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Seed Germination and Seedling Emergence of *Canavalia Lineata* (Thunb.) DC. (Fabaceae)

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Abstract

Canavalia lineata (Thunb.) DC. (Fabaceae) is a perennial trailing vine that flowers in the summer, and its seeds are sea-dispersed. It grows under various environmental conditions in coastal areas of the temperate and subtropical regions of Asia. Plant species with a wide distribution tend to differentiate in different environmental conditions; therefore, we conducted this study to find whether *C. lineata* has regional differentiation in seed germination and seedling emergence. Seeds of *C. lineata* collected from different areas of Japan were used to compare the seed-floating ability, the effect of low temperature on seed dormancy (0, 2, and 8 months) before germination, and the influence of temperature (4 ℃, 15 °C, 20 °C, and 25 °C) on seed germination. Seed-floating tests indicated that many *C. lineata* seeds can float on the sea without losing their germination ability, and there was no associated regional differentiation. In addition, our results showed that this species could germinate without being exposed to low temperatures, but the timing of germination in such cases was different from that in the seeds that experienced low temperatures. The optimal temperature for *C. lineata* germination was 20 °C or higher. These analyses did not reveal any regional differentiation. Our results suggest that sea-dispersed seeds of *C. lineata* that do not lose germination ability for a long period may germinate in relatively warm areas regardless of the presence of low temperature conditions. The study also indicates that as germination of this species requires a relatively high temperature; thus, their distribution has not expanded to the north of Japan.

Keywords: germination, sea, seed, *Canavalia lineata*

1. Introduction

Plants adapt in many ways to biotic and abiotic stresses in their surroundings to survive (Bohnert et al., 1995; Bartels & Sunkar, 2005). In coastal areas, many plants have developed characteristic morphologies to overcome stresses, such as strong winds, seawater droplets, and shifting sands (Greenway & Manns, 1980). Although seawater is often unnecessary for plant growth, it can be used for the seed dispersal by coastal plants. Seed dispersal by ocean currents is considered a long-distance dispersal mechanism and one of the characteristic properties of coastal plants (Nakanishi, 1988; Harwell & Orth, 2002). Many mangrove species have been documented to use seed dispersal by sea currents (Triest, 2008). In Japan, plants with sea-dispersed seeds have been reported in coastal areas. For example, using seed-floating and germination tests, Sawada and Tsuda (2005) reported 14 species of coastal plants, including three alien species, with sea-dispersed seeds. Gene flow had also been observed in the pantropical plants such as *Hibiscus tiliaceus* L. (Malvaceae) and *Ipomoea pes-caprae* (L.) R.Br. (Convolvulaceae) with sea-dispersed seeds (Takayama et al., 2008; Miryeganeh et al., 2014). Recently, Kuroda et al. (2018) suggested that the seeds of *Linaria japonica* Miq. (Plantaginaceae) are dispersed by sea currents as they can float in seawater for more than one month and germinate at an approximate rate of 40%. This indicates that in many plants of coastal areas of Japan, there is no insurmountable reduction in germination ability of seeds that float in seawater due to buoyancy.

Long-distance seed dispersal influences many key aspects of plant biology. It could spread invasive species in plant communities (Hovestadt et al., 1999). In particular, long-distance seed dispersal from south to north leads to the invasion of areas with significant climatic differences and temperature changes. Plant species undergo regional differentiation to adapt to these conditions. Some species of genus *Canavalia* (Fabaceae) have buoyant and impermeable seeds that can drift for long periods over great distances (Sauer, 1964); the genus *Canavalia* comprises approximately 60 species in subtropical and tropical regions (Sauer, 1964). Snak et al. (2016) explained that *Canavalia* achieved its present-day distribution through recent transoceanic dispersal of buoyant and impermeable seeds. Based on chloroplast and nuclear

DNA sequences, Vatanparast et al. (2011) suggested that Hawaiian *Canavalia* species arose by the colonization of sea-dispersed seeds. In Japan, three species of this genus are found: *Canavalia lineata* (Thunb.) DC., *C. rosea* (Sw.) DC., and *C. cathartica* Thouars (Ohashi, 1981). *C. lineata* is a perennial trailing vine that grows in coastal areas from in temperate and subtropical regions of Asia (Ohashi, 1981). Its leaves are compound, and leaflets are roughly circular; the flowers are small and reddish-purple, ranging from 2.5 to 3 cm in diameter (Figure 1). It blooms with great intensity between June and August and produces large fruits that are 6–12 cm long and 3-4 cm wide (Ohashi, 1981). The seeds are sea-dispersed (Nakanishi, 1987), and the distribution area is spread across the north of the three Japanese *Canavalia* species. Its distribution is limited to the north at Chiba Prefecture on the Pacific Ocean side and Shimane Prefecture on the Sea of Japan side (Tateishi, 1997) (Figure 2).

