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GROWTH AND GERMINATION CHARACTERISTICS OF THREE *AKEBIA* LIANA SPECIES FOR REVEGETATION

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ABSTRACT: Three woody climbing species of *Akebia* (Lardizabalaceae), *A. quinata* (AQ), *A.* × *pentaphylla* (AP) and *A. trifoliata* (AT), have been used for revegetation applications. However, the optimal applications of each species have not been established, as their seed germination and growth characteristics are poorly known. In the present study, we clarified their seed germination and stem growth characteristics to evaluate their potential for use as revegetation materials. Seed germination was conducted under continuous light (24 hours) with alternating temperature conditions (28 ºC/ 15 ºC, each for 12 hours) using five different pretreatments on seeds harvested in 2023. In assessments of stem growth, the number of climbing and creeping individuals, and the basal diameters of each were surveyed in 17 habitats in Nagano Prefecture, Central Japan, in 2023. In all pretreatments, the germination percentage was high in AP (maximum: 73%), but poor in AQ and AT (maximum: 5% and 10%, respectively). The development of cotyledons after germination was also faster in AP than in AQ or AT. The ratio of climbing individuals was significantly lower in AP (0.34) than in AQ and AT (0.67 each), and the basal diameter was significantly larger in climbing individuals than creeping individuals in all species. Consequently, it is considered AP is better suited for rapidly covering the ground surface by direct seeding, whereas AQ and AP would be better suited for climbing along pergolas and forming hedges by planting seedlings.

Keywords: Akebia, Revegetation, Germination, Climbing growth, Creeping growth

1. INTRODUCTION

Recent, the ecological conservation strategies employed for revegetation purposes have shifted to using native species. Formerly, exotic pasture grasses, with rapid and homogeneous germination and growth, were frequently used for revegetation across Japan. However, the use of native plant species can be challenging, mainly because the germination and growth of native species lack uniformity and their growth characteristics are typically inferior to exotic pasture grasses [1].

In addition, the selection of appropriate native plant species for specific revegetation purposes can also be problematic, as accurately identifying native plants to species is often difficult. In Japan, the naturalization of exotic species similar to the intended native species has been reported. For example, *Artemisia sacrorum* Ledeb., which is distributed on coastal cliffs in northern Japan, has been unexpectedly observed on cut-slope revegetation sites further inland in Japan [2]. This occurrence is likely due to seed contamination, where seeds are confused with *A. indica* Willd., a native species that is commonly used for revegetation.

Among the wild plant species that are used for revegetation, climbing plants typically show rapid growth, as they do not need to support themselves and hence invest more energy in stem elongation and less energy on stem thickness [3]. Climbing plants often outcompete other plants by smothering them [4]. Therefore, some species of climbing plants, such as kudzu vine (*Puelaria lobata* (Willd.) Ohwi), can become difficult to manage, while other climbing plants, such as wisteria (*Wisteria* spp.) or ivy (*Hedera* spp.), are widely used for revegetation as they require less maintenance.

Akebia (Lardizabalaceae) is a genus of deciduous woody climbing plants, with three species, *A. quinata* (Houtt.) Dence. (AQ), *A. trifoliata* (Thunb.) Koidz. (AT) and *A.* × *pentaphylla* (Makino) Makino (AP) growing naturally in Japan. Outside of Japan, AQ is naturally distributed in the Korea Peninsula and China and AT in China, whereas AP is endemic to Japan [5]. The key distinguishing characteristic of these species are their palmate leaf morphology [5]; each AQ leaf is comprised of five leaflets without serration, while those of AT and AP have three and five leaflets with serration, respectively. Since AP is presumed to be a hybrid between AQ and AT [6], the leaves of AP share aspects of the leaf morphology of these two species (Fig. 1). However, the variation observed in the serration of AP leaves is so large that AP is often indistinguishable from AQ [7].

