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Enhanced Summer Planting Survival of Japanese Larch Container-Grown Seedlings

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Abstract: A previous study revealed low survival rates for Japanese larch (*Larix kaempferi*) summer-planted seedlings grown in Hiko-V-120 containers. This study examines nursery practices that could potentially prevent deterioration of the seedling water balance after planting to improve the survival rate of this species, which has a low drought tolerance. During summer planting, we tested (1) drought hardening or high-potassium fertilization for two months before planting, (2) antitranspirant or topping treatment at planting, and (3) the use of the JFA-150 container with a larger capacity and lower growing density than the Hiko-V-120 container. Drought hardening increased seedling drought tolerance because of the low leaf:root ratio, due to lower leaf mass production, resulting in increased survival from 74% to 93% in Hiko-V-120 containers. When JFA-150 containers were used, the leaf:root ratio was lower because of higher root mass, resulting in an increase in survival to 87%, with the highest survival of 97% when combined with drought hardening. The application of antitranspirant increased survival to over 90%, whereas topping did not, probably because of severer competition from weeds. High-potassium fertilization did not affect seedling traits or survival. For better survival of summer-planted container-grown Japanese larch seedlings, it is recommended that they be grown in containers providing sufficient cell volume and density for root growth while the seedlings are in the nursery and that irrigation be withheld for two months before planting. In addition, to obtain higher survival, an antitranspirant can be applied at planting at a cost.

Keywords: antitranspirant; container cell volume; drought hardening; leaf:root ratio; osmotic adjustment

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1. Introduction

The quality of planted seedlings is one of the most influential factors affecting seedling survival and growth after reforestation [1–4]. Seedling quality is strongly associated with several morphological and physiological attributes of roots, stems, and leaves. It can be influenced by stock types, such as bare-root or container-grown [5–7], and nursery cultivation practices [8–11]. It is crucial to plant seedlings with attributes suited to the environment to achieve better performance after planting [3,11,12]. Increasing drought- and heat-induced tree mortality has been observed worldwide, and global climate change is accelerating this trend [13,14]. Therefore, the drought tolerance of seedlings is becoming more important for reforestation.

In Japan, unlike in European and North American countries, until recently, only bare-root seedlings had been used for afforestation. Container-grown seedlings have only been produced and planted in the last decade [15]. Therefore, best practices for producing container-grown seedlings are still being developed in Japan [16,17]. Container-grown seedlings generally tend to have a higher survival rate than bare-root seedlings, especially under drier soil conditions at the time of planting, primarily because of the growing media surrounding the root system of container-grown seedlings can contain substantial amounts of water. In addition, container-grown seedlings typically have a lower shoot-to-root ratio and rapid root development after their planting, resulting in an improved plant water status [7]. These traits also extend the planting period in container-grown seedlings. In Japan, the planting season for bare-root seedlings is generally limited to a short period of approximately a month over spring and autumn. The current challenge in Japan is that the area in need of reforestation is increasing because planting activities cannot keep up with the increase in clear-cut areas, due in part to the short planting season for bare-root seedlings and a decrease in the number of forest workers. Extending the planting period of container-grown seedlings to include the summer provides a possible solution. However, in Hokkaido in northern Japan, the precipitation in early summer is at its lowest during the year [18], making it an unsuitable period for planting.

Japanese larch (*Larix kaempferi* [Lamb.] Carrière) is an important tree species for timber production and as a pollen parent of hybrid larch in the cool temperate region of Japan [19], as well as Europe [20,21] and North America [22,23]. Larch species are sensitive to drought stress [24–26]; therefore, the survival and performance of the seedlings after planting can be particularly affected. In our previous study, in which container-grown seedlings of Japanese larch were planted monthly from May in spring to October in autumn in Hokkaido, the survival of seedlings planted in the summer (July) with less precipitation before and after planting was significantly lower (22%) than that of seedlings planted in the spring (80%). This result is attributed to the seedlings' low physiological and morphological drought tolerance in the early summer season [27]. To improve the survival of container-grown Japanese larch seedlings planted in the summer, it is necessary to increase their drought tolerance and/or reduce water loss through transpiration after planting.

The application of drought stress in the nursery, known as drought hardening, can potentially enhance the drought resistance of seedlings through physiological acclimation, osmotic adjustment, stomatal control, reduction of susceptibility to xylem embolism, and morphological acclimation, including a decrease in the leaf:root (or shoot:root) ratio [2,28–30]. Adjustment of the fertilization regime can also enhance stress tolerance [2,31]. In particular, potassium (K) fertilization can potentially promote root growth [32,33] and alleviate drought stress [34,35]. Many crop plants show an increase in yield with K fertilization under mild drought stress [34]. Additionally, treatments that reduce water loss through transpiration after planting, including applying an antitranspirant [36–38], and leaf removal [39,40] at outplanting, can also be effective for minimizing drought stress and planting shock, resulting in increased survival. Furthermore, the container's cell volume and growing density can affect the height, root collar diameter, root mass, and the leaf:root ratio of the seedlings produced [41], which in turn can affect their survival during drought after planting [2].

