, *H. minor*, and *H. vespertina* collected from the Hantaek Botanical Garden, Korea were sown in a moist germination medium (60% moisture) and received no CS (control) or 2 weeks of CS at 5°C followed by 2 weeks at 20°C and germinated at 2°C. In another test, *H. hongdoensis* seeds collected from Korea were sown and given 2 weeks of CS, followed by storage at 10, 15, 25, and 30°C and germination at 20°C. The number of seeds that germinated was recorded weekly for 7 weeks, and the number of days to germination was recorded from the start of temperature treatment.

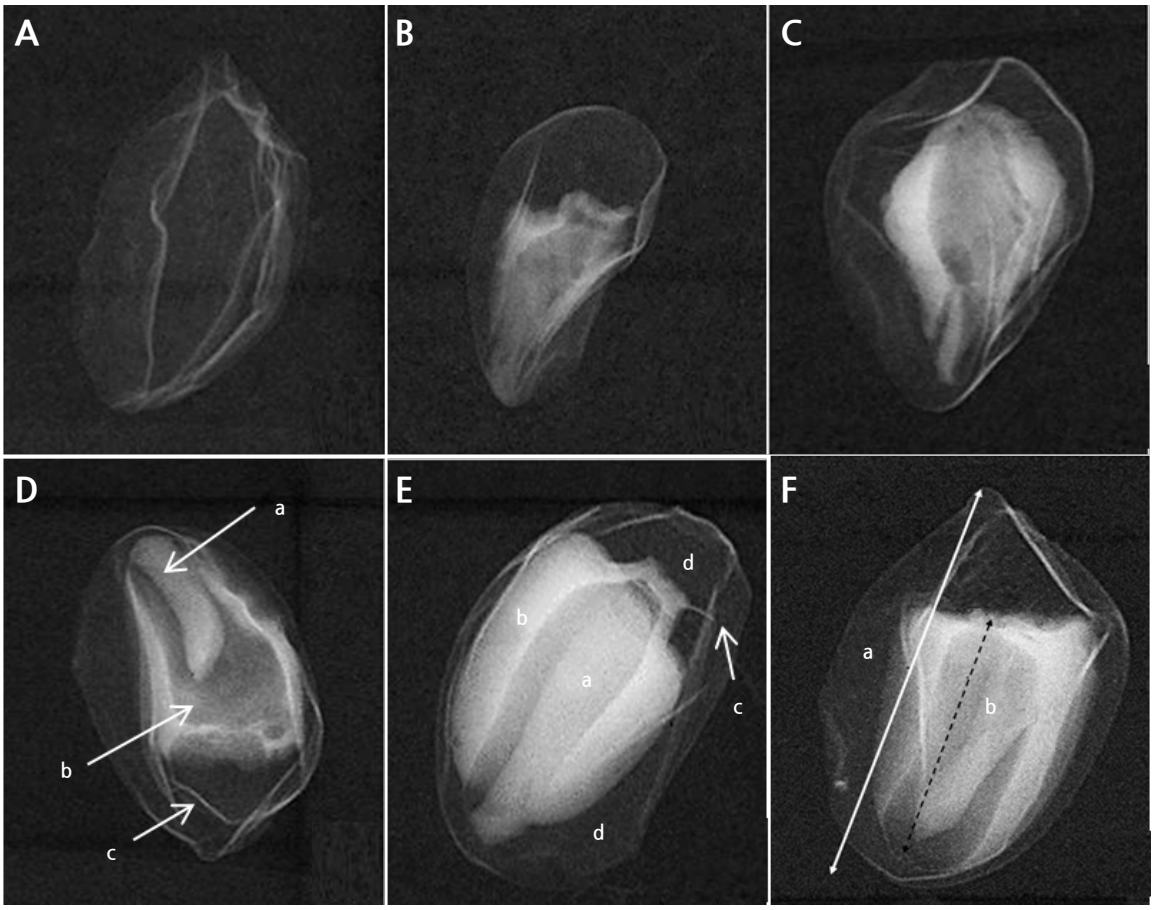

Fig. 3. Seed development of *Hemerocallis* 'Stella de Oro' revealed by X-ray imaging. A, no endosperm formed (empty), B, partial development of endosperm without clear formation of embryo, C, partial development of endosperm but degenerated embryo, D, partially developed embryo (a), degenerated endosperm (b), and endospermal membrane (c), E, developed embryo (a), endosperm (b), and endospermal membrane (c), air space (d), endosperm, and F, entire length of seed (a), and length of embryo and endosperm within seed (b).

