Non-deep physiological dormancy in seeds of *Euphorbia jolkinii* Boiss. native to Korea

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**Abstract**

**Background:** *Euphorbia jolkinii* Boiss. is a perennial species native to Jeju Island and the southern coastal area of Korea. Particularly on Jeju Island, the yellow flowers of *E. jolkinii* Boiss. have a high ornamental value because of their contrast with basalt. This study was conducted to investigate the effects of different temperatures (5, 15, 20, and 25 °C) and gibberellic acid (GA$_3$) concentrations (0, 10, 100, or 1000 mg/L) on seed dormancy and germination of *E. jolkinii*. In addition, we classified the seed dormancy type and compared types with those of other species in the same genus.

**Results:** The number of seeds with viable embryos and endosperms was approximately 66%. The final germination percentages at 5, 15, 20, and 25 °C were 51.7%, 83.5%, 2.6%, and 0.0%, respectively. In GA$_3$ concentration experiments, the final germination percentages of 0, 10, 100, and 1000 mg/L were 83.5%, 91.7%, 79.1%, and 83.4%, respectively, at 15 °C conditions, and 0.0%, 6.9%, 13.2%, and 27.3%, respectively, at 25 °C.

**Conclusions:** Germination improved at temperatures of 15 °C or lower. Furthermore, GA$_3$ treatment effectively reduced germination times. Thus, the seeds of *E. jolkinii* were classified as having non-deep physiological dormancy.

**Keywords:** Germination temperature, Gibberellic acid, Native plant, New ornamental plants, Physiological dormancy

**Background**

*Euphorbia jolkinii* Boiss. is a perennial herbaceous plant belonging to the Euphorbiaceae family (KPNI 2019). This plant is mainly distributed along the coasts of Jeju, Jeonnam, and Gyeongnam in Korea (Steinmann and Porter 2002). The genus *Euphorbia* has 1000–1600 species worldwide and is mainly distributed in subtropical and temperate zones (Steinmann and Porter 2002). Plants of the genus *Euphorbia* produce poisonous sticky white sap when damaged, and their flower structure is unique; therefore, they are grown for ornamental purposes or used for landscape decoration (Euphorbia PBI 2012). There are approximately 19 species native to Korea, including *E. jolkinii*, *E. esula* L., *E. helioscopia* L., and *E. humifusa* Willd. Ex Schltdl (Park 2007; KNA 2017). *E. jolkinii* grows to 40–80 cm in height and forms a colony with more than 30 stems per population (Bae 2000; Shin et al. 2018a).

The inflorescence is cyathium, consisting of a number of stamens and one pistil, and yellow-green flowers bloom from March to May. The fruit is a capsule, there are bumps on the surface, and the seeds are round and flat, approximately 3 mm in diameter. Stems with green leaves grow in winter, and flowering begins in early spring and lasts for 3 months. In particular, the colorful yellow-green flowers contrast with basalt and have a high ornamental value in the native habitat on Jeju Island.

With the enactment of the Nagoya Protocol, biodiversity and biogenetic resources are highly valued, and efforts are being made to develop and industrialize native plants (Oh et al. 2018). *E. jolkinii* is a medicinal plant material, and leaves and stems have been used as...
medicinal materials in oriental medicine. To verify and exploit these effects, studies on the pharmacological effects of extracts including their antioxidant and antibacterial activities, as well as melanin synthesis in melanoma cells have been conducted (Park et al. 2005; Kim et al. 2006; Kim et al. 2017). In addition, the unique, large flower structure and herbivorous form of *E. jolkinii* are considered to be of high ornamental value. To this end, researchers have evaluated the environment and growth characteristics of native habitats (Shin et al. 2018a), the vase life of cut flowers (Oh et al. 2018; Song et al. 2019a), cuttings for propagation (Shin et al. 2017), and cultivation according to pot size and nutrient conditions (Shin et al. 2018b). However, the dormancy characteristics of seeds and the conditions and characteristics of seed germination have not yet been reported.