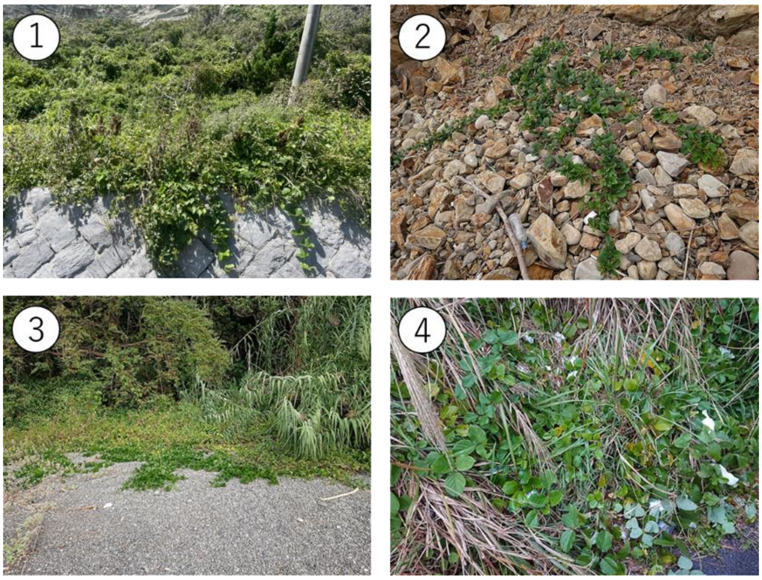

Figure 1. *Canavalia lineata*

1: Chiba; 2: Minamiawaji; 3 Aki; 4: Kochi. Figure numbers and locality names are corresponded in Table 1.

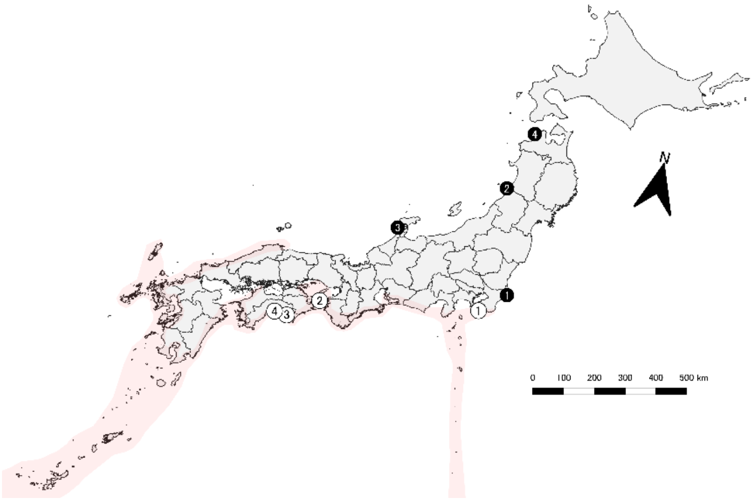

Figure 2. Distribution of *Canavalia lineata* based on Tateishi (1997)

Collecting localities indicate open circles. Numbers in the open circles are corresponded in Table 1. The localities where seedlings of this species were reported are indicated by solid circles. The numbers in the solid circle are based on the following reports; 1: Wada (1980), 2: Mori (1954), 3: Nakanishi (1980), 4: Nakanishi (2016).