The fruit of *Akebia* species has a thick oval pericarp with a white banana-like flesh that contains numerous black glossy seeds, and each of the seeds has a white waxy appendage (elaiosome) on its surface (Fig. 2). In general, elaiosome of plant seed works to inhibit seed germination and attracts ants as bait to promote seed dispersal by them [8]. In autumn, the pericarp of mature fruit acquires a purple tinge and dehisces, which is when the white flesh appears. The raw flesh is sweet, and the selection of favorable strains and domestication of *Akebia* has been promoted in Japan [9]. Despite having a bitter taste, the pericarp and young shoots are also consumed in Japan as wild vegetables [10]. In addition, the dried stem of *Akebia*, also referred to as 'mokutsu' in Japanese, is used as a traditional Chinese medicine [11]. Only AQ and AT are considered as 'mokutsu' in the Japanese Pharmacopoeia at present [12], but the contamination of plant materials derived from AP and its distribution in the Japanese market have been reported [13].

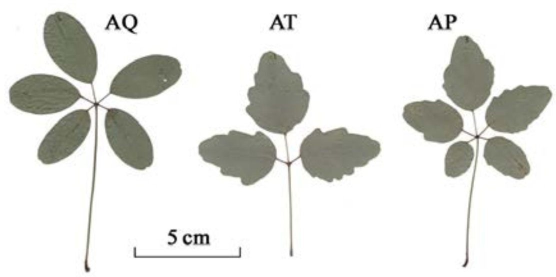

Fig. 1 Leaf morphologies of three species of *Akebia*

Fig. 2 Seed of *Akebia*

It is considered that the edible and medicinal uses of *Akebia* species have promoted the continued use of the plants in this genus for revegetation purposes. Indeed, pergolas and hedges trellised with *Akebia* (Fig. 3) can be seen widely in Japan. In addition, a case study of the application of this genus for use as green shade for parking lots has been reported [14]. In the event that *Akebia* plants do not succeed in climbing up some form of support, they spread on the ground surface and produce roots from stem nodes like other cover plants. An example of *Akebia* covering the ground surface densely is shown in Fig. 4.

Thus, in addition to being edible and having medicinal uses, *Akebia* species are considered to be well suited for revegetation applications. However, few studies have been published to date on the germination and growth characteristics of these species in Japan. In the present study, we investigated the germination and growth characteristics of three species of *Akebia*. In addition, we examined the potential application of these species for revegetation based on differences in the observed characteristics.

Fig. 3 The use of *Akebia* for revegetation purposes in Japan (A: pergola, B: hedge)

Fig. 4 An example of *Akebia* covering the ground surface on a roadside slope (the length of the pole with white and red stripes = 2 m)

2. RESEARCH SIGNIFICANCE

The significance of this study is to contribute to revegetation programs using *Akebia* species. The results will promote the application of appropriate species according to the intended aims of such revegetation programs, whether these are for pergolas and trellised hedges with climbing growth or for covering ground surfaces with creeping growth. In addition, basic information on germination and growth characteristics will facilitate the identification of indistinguishable *Akebia* species.

3. METHODS

3.1 Seed Germination Experiment

A seed germination experiment was conducted to clarify the differences in germination characteristics among the three *Akebia* species that are native to Japan. Mature fruit were harvested in October 2023 in areas of natural habitat where growth characteristics were recorded (Table 1). After washing the seeds in tap water and removing immature seeds that floated, mature seeds were disinfected for an hour by soaking in a solution prepared by diluting benomyl wettable powder (Sumitomo Chemical Garden Products Inc., Tokyo, Japan) in water at a 1:500 (w/v) ratio. The disinfected seeds were then wrapped in wet tissue, placed in polyethylene bags, and stored in a laboratory at room temperature for approximately 3 months.

Table 1 Elevation and transect length in each natural habitat for the survey of the growth property of *Akebia* species

Habitat No.	Elevation (m)	Transect length (m)
1	775	175
2	766	225
3	767	125
4	774	200
5	969	200
6	1022	100
7	1161	130
8	1377	70
9	1036	160
10	1092	150
11	1391	110
12	1212	120
13	795	85
14	1079	150
15	753	90
16	1086	60
17	1056	100

Five pretreatment methods were employed for seeds prior to sowing: control (without any treatment), cold stratification (keeping seeds in wet paper in a plastic bag at 4 ºC for a week), elaiosome removal using sandpaper, immersion in hot water (at 70 ºC for 2 minutes), and forced-air drying (at 30 ºC for 24 hours).