Seed dormancy refers to the plant’s self-suppression of germination under adverse environmental conditions for survival and is classified as physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY), and combinational dormancy (PY + PD) (Baskin and Baskin 1998; Baskin and Baskin 2004; Fenner and Thompson 2005). In PD, dormancy is affected by cold stratification, after-ripening, and gibberellic acid (GA) treatment. PD can be divided into three types: non-deep PD, intermediate PD, and deep PD, depending on the depth of dormancy (Nikolaeva 1977; Baskin and Baskin 2014). Non-deep PD is the most common form of seed dormancy. This type of dormancy can be broken by short periods of chilling stratification, after-ripening (dry storage), or gibberellin treatment. Intermediate PD usually requires at least 2 months of chilling or gibberellin application as a substitute for chilling, whereas deep PD requires a long period of at least 3 months of chilling stratification to relieve dormancy and generally does not respond to exogenous gibberellin application (Geneve 2003; Baskin and Baskin 2004). MPD and MD are ecophysiological characteristics that occur because seeds have immature embryos at the time they are separated from the parent plant (Adams et al. 2005a, 2005b). Immature embryos are defined not by the size of the embryo, but by how much the embryo must still grow before germination (or before the seed coat is split) (Baskin and Baskin 2005; Chen et al. 2015). Seeds in the genus *Euphorbia* have been reported to be mostly nondormant or to show PD (Best et al. 1980; Sen and Chatterji 1966; Gómez and Espadaler 1997; Narbona 2002). *E. esula* can break dormancy after a 3-month period of after-ripening treatment (Best et al. 1980), and *E. caducifolia*, *E. characias*, and *E. nicaensis*, which are not native to Korea, do not exhibit dormancy. However, none of these studies have clearly revealed the morphology and development of the embryo. Therefore, detailed research is needed to determine the characteristics of non-dormancy and PD.

Accordingly, in this study, we aimed to obtain basic data for the development of new ornamental plants by identifying optimal germination conditions and classifying dormancy types for *E. jolkinii* seeds.

**Materials and methods**

**Plant material**

The *E. jolkinii* seeds used in this experiment were collected on June 14, 2018, in Seongsan-eup, Seogwipo-si, Jeju (33° 18′ 23.0″, 126° 49′ 36.0″). The collected seeds were dried at room temperature (approximately 25 °C) for 2 weeks in the laboratory and then used in experiments.

**Morphological characteristics of seeds**

To observe the morphology of the seeds, 20 seeds were randomly selected. The seeds were cut in half with a double-edged razor (stainless blade; Dorco, Seoul, Korea). The sections were photographed at 50–60× magnification using a Dino-Life Edge Digital Microscope (AM 3111 Dino-Lite Premier; AnMo Electronics Co., Taiwan), and the secession, internal and external shapes of the seeds, and germination process were observed.

**Water uptake test**

To investigate whether seeds showed PY, the water uptake rate was checked in three replicates of 30 seeds. Seeds were placed in plastic Petri dishes (90 mm × 10 mm) on top of two filter papers (Whatman No.1; GE Healthcare, Buckinghamshire, UK) moistened with distilled water at room temperature. Seeds were weighed after 0, 3, 6, 9, 12, 24, 48, 72, and 96 h of incubation. Percent water uptake was calculated as \[\%W_u = \left(\frac{W_h - W_i}{W_i}\right) \times 100\], where \(W_h\) is the increase in mass of the seed, \(W_i\) is the seed mass after a given interval of imbibition, and \(W_i\) is the initial seed mass at 0 h.

**Temperature treatments**

Seeds were soaked in a solution of 1000 mg/L benomyl (Dongbu Benomy; Farm Hannong, Seoul, Korea) for 1 h. After sterilization, the samples were washed with distilled water five times. Seeds were placed in plastic Petri dishes (90 mm × 10 mm) on top of two filter papers (Whatman No.1; GE Healthcare) moistened with distilled water. To determine the optimum temperature for germination, seeds were incubated at 5, 15, 20 and 25 °C with light (16 h, cool white fluorescent lamp, approximately 25 μmol/m²/s) or darkness in a constant temperature growth chamber (Multi-Room Incubator; WiseCube, Wonju, Korea).
**GA₃ treatments**

To promote germination, seeds were soaked in GA₃ solutions of different concentrations (0, 10, 100, and 1000 mg/L) for 24 h at room temperature. Then, seeds were incubated at 15 °C and 25 °C under light conditions of 16 h light/8 h dark. All treatments were conducted in triplicates with 30 seeds. Germination was checked at day 3 of the experiment. In growth chambers, the photosynthetic photon flux was approximately 25 μmol/m²/s during the light period, and a 16-h light/8-h dark photoperiod was used. Sterile water was periodically administered to keep the filter paper moist.

**Cold stratification treatments**

Sterilized seeds were treated for 0, 4, 8, and 12 weeks at 4 °C and then incubated at 25 °C. The experiment was performed in three repetitions of 30 seeds, and germination was measured at 1-week intervals.