However, some reports have suggested that *C. lineata* has expanded ahead of these northern limits. For example, Nakanishi (1987) observed *C. lineata* to be distributed in Ibaraki Prefecture located north of the Chiba Prefecture. Moreover, Mori (1954) and Nakanishi (1980) showed them to be present in Yamagata Prefecture and Ishikawa Prefecture, located at the north of Shimane Prefecture, on the Sea of Japan side. Tateishi (1997) suggested that the northern limit of *C. lineata* on the Sea of Japan side was Shimane Prefecture because seedlings that colonized Yamagata and Ishikawa Prefectures did not reach their reproductive phase. A recent report indicated the presence of *C. lineata* seedlings in Aomori Prefecture on the Sea of Japan side, which is the northernmost part reported to date to have these seedlings (Nakanishi, 2016); however, this seed population also lacked reproductive ability. Based on these reports, the northern limits of this species remain unclear. Environmental constraints in the northern limits at early life-history stages of seed dispersal or seedling establishment may be critical in restricting the colonization of this species. Compared to the center of the distribution, the species distribution in the north may be limited by several biotic and abiotic environmental factors and differences in available resources, such as light, soil water, and nutrients. In addition, the wide distribution from south to north was expected to cause regional differentiation in *C. lineata*.

Understanding reproductive biology traits, seed germination behavior, and the influence of environmental factors on germination is crucial to conserve and manage coastal plant species (Evans et al., 2003; Heywood & Iriondo, 2003). The function of a seed is to establish a new plant, but it can do this once because the completion of germination is essentially an irreversible process. The specific environmental sensitivity of seeds may have implications for dispersal, colonization, plant demography, speciation, and extinction (Wills et al., 2014). The timing of seedling emergence is a crucial event in a plant's life cycle, affecting its chances of becoming established and reaching the reproductive phase (Vandelook & Van Assche, 2008; Donohue et al., 2010). Therefore, various plant species have evolved several dormancy mechanisms to optimize the seed germination time (Foley, 2001). Seedlings of most species emerge shortly after germination. The timing of seedling emergence is mainly regulated by dormancy breaking and the germination requirements of the seed. Various legumes have seeds that are physically dormant because of the development of water-impermeable seed coats (Baskin et al., 2000). This type of defense is also present in the wild progenitors of cultivated legumes (Abbo et al., 2014). The legume seeds exit dormancy in response to temperature changes in the habitat, by making the seed coat water-permeable (Smýkal et al., 2014). Temperature is one of the most important abiotic factors affecting seed germination (Baskin & Baskin, 1985; Milbau et al., 2009); it also controls germination timing in seasonal climates (Shafii & Price, 2001; Rajasekaran et al., 2002). Each plant species has a base and ceiling temperature that represent the extremes at which germination can occur (Finch-Savage & Leubner-Metzger, 2006). Seed characteristics generally remain highly conserved within a species, but even small changes in these characteristics can affect almost every aspect of their morphology, ecology, and physiology (Harper et al., 1970). However, some studies have examined variations in seed characteristics within species (Buckley et al., 2003). The germination response to temperature can vary among populations across the distribution range of a species (Seguí et al., 2021). Understanding how populations vary in their sensitivity to temperature along climate gradients is important for determining their vulnerability to climate variability and change. As *C. lineata* has a wide distribution area ranging from south to north, its seeds may show differentiation in ecological and physiological characteristics to regulate germination time along climate gradients. Therefore, this study aimed to elucidate and compare seed germination and seedling emergence in *C. lineata* seeds from different areas.

2. Method

2.1 Plant Material Collection

Seeds of *Canavalia lineata* were collected from four allopatric populations during November 2020 and September 2021. Seed populations used in our study were named after locations (Chiba, Minamiawaji, Kochi, and Aki) (Table 1). The seed collecting localities are shown in Table 1. Seeds were manually separated from inflorescences. Then, healthy seeds were selected and placed in envelopes. Germination tests without subjecting to low temperatures began immediately after seed collection. The remaining seeds were dry-stored in a refrigerator (MPR-312D, SANYO, Japan) at 4 °C until further use.

Table 1. Sampling localities used in this study. Locality numbers are corresponded in Figure 2

Locality name and number		Locality	Latitude and longitude	
Chiba	1	Iwaifukuro, Kyonan-machi, Awa-gun, Chiba Prefecture	35°10'N	139°82'E
Minamiawaji	2	Nadanigoro, Minamiawaji City, Hyogo Prefecture	34°19'N	134°74'E
Aki	3	Akano, Aki City, Kochi Prefecture	33°51'N	133°84'E
Kochi	4	Nino, Haruno-cho, Kochi City, Kochi Prefecture	33°47'N	133°50'E