The experimental design employed a two-way layout with two main factors (i.e., species with three levels and pretreatment with five levels); all experiments were performed in triplicate. After pretreatment, the seeds were placed on 0.5% agar medium in 9 cm-diameter petri dish (20 seeds per petri dish, and 3 petri dishes per pretreatment method) in January 2024. The petri dishes were incubated using an altering temperature regime (28 ºC / 15 ºC each for 12 hours) under constant light conditions (i.e., 3,000 lux provided by a white, fluorescent light).

The number of germinated seeds in each petri dish was checked at intervals of one or two days. Germination was determined to have occurred based on the appearance of the radicle breaking through the seed coat. The germinated seeds were removed from the petri dish and planted in pots, which were filled with a mixture of Akadama soil (natural granular clay), Kanuma soil (naturally weathered pumice), vermiculite, and peat moss at a ratio of 3:3:3:1. The pots were kept in a laboratory at room temperature and maintained under natural light conditions. The initial growth characteristics of the seedlings were observed for three months.

3.2 Survey of Growth Characteristics in Natural Habitat

To clarify the growing characteristics (i.e., climbing and creeping ability) in each *Akebia* species, we surveyed 17 natural habitats where the three species were sympatrically distributed in Nagano Prefecture, Central Japan. The survey was conducted from August to October 2023. The habitats were primarily surveyed along the edges of forest roads as *Akebia* species were reported to be distributed at the edge of deciduous forests [15]. Plants were surveyed along a 100 m transect with the length of the transect increased or decreased depending on the extent of favorable habitat. Elevation and transect length for each habitat are shown in Table 1.

To compare plant growth characteristics, the number of climbing and creeping individuals was counted and the basal diameter of each individual was measured. The number of either climbing or creeping individuals relative to the total number of individuals was then used to compare habitats.

4. RESULTS

4.1 Seed Germination Characteristics

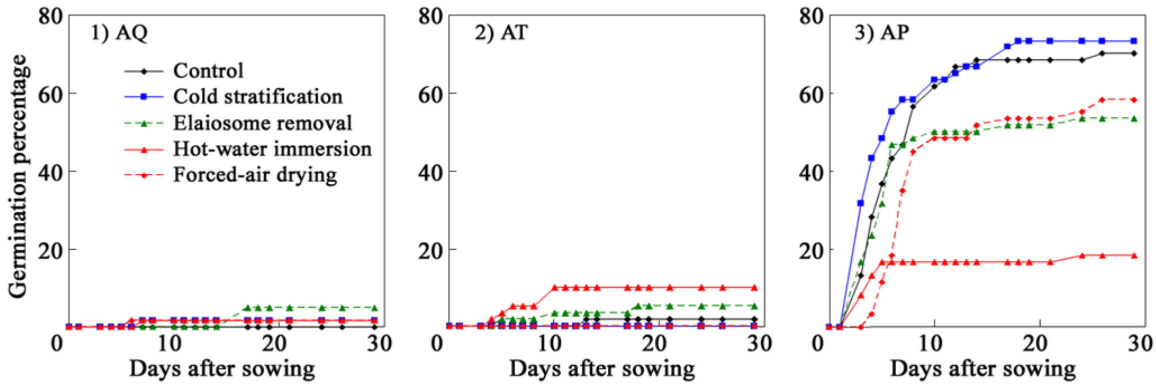

Fig. 5 Germination percentages in three *Akebia* species after sowing

Table 2 Germination percentages for three *Akebia* species (mean ± standard deviation)