**After-ripening treatments**

The harvested seeds were dried and stored at room temperature for 0, 3, and 6 weeks. At the end of the treatment period, the seeds were sterilized and incubated at 25 °C. The experiment was performed in three repetitions of 30 seeds, and germination was measured at 1-week intervals.

**Germination investigation**

Germination was defined as the point at which radicles emerged at least 1 mm beyond the seed coat. Radicle emergence was checked every 3 days. After the final germination observation, poor-quality seeds were excluded from calculation of the germination percentage. The final germination was measured after 16 weeks of seed incubation. The germination characteristics, final germination percentage (FGP), day of germination, mean incubation, and days to 50% germination (T₅₀) in days were calculated, as follows:

\[
\text{FGP} = \frac{N_g}{N_i} \times 100
\]

where \(N_g\) is the number of final germinated seeds and \(N_i\) is the total number of seeds tested (Al-Mudaris 1998; Gharineh et al. 2004).

\[
T_{50} = T_i + \left[ \frac{(N + 1)/2 - N_i}{(N - N_i)} \right] \times (T_j - T_i)
\]

where \(N\) is the final number of seeds germinating, and \(N_i\) and \(N_j\) are the total numbers of seeds germinated by adjacent counts at times \(T_i\) and \(T_j\) (Coolbear et al. 1984).

\[
\text{MGT} = \sum (N_i \times T_i)/N
\]

where \(N_i\) is the number of seeds germinated, \(T_i\) is the number of days from the start of the experiment, \(N\) is the total number of germinated seeds at the end of the experiment (Naylor 1981).

**Data analysis**

Data were subjected to statistical analysis using SAS 9.4 software (SAS Inst. Inc., Cary, NC, USA). Statistical evaluation was conducted using analysis of variance, and significant data were analyzed further using Tukey’s honestly significant difference test (\(p \leq 0.05\)).

**Results**

**Morphological characteristics of seeds**

E. jolkinii seeds are surrounded by a hard pericarp with bumps (KBIS 2019). The seeds were dark brown and globose or spherical (Fig. 1A). The surface appeared smooth, but had a mesh structure (areolate) (KNA 2017). The collected seeds were 2.12–2.85 mm in length and 2.10–2.35 mm in width, with an average of 2.18 mm. The weight of 100 seeds was approximately 473 mg, and the percentage of sound seeds was approximately 66%. After cutting the seeds, no endosperm vigor was observed in some of the seeds (Fig. 1D).

**Water uptake rate**

The weight of the seeds increased by 21% compared to the initial dry weight after 6 h of water absorption and by 39% after 24 h of water absorption (Fig. 2). After showing a 50% increase in water uptake, the seeds were saturated and did not increase further in weight after 72 h.

**Temperature control of seed germination**

The FGPs at 5, 15, 20, and 25 °C were 51.7%, 83.5%, 2.6%, and 0.0%, respectively (Fig. 3). No seeds germinated under the four temperatures for four weeks. Less than 5% of the seeds germinated at 20 °C and 25 °C. At 5 °C and 15 °C, germination started after 47.3 and 39.0 days, respectively (Table 1). The mean germination times at 5°C and 15 °C were 53.8 and 82.3 days, respectively. The FGP was higher at 15 °C, but the germination progression was faster at 5 °C. In addition, \(T_{50}\) values were 48.7 and 85.0 days at 5 and 15 °C, respectively.

**Effects of GA₃ treatment on seed germination**

GA₃ effectively breaks seed dormancy, promotes embryo development, and enhances germination (Finch-Savage 2006; Baskin and Baskin 2014; Cho and Lee 2017). GA₃ treatment affected the germination of E. jolkinii seeds at 15 °C and 25 °C; in particular, the germination was promoted effectively at 15 °C. Following treatments with 0, 10, 100, or 1000 mg/L of GA₃, the germination percentages were 83.5%, 91.7%, 79.1%, and 83.4%, respectively, at 15 °C and 0%, 6.9%, 13.2%, and 27.3%, respectively, at 25 °C (Fig. 4). There were significant differences in the day of germination according to GA₃ concentration.
Fig. 1 Germination process of *Euphorbia jolkinii* Boiss. seeds. **A** A fruit and a seed. **B** Inner morphology. **C** A radicle. **D** A festered seed. **E**, **F** Seedling emergence. **G**, **H** Flowering of *E. jolkinii* in a native habitat. en, endosperm; em, embryo. Scale bars = 1 mm.