2.2 Seed-Floating Test Followed by Germination Experiment

Floating tests were performed in the summer of 2021 using the seeds incubated at low temperatures in a refrigerator for eight months. Four groups (Chiba, Minamiawaji, Kochi, and Aki) of thirty *C. lineata* seeds were placed in glass basins (30 cm depth × 30 cm diameter) three-quarter filled with saline water (3.45 % NaCl solution) and subjected to three weeks floating period. During the floating test, sunken seeds were removed from the glass basins. After the floating period, the seeds were washed with water, and the integrity of the water-impermeable seed coat was breached by artificially cutting a hole in the seed coat. These seeds were then used for subsequent germination experiments. The germination experiments were carried out in flowerpots (10 cm depth × 10 cm diameter) filled with three-quarters of vermiculite (KA09, Kohnan Co., Japan) in distilled water. The seeds were sown in the flowerpots and then the pots were placed in an incubator (A5501, AS ONE, Japan) at 25 ℃. All four groups of floating seeds (Chiba, Minamiawaji, Kochi, and Aki) were treated in this way. Seeds were considered germinated at the seedling emergence. The percent germination was recorded every day for two weeks. All values were expressed as mean ± SD. Tukey’s multiple comparison test was used to determine significant differences in germination between floating tests done on seawater and distilled water, for seed collected from different areas; p-values < 0.05 were considered significant.

2.3 Germination Experiments After Different Low-Temperature Incubation Periods

In general, a temperature around 5 ℃ is considered optimum for breaking seed dormancy. This temperature is often used in experiments involving pre-chilling treatments (Baskin & Baskin, 2014). However, in some cases, temperatures below 5 ℃ can be more effective in breaking dormancy. To reveal the effect of low temperature on the dormancy, *C. lineata* seeds were placed in a refrigerator at 4 ℃ for three different periods: 0 months (non-dormancy), 1 month, and 8 months. Thirty seeds collected from Chiba Prefecture were used in this experiment. The seeds were then taken out from the refrigerator and their seed coats were pierced. Then, the germination experiments were performed in flowerpots (10 cm depth × 10 cm diameter) filled three-quarters with vermiculite (KA09, Kohnan Co., Japan) and distilled water. The pots were kept in an incubator (A5501, AS ONE, Japan) at 25 ℃. The percent germination was recorded daily for two weeks. All values were expressed as mean ± SD. Tukey’s multiple comparison test was used to determine significant differences in germination among different incubation periods of low temperature and p-values < 0.05 were considered significant.

2.4 Germination Experiments at Different Incubation Temperatures

To determine the effect of temperature, seeds were germinated in incubators under two temperature regimes: 20 °C and 25 °C (20 °C: THS-020DB, ADVANTEC, Japan; 25 °C: A5501, AS ONE, Japan). In this experiment, the seeds collected from Chiba Prefecture (n=30) or Minamiawaji (n=10) were pre-exposed to 4 ℃ for one month and one week, respectively. Then, they were taken out from the refrigerator, and their seed coats were pierced. Germination experiments were conducted using the method described above, and the percent germination was recorded every day for two weeks.

Similarly, additional experiments were performed on seeds collected from Minamiawaji (n=10) in the incubator at 4 °C and 15 °C (4 °C: MPR-312D, SANYO, Japan; 15 °C: THS-020DB, ADVANTEC, Japan). Two weeks later, the temperature was raised to 25 ℃, and the germination rates were measured. All values were expressed as mean ± SD. Tukey’s multiple comparison test was used to determine significant differences in germination at different incubation temperatures, and p-values < 0.05 were considered significant.

3. Results and Discussion

Legumes have soft-to-hard seed coats that play a critical role in the lateral transfer of assimilates and other nutrients before their release into the developing embryo (Lush and Evans, 1980; Offler & Patrick, 1984, 1993). Ma et al. (2004) reported that small cuticular cracks, which are consistently correlated with seed coat permeability to water, are not present in the hard seed coat. *Canavalia* species also have a water-impermeable seed coat (Mendoza-González et al., 2014). Dalling et al. (2011) hypothesized that the hard seed coat should depend on the likelihood of seed predation and that non-hard coats might evolve as part of a predator avoidance strategy. Bean weevils are seed predators that feed on legumes; their adults deposit eggs on seeds, then the larvae chew their way into the seed and feed on the bean endosperm and embryo (Janzen, 1977). The perforation of the seed coat by bean weevils is considered to play an important role in water permeation into the embryo and endosperm, and trigger germination of legume seeds (Fox et al., 2012). Therefore, in our study, we used an artificial method to break the seed coat. The integrity of the water-impermeable seed coat was compromised by cutting a hole in the seed coat; the floating tests and the tests after incubating seeds at low temperatures were performed without modifying the seeds.