This study aimed to examine whether two months of irrigation or K fertilizer management in the nursery before planting, the application of antitranspirant and leaf removal at planting, and the use of containers with high capacity and low density could improve the survival of container-grown 1-year-old Japanese larch seedlings following summer planting. We evaluated (1) the morphological attributes of growth, biomass allocation, leaf morphology, and physiological acclimation of leaf water relations just before summer planting, (2) photosynthetic response after summer planting, and (3) survival and growth after a growing season of seedlings planted during the summer.

2. Materials and Methods

2.1. Plant Materials

The seedlings were grown in a glass greenhouse with no supplemental lighting system at the Forestry Research Institute, Hokkaido Research Organization, in Bibai, Hokkaido, Japan (43°17′ N, 141°51′ E, 42 m a.s.l.). According to the data from the nearest meteorological station (Bibai meteorological station, Japan Meteorological Agency, Tokyo, Japan) located 9 km from the study site, during the study period, 2014–2015, the annual mean temperature was 7.4 °C, the monthly mean daily maximum temperature in the warmest months (July 2014 and August 2015) was 27 °C, and the monthly mean daily minimum temperature in the coldest months was −14 °C (January 2014) and −11 °C (January 2015). Annual precipitation was approximately 1100 mm, and the maximum snow depths were 142 cm (March 2014) and 86 cm (January 2015).

We used two types of containers with different cell capacities and densities. One was the Hiko-V-120 SideSlit (BCC, Landskrona, Sweden) with a density of 526 cells m^{-2}, a cell volume of 120 mL, a cell height of 110 mm, and side slits in the cell wall, which was used in our previous study with extremely low survival for summer planting, as mentioned previously [27]. The other container was the JFA-150 (Zenbyouren, Tokyo, Japan), the most common container type in Japan, with a density of 296 cells m^{-2}, a 150 mL volume, a 130 mm height, and no side slits but a ribbed cell wall to prevent root spiraling. For the first year, we followed the seedling growing protocol from our previous study [27]. Containers were filled with a growing medium of peat moss mixed with slow-release fertilizer (Osmocote Exact Standard M3-4, N:P:K = 16:9:12, HYPONeX JAPAN CORP., Osaka, Japan) at 4 g per liter. Seeds used for this study were collected from the town of Aibetu in Hokkaido in 2013. Filled seeds were selected using the density method of immersing them in water and treating them with cold stratification at 2 °C for 21 days to promote germination. We sowed three seeds per cell on 24 April 2014. If more than one germinated, we immediately removed them, leaving one seedling per cell. Germination peaked two weeks after sowing and was generally complete within a month. Irrigation was conducted once or twice a day using mist sprinklers to field capacity to prevent the growing medium from drying out. Once a week, liquid fertilizer was applied instead of irrigation. Each cell received liquid fertilizer to fill the field capacity, i.e., approximately 70 mL per liter of growing medium, with 50 ppm nitrogen (N) (HYPONeX standard, N:P:K = 6:10:5, HYPONeX JAPAN CORP., Osaka, Japan) from 4 to 8 weeks after sowing at the initial growth phase, with 100 ppm N (Professional HYPONeX, N:P:K = 20:20:20, HYPONeX JAPAN CORP., Osaka, Japan) from 8 weeks after sowing to mid-August at the rapid growth phase and with 25 ppm N (Universol Violet, N:P:K = 10:10:30, HYPONeX JAPAN CORP., Osaka, Japan) from late August to late September at the dormant growth phase. The air temperature inside the greenhouse from May to August generally ranged between 15 °C and 35 °C. We moved the containers from the greenhouse to outdoors on 1 September 2014, for cold winter hardening, stopped irrigation in late October, and then stored them under snow during the winter from November to April.

In late April 2015, after winter storage, the containers were returned from the snow to the outdoors. Half the number of seedlings in each container was pulled out of the container together with the growing medium. The empty and seedling-filled cells were arranged alternately in the container. The pulled-out seedlings with the growing medium were placed into other containers in the same arrangement pattern, following the protocol of Harayama et al. [27]. As a result, seedlings were grown at a density of 263 cells m^{-2} in the Hiko-V-120 containers and 148 cells m^{-2} in the JFA-150 containers for two months prior to planting. Reducing seedling density in a container is sometimes done by general nursery growers in Japan to reduce competition between seedlings, and thus, promote diameter growth and prevent lower branch dieback.