Days after sowing	Pretreatment	AQ		AT		AP	
5	Control	0.0 ± 0.0	d	0.0 ± 0.0	d	36.7 ± 5.8	b
	Cold stratification	0.0 ± 0.0	d	0.0 ± 0.0	d	48.3 ± 2.9	a
	Elaiosome removal	0.0 ± 0.0	d	1.7 ± 2.9	d	31.7 ± 15.3	b
	Hot-water immersion	0.0 ± 0.0	d	3.3 ± 5.8	d	16.7 ± 5.8	c
	Forced-air drying	0.0 ± 0.0	d	0.0 ± 0.0	d	11.7 ± 2.9	d
29	Control	0.0 ± 0.0	d	1.7 ± 2.9	d	70.0 ± 8.7	a
	Cold stratification	1.7 ± 2.9	d	0.0 ± 0.0	d	73.3 ± 5.8	a
	Elaiosome removal	5.0 ± 5.0	cd	5.0 ± 0.0	cd	53.3 ± 20.2	b
	Hot-water immersion	1.7 ± 2.9	d	10.0 ± 13.2	cd	18.3 ± 7.6	c
	Forced-air drying	1.7 ± 2.9	d	0.0 ± 0.0	d	58.3 ±17.6	b

Different letters in columns corresponding to 5 days after sowing (das) and those in columns of 29 das denote significantly different means as determined by Tukey's HSD (p <0.05).

Figure 5 shows the changes in the mean germination percentage after sowing. The mean germination percentage in the early period (i.e., 5 days after sowing (das)) and in the final period (29 das) are shown in Table 2, as these are important phases of seed germination.

In AQ, seed germination was generally poor, and no seeds had germinated at 5 das (Fig. 5 and Table 2). The maximum final germination percentage was 5.0% in the elaiosome removal treatment, and 0.0 to 1.7% in the other pretreatments. In AT, seed germination was also generally poor (Fig. 5 and Table 2), with some seed germination observed at 5 das in the elaiosome removal and hot-water immersion pretreatments. The maximum final germination percentage was 10.0% in the hot-water immersion pretreatment, and 0.0 to 5.0% in the other pretreatments.

Conversely, in AP, seed germination was generally markedly higher and occurred sooner than in AQ and AT (Fig. 5 and Table 2). The maximum final germination percentage reached 73.3% in the cold stratification pretreatment. However, since the final germination percentage was 70% in the control treatment (i.e., seeds were not subjected to any pretreatment), no significant difference was observed compared to the maximum germination percentage. The germination percentage in the forced-air drying (58.3%), elaiosome removal (53.3%), and hot-water immersion (18.3%) pretreatments was significantly lower than that in the control (Tukey’s HSD, p<0.05). In AP, germination started at 3 das, and the maximum germination percentage at 5 das reached 48.3% in the cold stratification pretreatment, which was significantly higher than that recorded under the other pretreatment conditions (Tukey’s HSD, p<0.05).

In addition, the earliest development of cotyledons in germinated seeds was observed in AP at 29 das, while in AQ and AT, cotyledon development was observed at 61 das. The seedlings of all species had reached a height of at least 10 cm by 123 das.

Table 3 Number of individuals surveyed and ratio of climbing individuals for three *Akebia* species in natural habitat

Site No.	Creeping individuals			Climbing individuals			Ratio of climbing individuals to the total individuals		
	AQ	AT	AP	AQ	AT	AP	AQ	AT	AP
1	5	1	8	6	9	4	0.55	0.90	0.33
2	6	8	12	14	7	4	0.70	0.47	0.25
3	4	0	7	17	10	9	0.81	1.00	0.56
4	8	3	10	19	5	7	0.70	0.63	0.41
5	1	7	12	6	8	3	0.86	0.53	0.20
6	3	17	11	2	7	4	0.40	0.29	0.27
7	10	4	15	6	10	2	0.38	0.71	0.12
8	3	2	9	10	11	6	0.77	0.85	0.40
9	1	6	9	7	15	4	0.88	0.71	0.31
10	4	11	12	7	10	2	0.64	0.48	0.14
11	2	4	6	12	7	4	0.86	0.64	0.40
12	4	2	13	11	8	9	0.73	0.80	0.41
13	11	5	17	7	1	4	0.39	0.17	0.19
14	6	6	8	7	11	7	0.54	0.65	0.47
15	7	5	9	14	6	5	0.67	0.55	0.36
16	4	0	7	10	7	6	0.71	1.00	0.46
17	3	0	13	15	4	10	0.83	1.00	0.43
Total	82	81	178	170	136	90			
Mean ± SD							0.67 ± 0.17 a	0.67 ± 0.24 a	0.34 ± 0.13 b

Different letters in columns denote significantly different means as determined by Tukey's HSD (p <0.05).