3.1 Seed-Floating Ability of Seeds From Different Collecting Areas

The floating test in saline water indicated that seeds of *C. lineata* collected from various areas had a high probability to continuously float for three weeks or more (Table 2). Moreover, no significant differences were found between seeds collected from the different localities (Table 2).The question remains: how far could the seeds of *C. lineata* disperse in the floating period of three weeks or more? Although the speed of movement of seeds in the sea is unknown, an interesting phenomenon in 2021 provided clues regarding the movement of floating objects in the ocean current. Pumice stones were ejected from Fukutoku-Oka-no-Ba, which is an undersea volcano on the Ogasawara Islands, in an eruption in mid-August (Geological Survey of Japan, 2021). The pumice stones were found in Okinawa in mid-October and along the coast of Chiba Prefecture in early November (Geological Survey of Japan, 2021). Although the movement of pumice and seeds cannot necessarily be the same, it was shown that the distance from Okinawa to Chiba could be covered by floating objects in the ocean current in approximately a few weeks, suggesting that seeds of *C. lineata* floating on the sea for more than three weeks can be dispersed at long distances.

Table 2. Comparison of seed floating ratio in saline water among collecting localities

	localities							
days	Chiba		Minamiawaji		Aki		Kochi	
0	100.0±0.00	a, A	100.0±0.00	a, A	100.0±0.00	a, A	100.0±0.00	a, A
5	95.24±4.76	ab, A	100.0±0.00	a, A	100.0±0.00	a, A	82.61±8.08	b, A
10	95.24±4.76	ab, A	100.0±0.00	a, A	100.0±0.00	a, A	78.26±8.79	b, A
15	95.24±4.76	ab, A	100.0±0.00	a, A	100.0±0.00	a, A	73.91±9.36	b, A
20	85.71±7.82	ab, A	100.0±0.00	a, A	100.0±0.00	a, A	73.91±9.36	b, A
23	85.71±7.82	ab, A	100.0±0.00	a, A	93.33±6.67	ab, A	73.91±9.36	b, A

Locality names are corresponded in Table 1. Columns marked by different letters differ significantly according to the Tukey's HSD test ($p < 0.05$). Small alphabets indicate the results of tests among localities, and large alphabets indicate the results of tests among days within localities.

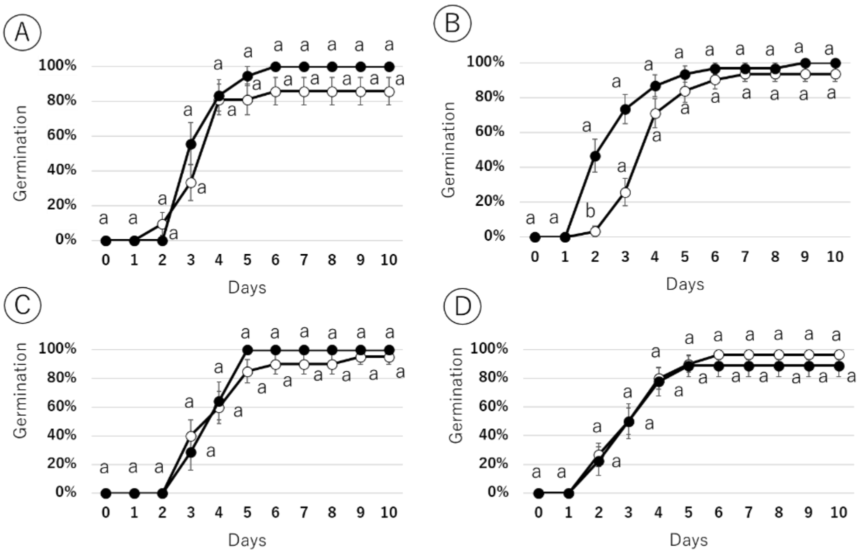

Figure 3. Percentage of seed germination of *Canavalia lineata* versus days in saline water (solid circle) and distilled water (open circle)

A: Chiba; B: Minamiawaji; C: Aki; D: Kochi. Locality names are corresponded in Table 1.