Germination test

There were 50 seeds per replication and two replications per treatment. Seeds were sown individually in 32-cell trays filled with growing medium to a depth of 0.5 cm and germinated in a growth chamber maintained at 20°C. Once the cotyledons began to emerge from the medium, the number of seeds germinated was counted weekly for 6 weeks. The number of days to germination was counted from the date of sowing or stratification of seeds in moist medium, as indicated in each test. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System program, and means were compared with Duncan's multiple range test at $p<0.01$.

Results

Order of flower bud opening and capsule development per scape

Each 'Stella de Oro' flower stayed open for one day, and capsules developed along with the development of new flower

buds on the same scape (Fig. 1). The order of flower opening depended on the branching pattern of the scape. Based on anthesis as day 0, flower buds are labeled with regard to the number of days until anthesis (+1, +2, etc.). Spent flowers and developing seed capsules based on the number of days after anthesis (-1, -2, etc.). However, capsules did not form from every flower following anthesis.

Sizes of capsules and the number of seeds based on the capsule size and color of seeds

Generally, large capsules in categories 1 [capsule 1; 3.5 cm × 2.8 cm (length × width)] and 2 (3.2 cm × 2.4 cm) formed from scapes that did not branch (Fig. 1-A) and that developed at 35–40 DAA, while small capsules in categories 5 and 6 (2.4 cm × 1.9 cm and 2.3 cm × 1.8 cm, respectively) formed from scapes that formed branches or that developed at 15–20 DAA (Fig. 2). When the carpel of category 1 and 2 capsules were split open, 14.0 and 13.6 seeds were lined up along the placenta and 13.5 and 11.3 seeds were black, respectively, while some seeds from category 5 and 6 capsules were creamy white to yellow. There were significantly fewer black seeds from category 5 (3.3 seeds) and 6 (2.5 seeds) capsules than from category 1 and 2 capsules. Seed formation along the placenta in one ovary of a given category of capsules was not symmetrical (Table 1).

The fewest number of seeds (8.6 seeds) was harvested from category 6 capsules, which were the smallest capsules. Seeds collected from category 3 capsules were of various colors, including black, dark brown, light brown, and white (Fig. 4-G). The number of white seeds was significantly higher in category 5 (5.0 seeds) and 6 (4.3 seeds) capsules compared to category 1 and 2 capsules.

Categories of seeds based on the X-ray images and germination

Using X-ray imaging, seeds showing different stages of embryo and endosperm development (Fig. 3) were classified into