Fig. 2 Water uptake by seeds of *Euphorbia jolkinii* Boiss. as represented by an increase in mass. Seeds were incubated at room temperature for 96 h. Error bars indicate means ± standard errors of three replicates of 30 seeds.
At 15 °C, the GA3 control started to germinate 39 days after the start of the experiment, whereas seeds treated with 100 and 1000 mg/L GA3 started germination on days 13 and 7, respectively. The mean times to germination after 0, 10, 100, and 1000 mg/L GA3 treatment were 82.3, 84.7, 34.8 days, and 34.8 days, respectively, and the $T_{50}$ values were 85.0, 95.0, 47.7, and 41.3 days, respectively (Table 2).

**Cold stratification treatments**

To assess the dormancy of *E. jolkinii* the germination percentage was observed for 9 weeks after cold stratification treatment for 4, 8, and 12 weeks. However, the germination percentage was less than 10% irrespective of the treatment duration.

**After-ripening treatments**

Germination was observed for 17 weeks after 3 and 6 months of after-ripening treatment at room temperature; however, the seeds did not germinate.

**Discussion**

The seeds were divided into basal, peripheral, and axile, according to the shape of the embryo (Martin 1946). In the basal type, the embryo does not exceed half the size of the seed and is located at the lower end. The peripheral type has a large embryo and is curved along the seed coat. In the axile type, the embryo varies and is located in the center of the seed (Song et al. 2019b). *E. jolkinii* seeds were axile, with the embryo located along the center of the seed (Fig. 1B). Seeds of plants native to the temperate regions are often separated from the parent without developing enough embryos, and seeds with such immature embryos are MD- or MPD-type seeds with a ratio of 0.5% or less. We confirmed that the length of the embryo in *E. jolkinii* seeds accounted for approximately 80–90% of the length of the seed, and the radicles protruded without any difference in the length of the embryo just before germination (Fig. 1C). Therefore, there was no MD in *E. jolkinii* seeds.

If germination does not occur because of the impermeability of the seed coat or pericarp, the seed is classified as showing PY (Baskin et al. 2000). If the water

**Table 1** Effect of temperatures on the germination of *Euphorbia jolkinii* seeds

| Temperature (°C) | Days to germination | Mean times to germination | $T_{50}$ |
|-----------------|---------------------|---------------------------|---------|
| 5               | 47.3 ± 1.3b         | 53.8 ± 1.8b               | 48.7 ± 1.3b |
| 15              | 39.0 ± 4.0b         | 82.3 ± 2.9a               | 85.0 ± 3.0a |
| 20              | 102.0 ± 0.0a        | --                        | --       |
| 25              | --                  | --                        | --       |
| $P$-value       | 0.0012              | 0.0011                    | 0.0004   |

*The different letters are significantly different ($p \leq 0.05$) using Tukey’s HSD test ($n = 3$)*
uptake rate is more than 20% within 24 h, the seed is judged to have water permeability (Baskin and Baskin 2003). Therefore, we found that there was no PY in *E. jolkinii* seeds, which had increased water absorption within 6 h.

*E. jolkinii* had the highest germination percentage at 15 °C. In comparison, *E. boetica* had a germination percentage of 73% at 17/21 °C (night/day, 13 h/11 h) and a *T*<sub>50</sub> of 7.4 days, and *E. heterophylla* had a germination percentage of about 80% at 25 °C and 30 °C (Cecilia and Jarbas 2000; Narbona et al. 2007). *E. boetica* grows in lowland pine forests or acidic soils (Benedí et al. 1997), and *E. heterophylla* grows as weeds in tropical and subtropical America (Hutchinson and Dalziel 1958; Wilson 1981). Different plants have different germination conditions, and even similar plants may show major differences in the germination characteristics when their native habitats differ at the national level (Baskin et al. 1989). Thus, we confirmed that the ideal environmental conditions for the germination of *E. jolkinii*, which is distributed only along the southern coast of Korea, were different from other species of the genus *Euphorbia*.

In non-deep PD, dormancy of seeds can be broken by various treatments, including 1–2 months of cold stratification treatment, after-ripening, and GA treatment. In intermediate PD, dormancy breaking through GA treatment is limited to some species, and it is possible to break dormancy through cold stratification treatment for 2–3 months (Geneve 2003; Baskin and Baskin 2004). In addition, it is possible to partially satisfy the low-temperature requirement for breaking dormancy through dry storage such as post-aging. In deep PD, the dormancy of seeds cannot be broken by GA treatment, but it is possible to break dormancy through 3–4 months of low-temperature or high-temperature wet treatment (Geneve 2003; Baskin and Baskin 2004). In this study, we showed that GA<sub>3</sub> treatment could effectively improve the germination percentages of *E. jolkinii* seeds. The germination percentage was 79.1–83.4% at 100 and 1000 mg/L, but there were no significant differences between these two concentrations. In addition, the day of germination, mean time to germination, and *T*<sub>50</sub> were shortened in the presence of 100 and 1000 mg/L GA<sub>3</sub>. Indeed, at these concentrations, the day to germination was 3–5 times earlier, the mean time to germination was shortened 2 times, and the *T*<sub>50</sub> was shortened by 2.0–2.3 times compared with the control. Therefore, GA<sub>3</sub> treatment improved the germination percentage.