However, even if the seeds float in seawater for a long time, seed dispersal by sea currents cannot be effective if the seeds do not retain their germination ability. Our germination tests revealed that the seeds that floated on seawater had high germination rates; however, these rates were slightly lower than those of the seeds that floated on pure water (Figure 3). This finding suggested that invisible scratches on the seed coat may affect the penetration of saline water into the seed. Our results indicated that most seeds could germinate after floating in saline water, indicating that seeds of *C. lineata*, long which have dispersed by sea currents, maintain their germination ability. Moreover, no significant difference was observed between the seeds collected from different regions in floating experiments (Tables 3, 4), indicating that there was no regional differentiation in seed germination ability. These results suggested that *C. lineata* seeds could be dispersed over long distances, but the lack of differentiation between regions may inhibit the expansion of the distribution area.

Table 3. Comparison of seed germination ratio floated in saline water among collecting localities

	localities							
days	Chiba		Minamiawaji		Aki		Kochi	
0	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A
1	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A
2	0.00±0.00	b, A	46.67±9.26	a, AB	0.00±0.00	b, A	22.22±10.08	ab, AB
3	55.56±12.05	ab, AB	73.33±8.21	a, BC	28.57±12.53	b, B	50.00±12.13	ab, BC
4	83.33±9.04	a, BC	86.67±6.31	a, CD	64.29±13.29	a, C	77.78±10.08	a, CD
5	94.44±5.56	a, C	93.33±4.63	a, D	100.00±0.00	a, D	88.89±7.62	a, D
6	100.00±0.00	a, C	96.67±3.33	a, D	100.00±0.00	a, D	88.89±7.62	a, D
7	100.00±0.00	a, C	96.67±3.33	a, D	100.00±0.00	a, D	88.89±7.62	a, D
8	100.00±0.00	a, C	96.67±3.33	a, D	100.00±0.00	a, D	88.89±7.62	a, D
9	100.00±0.00	a, C	100.00±0.00	a, D	100.00±0.00	a, D	88.89±7.62	a, D
10	100.00±0.00	a, C	100.00±0.00	a, D	100.00±0.00	a, D	88.89±7.62	a, D

Locality names are corresponded in Table 1. Columns marked by different letters differ significantly according to the Tukey's HSD test ($p < 0.05$). Small alphabets indicate the results of tests among localities, and large alphabets indicate the results of tests among days within localities.

Table 4. Comparison of seed germination ratio among collecting localities

	localities							
days	Chiba		Minamiawaji		Aki		Kochi	
0	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A
1	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A	0.00±0.00	a, A
2	9.52±6.56	ab, A	3.23±3.23	b, AB	0.00±0.00	b, A	26.67±8.21	a, B
3	33.33±10.54	a, A	25.81±7.99	a, B	40.00±11.24	a, B	50.00±9.28	a, B
4	80.95±8.78	a, B	70.97±8.29	a, C	60.00±11.24	a, BC	80.00±7.43	a, C
5	80.95±8.78	a, B	83.87±6.72	a, C	85.00±8.19	a, CD	90.00±5.57	a, C
6	85.71±7.82	a, B	90.32±5.40	a, C	90.00±6.88	a, CD	96.67±3.33	a, C
7	85.71±7.82	a, B	93.55±4.49	a, C	90.00±6.88	a, CD	96.67±3.33	a, C
8	85.71±7.82	a, B	93.55±4.49	a, C	90.00±6.88	a, CD	96.67±3.33	a, C
9	85.71±7.82	a, B	93.55±4.49	a, C	95.00±5.00	a, D	96.67±3.33	a, C
10	85.71±7.82	a, B	93.55±4.49	a, C	95.00±5.00	a, D	96.67±3.33	a, C

Locality names are corresponded in Table 1. Columns marked by different letters differ significantly according to the Tukey's HSD test ($p < 0.05$). Small alphabets indicate the results of tests among localities, and large alphabets indicate the results of tests among days within localities.