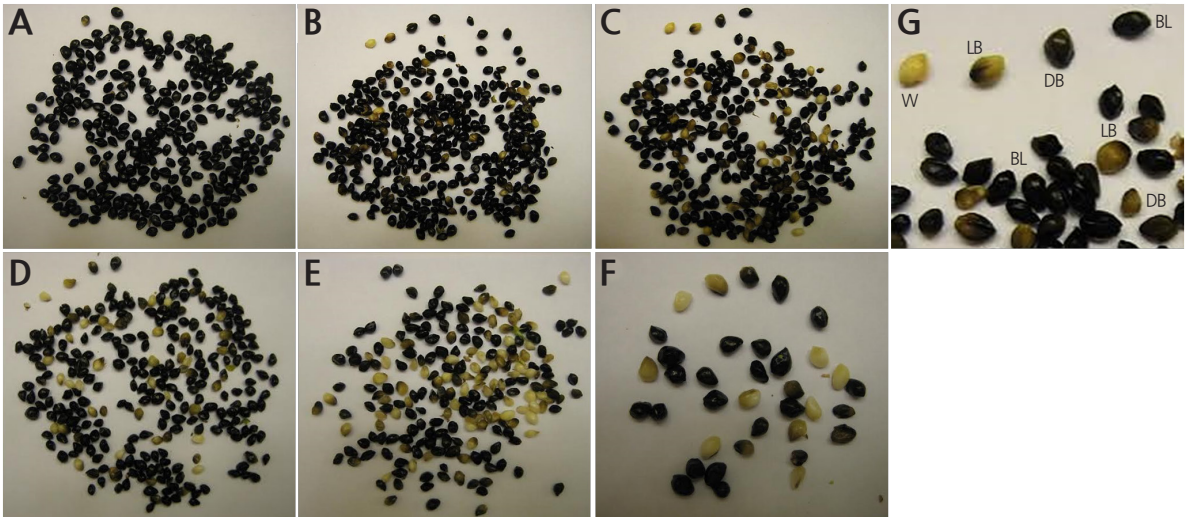

Fig. 4. Seeds of *Hemerocallis* 'Stella de Oro' from capsules harvested on Aug 7. Capsules were assigned to categories 1 through 6 (A through E, respectively), based on age, shown at 35-40 days after anthesis (DAA), 30-35 DAA, 25-30 DAA, 20-25 DAA, and 15-20 DAA (two different sizes), respectively. The majority of seeds from capsules A and B were black. Seeds from capsules C, D, E, and 6F were black (BL), light/dark brown (LB/DB), and milky white (W), as shown in G, which is a magnified view of part of image C.

various categories. Seed in category A in Fig. 3 (Fig. 3-A) were empty, as they lacked both endosperm and embryo, while seeds in category E had a fully developed embryo, endosperm, endospermal membrane, and air space (Fig. 3-E). Capsules in categories 5 and 6 produced seeds that had not undergone complete embryo and endosperm development (Fig. 3-A, B, and C), which did not germinate. Seeds with well-developed embryo and endosperm (Fig. 3-D and E) collected from category 6 capsules germinated at 11% and 32% for category D and E seeds, respectively, whereas those from category 1 capsules germinated at 7% and 72%, respectively. More than 84% (category 6) and 95% (category 1) of seeds belonging to category E germinated in 16 to 20 days regardless of capsule development.

The ratio of length of embryo and endosperm (b) over seed (a) ranged from 0.67, 0.70, and 0.72 in category C, D, and E seeds from category 6 capsules, respectively, to 0.69, 0.69, and 0.74 for seeds collected from category 1 capsules; these numbers are not significantly different from each other (Table 2).

Germination as influenced by *Hemerocallis* taxa

More than 74% of seeds of *H. lilioasphodelus*, *H. citrina* 'April Flower', and *H. minor* collected from China germinated in 16, 14, and 22 days, respectively, and *H. thunbergii*, seeds collected from Korea reached a maximum germination percentage of 80% in 13 days. Except for *H. citrina*, the germination percentage reached a plateau in 2 weeks, and the seeds took less than 16 days to germinate; however, other species took significantly longer than *H. minor* to germinate (Table 3).

The effects of 2 weeks of CS at 5°C on germination

Germination rate of *H. dumortieri* was less than 1% with or without 2 weeks of CS, and the germination rate of *H. minor* was very low (17%), even with 2 weeks of CS (Table 4). Germination rates of *H. vespertina* and *H. hongdoensis* increased

Table 2. Distribution of *Hemerocallis* 'Stella de Oro' seeds based on the X-ray image classification and the number of seeds germinated, with the final germination percentage and the number of days to germination.

Development of capsule[z]	X-ray image of seed[y]	Percentage of seeds		Final germination (%)	No. of days to germination[x]	Ratio[w] (LCE/LS)	Sample size
		Germinated	Not germinated				
Immature	A	0 c[v]	4 b	0 c	0 b	0.0 c	2
capsules 6	B	0 c	5 b	0 c	0 b	0.48 b	2.5
	C	0 c	19 a	0 c	0 b	0.67 a	9.5
	D	11 b	23 a	32 b	17 a	0.70 a	17
	E	32 a	6 b	84 a	16 a	0.72 a	19
Mature	A	0 c	4 b	0 c	0 b	0.0 c	2
capsules 1	B	0 c	4 b	0 c	0 b	0.56 a	2
	C	0 c	4 b	0 c	0 b	0.69 a	2
	D	7 b	5 b	58 b	20 a	0.69 a	6
	E	72 a	4 b	95 a	17 a	0.74 a	38

[z]Capsules of different sizes harvested on Aug. 7, 2014.
[y]X-ray images of seeds. A - no embryo or endosperm (empty), B - partial development of endosperm without clear formation of embryo, C - partial development of endosperm but degenerated embryo, D - partially developed embryo, E - developed embryo, endosperm, endospermal membrane, and air space.
[x]Number of days to germination.
[w]Ratio of the length of embryo and endosperm (LCE) over the length of the seed (LS).
[v]Mean comparison by DMR at $p \leq 0.01$, F-test. Means in a column with different letter are significantly different.