4.2 Growth Characteristics in Natural Habitat

Table 3 shows the number and ratio of climbing individuals along each of the transects studied. Approximately 30 to 50 individuals of each species were observed along each of the transects, which gave a total of 252, 217, and 268 individuals of AQ, AT, and AP, respectively. The mean ratio of climbing individuals to the total individuals was 0.67, 0.67 and 0.34 for AQ, AT, and AP, respectively, and the ratios obtained for AQ and AT were significantly higher than those for AP (Tukey's HSD, p<0.05).

Table 4 shows the relationships between the ratio of climbing individuals and environmental parameters in each habitat (i.e., elevation, population size, and population density of *Akebia*). Population size refers to the number of individuals encountered along each transect, as shown in Table 1. Population density was calculated by dividing the total number of each species (creeping and climbing individuals combined) by transect length. Correlation coefficients ranged from -0.265 to 0.215 (i.e., the maximum R^2 values was 0.07), and no significant correlations were detected (F-test, n =17) for any combination of species and parameters (Table 4).

The mean basal diameter, i.e., the average value obtained for the individuals along each of the 17 transects, is shown in Table 5. In AQ, AT and AP, the mean basal diameter of creeping and climbing individuals was 1.8 mm, 1.8 mm, and 1.6 mm, and 2.7 mm, 4.5 mm, and 2.3 mm, respectively. Within each species, the mean basal diameter of climbing individuals was generally larger than that of creeping individuals, but a significant difference was detected only for AT relative to the other species Tukey's HSD, p<0.05). Within the same growth form, the mean basal diameter of climbing individuals was significantly larger in AT than in either AQ or AP.

Table 4 Correlation coefficient between the ratio of climbing individuals to the total individuals and population parameters

Factor	Ratio of climbing individuals to the total individuals		
	AQ	AT	AP
Elevation	0.196	0.215	0.036
Population size	0.135	-0.169	-0.265
Population density	0.022	-0.260	0.186

Table 5 Basal diameter of *Akebia* species in natural habitat (the mean ±SD among 17 habitats)

Growth	Basal diameter (mm)		
Form	AQ	AT	AP
Creeping	1.8 ± 0.3 b	1.8 ± 0.8 b	1.6 ± 0.2 b
Climbing	2.7 ± 0.4 b	4.5 ± 2.9 a	2.3 ± 0.6 b

Different letters in columns denote significantly different means as determined by Tukey's HSD (p <0.05).

5. DISCUSSION

5.1 Seed Germination and Initial Seedling Growth

Of the three species, seeds from AP showed the lowest level of dormancy. This finding was supported by the fact that the germination percentage of AP seeds without any pretreatment was the highest (Fig. 5 and Table 2). Furthermore, cotyledon development occurred faster in the germinated seeds of AP than in the seeds of AQ and AT. These germination and seedling growth characteristics are favorable for plants intended for using in revegetation. Conversely, the seeds of AQ and AT appeared to have higher levels of dormancy. This difference might be attributed to heterosis, i.e., hybrid vigor. For example, the appearance of the radicle and subsequent seedling growth in maize occurred more quickly in hybrid seed than in seed from the parents, likely due to the difference in the activation rate of embryo cells after water absorption [16]. Since AP is presumed to be a hybrid between AQ and AT [6], it is considered that active growth of embryo cells by heterosis resulted in the appearance of the radicle in AP sooner than in AQ or AT.

Hot-water immersion is a widely used method to disrupt the dormancy of hard seeds with impermeable seed coats [16], and was reported to have both beneficial (disrupting seed dormancy) and deleterious (heat damage) effects on seed. Indeed, it has been reported that there is an optimal duration of immersion that is required to maximize the germination percentage in seeds [17]. From Table 2, the effect of hot-water immersion on the germination percentage appeared to be slightly beneficial compared to the control treatments in AQ and AT (1.7% vs. 0.0% and 10.0% vs. 1.7%, respectively), but deleterious in AP (18.3% vs. 70.0%). These results imply that the strength of seed dormancy and the sensitivity to heat treatment may differ among the three *Akebia* species; for example, hot-water immersion at 70 ºC for 2 minutes in the present study might be too low for disrupting dormancy in AQ and AT seeds, but too high for AP seeds.