3.2 Germination Rate With and Without Low-Temperature Stress

The low-temperature tests for various periods indicated that *C. lineata* seeds did not require low-temperature exposure for germination (Figure 4), and have the potential to germinate as soon as they were washed ashore in coastal areas. However, it remains unclear why *C. lineata* seeds do not need low temperatures for germination, despite being restricted to relatively seasonal environments, where favorable conditions occur over most of the year. However, it was very interesting to note that germination rates were significantly different among the low-temperature periods after seeds had absorbed water (Figure 4). Seeds that experienced low temperatures showed faster germination, implying that *C. lineata* seeds could select the time of germination to adapt to the optimal period for seedling emergence. Plants develop dormancy to overcome long periods of unfavorable growth conditions, such as dry hot summers and cold winters (Vegis, 1964). In general, dormancy is considered a mechanism for avoiding periods that are favorable for germination but unfavorable for subsequent seedling establishment (Vleeshouwers et al., 1995). For example, Langens-Gerrits et al. (2003) indicated that the longer the cold storage, the faster and more uniform leaf emergence occurred in lily, indicating that low temperature not only affects the number of sprouted bulblets but also the time of emergence. The most favorable period for seedling establishment varies according to geographical distribution and climatic conditions. Therefore, different climatic conditions are often reflected in the dormancy-breaking requirements of seeds (Skordilis & Thanos, 1995). Habitat preference and the life cycle of the species are other factors that determine the optimal period for seedling emergence and thus affect seed behavior (Nikolaeva, 1999). Snak et al. (2016) revealed the molecular phylogenetic relationships in *Canavalia* species, he observed that *C. lineata* was closely related to *C. rosea* (Sw.) DC., *C. cathartica* Thouars, *C. gladiata* (Jacq.) DC., and so on, which have a wide distribution range in the tropics without winters. Although *C. lineata* has a wide distribution area, each region has a winter season. We hypothesized that *C. lineata* has gained the ability to recognize low temperatures to improve fitness in the temperate zone, regardless of whether this species could germinate without exposure to low temperatures. To verify this hypothesis, it is necessary to conduct germination experiments on seeds of tropical *Canavalia* species exposed to low temperatures, in the future.

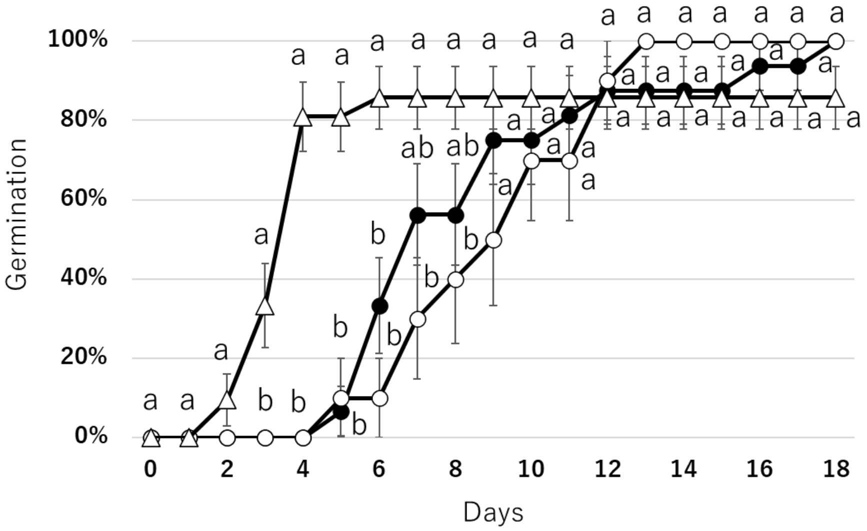

Figure 4. Influence of low temperature periods before seed germination of *Canavalia lineata* (0 month [solid circle], 1 month [open circle] and 8 months [open triangle])

3.3 Effect of Temperature on Seed Germination

Temperature is the most important driving force influencing plant development rate; for example, the effects of temperature on plant development are the basis for models used to predict germination timing. Although all of the above germination experiments on *C. lineata* seeds were performed at 25 ℃ based on the methods of Butler et al. (2014), it is unclear whether the temperature change could affect the germination and seedling emergence. Thus, the results of germination experiments at multiple temperatures using *C. lineata* seeds with holes cut revealed that they could germinate at temperatures 20 ℃ or above, but not at 15 ℃ or below (Figures 5, 6). Moreover, the increase in the temperature significantly accelerated the seedling emergence (Figure 6). Although their germination occurred at 20 ℃, not all individuals germinated (Figure 5), suggesting that this temperature was unlikely to be the optimum temperature for germination. These results suggest that *C. lineata* seeds require relatively high temperatures for germination and seedling emergence; therefore, this species occurs in warmer areas and is not distributed in the northern regions of