Table 3. Seed germination data for *Hemerocallis* taxa collected from China and Korea.

Species/Origin of collection	Germination (%)[z]				No. of days to germination
	1 wk	2 wk	3 wk	6 wk	
H. lilioasphodelus / China	3 a[y]	46 a	63 a	74 a	16 b
H. citrina / *China*	11 a	91 a	91 a	95 a	11 b
H. citrina 'April Flower' / China	0 a	65 a	76 a	83 a	14 b
H. minor / China	1 a	23 b	38 b	74 a	22 a
H. thunbergii / Korea	2 a	74 a	79 a	80 a	13 b

[z]Cumulative germination percentage.
[y]Mean comparison by DMR at $p \leq 0.01$, F-test. Means in a column with different letters are significantly different.

Table 4. The effect of cold stratification on seed germination of *Hemerocallis* species.

Species	Treatment (2 wk)[z]	Germination (%)[y]				No. of days to germination[x]
		1 wk	2 wk	3 wk	6 wk	
*H. dumortieri*i	20°C	0 a[w]	0 a	0 a	0 a	0
	5°C → 20°C	0 a	1 a	1 a	1 a	25 (11)
H. minor	20°C	3 a	7 a	10 a	12 a	15
	5°C → 20°C	5 a	13 b	17 a	17 a	26 (12)
H. vespertina	20°C	17 a	34 a	45 b	51 a	12
	5°C → 20°C	28 b	49 a	67 a	67 a	10 (10)
H. hongdoensis	20°C	33 a	55 a	77 a	80 a	12
	5°C → 20°C	63 b	78 b	81 a	81 a	20 (6)

[z]Seeds were treated at 20°C for 2 weeks (control) and t at 5°C for 2 weeks followed by 2 weeks at 20°C.
[y]Cumulative germination percentage
[x]Number of days to germination was counted from seed sowing or from the end of 5°C treatment (in parentheses).
[w]Mean comparison of temperature treatment in a given species by DMR at $p < 0.01$, F-test. Means in a row (20°C vs. 5°C → 20°C) in each species with different letters are significantly different.

during the first 2 weeks after sowing when preceded by 2 weeks of CS. However, germination of *H. vespertina* was higher, reaching 67% with5°C treatment. By contrast, the germination rate of *H. hongdoensis* was 33% vs. 63% in 1 week and 55% vs. 78% in 2 weeks without and with CS treatment, respectively, but they reached 77% and 81% without and with CS at 3 weeks; the latter values are not significantly different. Germination took 12 and 20 days after sowing without and with CS, respectively, but only 6 days in *H. hongdoensis*, where the number of days to germination was counted from the sowing date after CS.

Discussion

Seed maturity in relation to size of capsules, X-ray images, and germination

The failure of seeds to germinate could result from many factors, such as harvesting immature seeds prematurely or failure to provide CS or other environmental conditions required to break dormancy in mature seeds. *Hemerocallis* showing indeterminate inflorescence development produces the youngest flowers at the tips of scapes while the capsules are developing. Therefore, capsules harvested at any one time may be at different stages of development and contain both mature and immature seeds.

Hemerocallis capsules harvested at least 5 weeks after anthesis were considered mature, containing seeds with embryos capable of germinating (Arisumi, 1973), which is shorter than the 50–60 days to maturity suggested by other research (Griesbach and Voth, 1957). Although dormancy was reported as a factor of poor seed germination (Griesbach and Voth, 1957), it is evident that dormancy is not present in seeds of all *Hemerocallis* taxa evaluated in this study, and maturity is the major factor that affects seed germination. Immature seeds will fail to germinate as was reported in *Styrax japonicus* (Horimoto et al., 2011) and also in *Corylopsis coreana* Nakai. To our best knowledge, this may be the first report to prove that the previous report (Griesbach and Voth, 1957) may not accurately assess the dormancy in *Hemerocallis*, which is a significant finding to correct the earlier report.