Cold stratification has been reported to be required for disrupting dormancy and germination in numerous plant species [16]. AP seeds demonstrated minimal dormancy, achieving high germination percentages in the control treatment. Cold stratification further improved germination in the early period (Fig. 5 and Table 2). Consequently, cold stratification may accelerate germination in non-dormant seeds of AP. However, cold stratification was only slightly effective for disrupting dormancy in AQ and AT, implying that the cold stratification regime employed in the present study (i.e., seed storage at 4 ºC for a week) was insufficient, or that the cold temperatures during seed storage did not affect germination at all.

Removing the elaiosome is also a popular method for disrupting seed dormancy since the composition of the elaiosome typically inhibits seed germination [16]. Although elaiosome removal did not appear to have a distinct effect on increasing the germination percentage in AQ and AT, it did appear to suppress germination only in AP compared to the control (Fig. 5 and Table 2). Therefore, seed dormancy in AQ and AT did not appear to be affected by the elaiosome, and the removal may harm the seed.

The results obtained for the forced-air drying pretreatment were similar to those for elaiosome removal (Fig. 5 and Table 2). . Forced-air drying is not widely used as a pretreatment method, but it has been reported to disrupt dormancy in some leguminous species with hard seeds [17]. Although it has been suggested that drying *Akebia* seeds during storage may decrease germination percentage [10], the forced-air drying regime employed in the present study (i.e., 30 ºC for 24 hours) appeared to be deleterious, at least for AP seeds.

5.2 Growth Characteristics in Natural Habitat

The ratio of climbing individuals in natural habitat was lower in AP than in AQ or AT (Table 3). Since the ratio did not appear to be related to habitat type (Table 4), the growth characteristics of creeping or climbing were considered to be species dependent. It has been reported that AQ and AT have a tendency for prioritizing basal stem growth over canopy growth, with the foliage of the upper canopy tending to develop slowly [8]. Based on the diameter in climbing stems, this tendency of vigorous basal growth was more obvious in AT than in AQ (Table 5). Given that AP exhibited the opposite tendency (prioritizing canopy growth over basal stem growth) to AQ and AT, creeping growth in AP can be attributed to rapid stem elongation through vigorous canopy growth and slow basal growth, which resulted in the development of thin stems that were incapable of supporting the plant and creeping along the ground.

In each species, the mean basal diameter of climbing individuals tended to be larger than that of creeping individuals (Table 5). This observation suggests the potential occurrence of two contrasting processes: one where the stem thickens before initiating climbing and another where the stem thickens subsequent to climbing. The first process is supported by the observed trend in vigorous basal growth, which is consistent with the slower seedling growth observed in AQ and AT. Conversely, the second process may occur due to increased photosynthesis in the foliage of the upper layers after the climbing begins. Initiating climbing growth when the stem is young and slender could be advantageous for rapid revegetation of pergolas and hedges.

6. CONCLUSION

In the present study, we investigated the germination and growth characteristics of three species of *Akebia* to clarify their suitability for revegetation applications. The findings can be summarized as follows:

a) AP seeds exhibited minimal dormancy, with the germination percentage exceeding 70% without any pretreatment, and rapid cotyledon development was observed in seedlings. Conversely, AQ and AT seeds exhibited strong dormancy, with seedlings showing slower development compared to AP.

b) Regarding growth characteristics in natural habitats, AQ and AT typically exhibited climbing growth with a thicker basal diameter, whereas AP predominantly displayed creeping growth with a thinner basal diameter.

c) Based on these observations, we recommend specific applications for the three *Akebia* species: AP is ideal for rapid ground cover through direct seeding, while AQ and AP are better suited for vertical growth along structures such as pergolas and hedges through the planting of seedlings.

d) The observed differences in seed germination and growth characteristics provide a clear basis for distinguishing between AP and AQ.

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