Japan. In addition, *C. lineata* seeds that could not germinate at 4 ℃ and 15 ℃, germinated at 25 ℃ (Figure 6), indicating that the seeds did not die even if they were left at low temperature for a certain period. Therefore, *C. lineata* seeds may be present as drifting seeds and buried seeds along the coast of Japan, as seeds can only remain ungerminated in the ground if they are affected by predation or pathogenic infection or both.

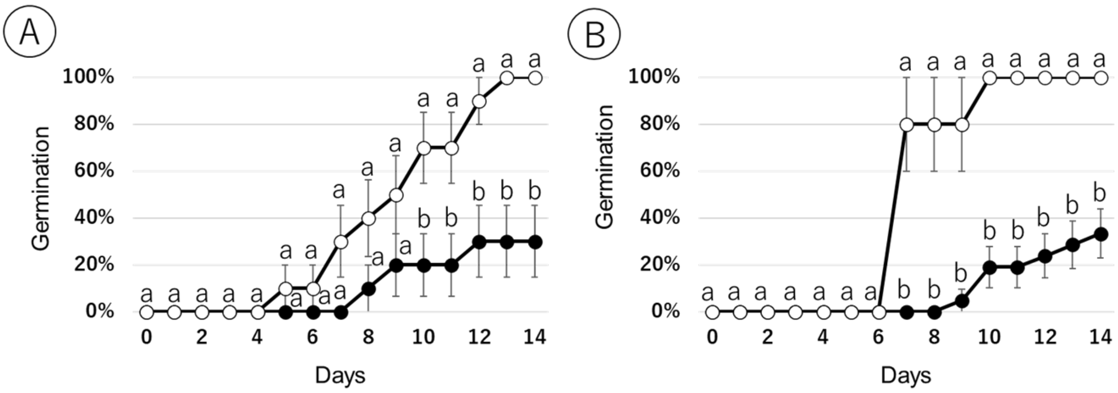

Figure 5. Comparison days of seedling appearance between 20 ℃ (solid circle) and 25 ℃ (open circle)

A: Chiba; B: Minamiawaji. Locality names are corresponded in Table 1.

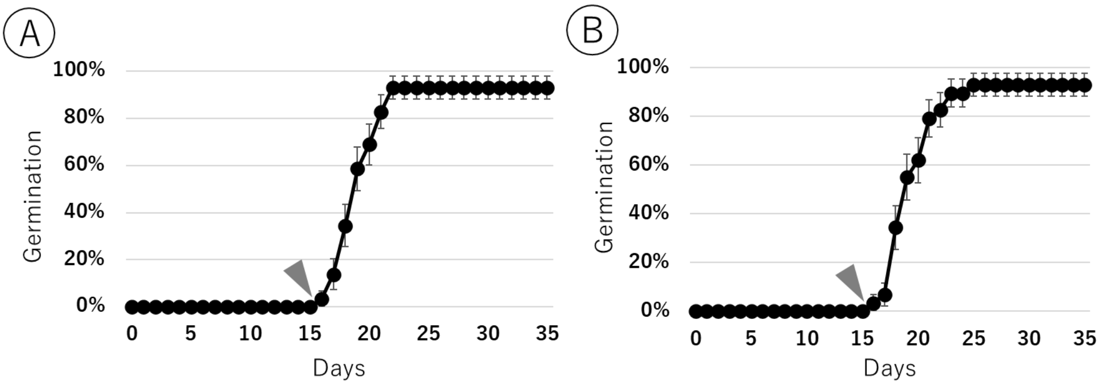

Figure 6. Cumulative seed germination percentage of *Canavalia lineata* after transferred (14 days) from 4 ℃ or 15 ℃ into 25 ℃ used in the seeds collected from Minamiawaji

Arrowheads indicate the day when the temperature was transferred from 4 ℃. Locality name is corresponded in Table 1. A: from 4 ℃ into 25 ℃; B: from 15 ℃ into 25 ℃.

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