Based on the results of our research, seed capsules at least 3.1 cm long and 2.4 cm wide harvested 35–40 days after anthesis can produce more than 11 mature and black seeds per capsule. Although it is recommended that seeds should be harvested when the distal end of the capsules starts to split (Munson, 1989), capsules harvested in early August did not show any sign of splitting, and the seeds were firmly attached to the placenta tissue. Timing of seed harvest is particularly important following hybridization so that seeds will not be dispersed prior to collection. However, the size of capsules cannot be used to predict the maturity of seeds, since smaller capsules (< 2.7 cm in length) produced a few black seeds and more brown and even white seeds. However, these black seeds were not mature, since they started to shrivel when they lost moisture and they failed to germinate.

X-ray images can be a good indicator of seed viability, as revealed in seeds with a partially developed embryo and degenerated endosperm encircled with an endospermal membrane and those with well-developed embryo and endosperm. A previous study showed that the endosperm and ovules of *Hemerocallis* seeds attained their maximum sizes by the third and fourth weeks of development, respectively, and the embryos reached their mature size by the fifth week (Arisumi, 1973). More than 93% of seeds from mature capsules germinated in 18 days, demonstrating that X-ray images can accurately indicate the maturity of viable seeds.

Mature *Corylopsis coreana* seeds with fully developed endosperm and cotyledons sink when immersed in water, as verified by X-ray images (Kim et al., 2016), with germination percentages higher than 85-90%. However, the presence of air space in mature dry seeds between the seed coat and endosperm or embryo may prevent seeds from sinking when immersed in water. Therefore, immersing 'Stella de Oro' seeds with shiny black seed coats may not be a practical way to separate fully mature and viable seeds from immature and non-viable seeds. The air space in 'Stella de Oro' seeds based on the ratio of endosperm to seed coat is larger than the air space shown in images of seeds of *H. dumortieri* var. *esculenta* and *H. fulva* var. *littorea* prepared by hand using a razor blade (Suzuki et al., 2003). In dry seeds, the ratio of embryo(s) to albumen (endosperm) expressed as a percentage was 40-56% following moist storage and 33% when the seeds were dry and separated from the endosperm, which is smaller than the 73.6% value detected in 'Stella de Oro'. Embryo deformation, as shown in X-ray images in the study or in tissues with less than 50% of endosperm reserves in castor bean, negatively affects the physiological potential of the seed (Carvalho et al., 2010).

The effect of taxa and CS on seed germination

Seeds from all taxa germinated higher than 74% in about 4-6 weeks, attaining > 50% of final germination rates in 2 to 3 weeks. This result clearly indicates that seeds do not exhibit dormancy and that germination of *H. dumortieri*, *H. minor*, *H. vespertina*, and *H. hongdoensis* seeds do not require CS. However, *H. dumortieri* var. *esculenta* and *H. fulva* var. *littorea*

seeds respond positively to CS; germination rate increased to 95% or higher when the seeds were stored under moist conditions (Suzuki et al., 2003). Seeds of *H. minor* and *Hemerocallis* hybrid of complex origin also had increased germination percentages after CS for 3 weeks at 3.5°C (Griesbach and Voth, 1957). Therefore, the requirement of CS to break dormancy, which may be induced by the endosperm membrane (Griesbach and Voth, 1957) to promote germination at 22°C, should be further tested in other species.

In conclusion, 'Stella de Oro' seed capsules with mature seeds with well-developed embryo and cotyledons as confirmed with X-ray imaging and seed germination test can be harvested at 35-40 days after anthesis, with seed germination rates higher than 94% in 17 days, and CS is not necessary for the seeds to germinate rapidly. Seeds of *H. thunbergii* and *H. citrina* germinated within 13 days without CS. Two weeks of CS at 5°C did not accelerate seed germination when the number of days to germination was counted from the beginning of CS treatment in *Hemerocallis* species, including *H. hongdoensis* and *H. thunbergii*. Seeds of *H. hongdoensis* germinated in 24-27 days with or without CS.

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