### Table 2

| GA<sub>3</sub> concentration (mg/L) | Days to germination | Mean times to germination | *T*<sub>50</sub> |
|-----------------------------------|---------------------|--------------------------|-----------------|
| 0                                 | 39.0 ± 4.0<sup>b</sup> | 82.3 ± 2.9<sup>a</sup> | 85.0 ± 3.0<sup>a</sup> |
| 10                                | 83.5 ± 1.5<sup>a</sup> | 84.7 ± 2.4<sup>a</sup> | 95.0 ± 0.0<sup>a</sup> |
| 100                               | 13.0 ± 1.0<sup>c</sup> | 34.8 ± 2.8<sup>b</sup> | 47.7 ± 6.2<sup>b</sup> |
| 1000                              | 7.0 ± 0.0<sup>c</sup> | 34.8 ± 9.1<sup>b</sup> | 41.3 ± 8.4<sup>b</sup> |
| P-value                           | 0.0001              | 0.0004                   | 0.0011          |

<sup>b</sup>The different letters are significantly different (p ≤ 0.05) using Tukey’s HSD test (*n* = 3)
and shortened the mean time to germination, confirming that the seeds showed non-deep PD.

Among native plants in Korea, gibberellin treatment has been shown to improve or promote seed germination. Germination percentages of Primula modesta var. hannasanensis T.Yamaz. were 95.0% and 95.5% at GA3 concentrations of 200 and 500 mg/L, respectively, which were 56.0–69.0% higher than those of the control treatment (Cho and Lee 2017). Additionally, the germination percentage (72.5%) of Tiarella polyphylla D. Don was improved by GA3 treatment at a concentration of 1000 mg/L compared with the control treatment (33.3%) (Choi et al., 2018). Moreover, the germination percentages of Veronica kiusiana var. diamantiaca (Nakai) T. Yamaz. were enhanced by GA3 treatment to 71.3%, 87.2%, and 91.7% at GA3 concentrations of 50, 100, and 1000 mg/L, respectively, compared with that of the control (44.7%) (Song et al. 2019b). Zanthoxylum piperitum (Kim et al. 1997), Exochorda serratifolia (Lee et al. 2006), Pinus pumila (Lim et al. 2015), Lychnis wilfordii (Regel) Maxim (Ryu et al. 2017), and many other species have also been shown to have improved germination percentages following gibberellin treatment, suggesting that this treatment breaks the PD of seeds. Accordingly, these findings showed that the concentration and time of gibberellin treatment were critical factors for improving germination percentages.

**Conclusion**

Wild native species have a wide distribution range, and it is not easy to establish an appropriate environment for seed germination. *E. jolkinii* Boiss. seeds require a longer time to germinate than other species in the same genus *Euphorbia*. In this study, we confirmed that these seeds showed no MD or PY, as demonstrated by embryo observation and water permeability. When germination was observed at various temperatures, we found that the optimum temperature for germination was 15 °C, although the mean germination time at 15 °C was very late (82.3 days), and PD was confirmed. Additionally, the days to germination, mean time to germination, and T50 were shortened following GA3 treatment at concentrations of 100 and 1000 mg/L, thus classifying *E. jolkinii* seeds as showing non-deep PD. Overall, we confirmed that the germination of *E. jolkinii* seeds was improved at temperatures below 15°C. In addition, cold stratification and after-ripening, GA3 individual treatments were not effective for germination. However, treatment with GA3 at 15°C reduced the mean germination time. Scientific conclusions on these environmental conditions and dormancy will be useful for establishing a mass production system for commercial use and ecological management.

**Abbreviations**

PD: Physiological dormancy; MD: Morphological dormancy; MPD: Morphophysiological dormancy; PY: Physical dormancy; FGP: Final germination percentage; T50: Days to 50% germination; MGT: Mean germination time

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**Authors’ contributions**

OHJ and SUS carried out the study, analyzed the data, and wrote the manuscript. LSY conceived the study design, analyzed the data, and reviewed the manuscript. KSY collected and analyzed the data. JMJ participated in the study and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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