2.2. Irrigation and Potassium Fertilizer Treatments

Irrigation and K fertilizer treatments were applied for approximately two months, from late April 2015 after winter storage to early July 2015 just before summer planting, to the 1-year-old container seedlings. For the irrigation treatment, as a control, we irrigated the seedlings in eight Hiko-V-120 containers and six JFA-150 containers with sprinklers twice daily in the morning and evening following the protocol of Harayama et al. [27]. For the drought hardening treatment, we irrigated seedlings in two containers per type

(n = 40 seedlings for each container type) twice a week; however, if there was rainfall that sufficiently moistened the growing medium between irrigation, the irrigation was skipped. We used bottom irritation rather than sprinklers for the drought hardening treatment because the dried growing medium of peat moss was water repellent, and sprinkler irrigation might not thoroughly wet the growing medium. As a result of these irrigation protocols, the soil moisture content in the drought hardening treatment repeatedly and progressively decreased to approximately 59% (wet basis) in the Hiko-V-120 containers and approximately 75% (wet basis) in the JFA-150 containers before each of the 14 irrigation opportunities without rainfall out of a total of 20 irrigation opportunities during the treatment period. Alternatively, soil moisture was generally maintained at approximately 85% in both containers with the control irrigation treatment.

A high-K fertilizer treatment was applied to seedlings (n = 40) receiving the control irrigation treatment in two Hiko-V-120 containers. Owing to the insufficient number of seedlings, the high-K treatment was not applied to JFA-150 containers or combined with the drought hardening treatment in Hiko-V-120 containers. We applied 1.5 times the K and 0.5 times the N of the control treatment as a high-K treatment, i.e., 150 ppm K and 50 ppm N of the liquid fertilizer used in the dormant growth phase of the previous year (Universol Violet, N:P:K = 10:10:30, HYPONeX JAPAN CORP., Osaka, Japan) for high-K treatment, whereas we applied 100 ppm K and 100 ppm N as a control using the liquid fertilizer (Professional HYPONeX, N:P:K = 20:20:20) employed in the rapid growth phase of the previous year. The liquid fertilizer was applied once a week by hand to reach the field capacity. Each seedling in a Hiko-V-120 container receiving the high-K treatment was given a total of approximately 13.9 mg K (equivalent to 9.5 g K m^{-2}) and 4.6 mg N (equivalent to 3.2 g N m^{-2}) for two months, compared with approximately 9.2 and 11.6 mg K (equivalent to 6.4 and 7.3 g K m^{-2}) and 9.2 and 11.6 mg N (equivalent to 6.4 and 7.3 kg N m^{-2}) for the control treatment in Hiko-V-120 and JFA-150 containers, respectively.

After two months of treatments, just before summer planting, five seedlings from each treatment were harvested: The control irrigation treatment and fertilization in the Hiko-V-120 (HC) and JFA-150 containers (JC), drought hardening treatment with control fertilization in the Hiko-V-120 containers (HD) and JFA-150 containers (JD), and high-K fertilization with control irrigation in the Hiko-V-120 (HK). The harvested seedlings were then separated into leaves, stems with branches, and roots. Roots were washed gently with tap water to remove the growing medium. Samples were then dried in an oven at 70 °C for more than 72 h to constant mass. Dry mass was measured for leaves (DM_{leaf}), stems with branches (DM_{stem}), and roots (DM_{root}), and the leaf:root ratio was calculated by dividing DM_{leaf} by DM_{root}. We sampled 10 to 22 needles from the leaves of each seedling before drying, and the projected area of needles (A_L) was measured using a scanner and ImageJ 1.51s software (NIH, Bethesda, MD, USA). The needle samples were then dried, and leaf mass per area (LMA) was calculated as the dry mass of needles divided by A_L.

2.3. Root Membrane Integrity and Leaf Water Relations

Relative electrolyte leakage from fine roots (REL) was measured to evaluate whether root viability was impaired by treatments, especially drought hardening that caused drying of the growing medium [42,43]. Two or three fresh fine roots (<2 mm diameter) were collected from each seedling (n = five per treatment) before measuring the dry mass described previously, cut to approximately 2 cm lengths, and submerged in 16 mL of ultrapure water in a 30 mL screw-top bottle for 24 h at 25 °C. The bathing solution conductivity (EC_{live}) was measured using a portable electrical conductivity meter (B-771, HORIBA Ltd., Kyoto, Japan), after which the solution containing the fine roots was autoclaved for 10 min at 110 °C. The electrical conductivity was measured again when the temperature of the solution decreased to room temperature (EC_{dead}). REL was calculated as follows:

$$REL = (EC_{live} - EC_{pw})/(EC_{dead} - EC_{boil}) \times 100, \quad (1)$$

where EC_{pw} and EC_{boil} were the electrical conductivity of the ultrapure water before and after autoclaving, respectively.

Leaf water relations were analyzed using a pressure–volume technique [44] on a current-year long shoot sampled from the seedling before measuring the dry mass (n = five per treatment) to evaluate the effects of irrigation and fertilization on the drought tolerance of leaves [45,46]. After irrigating, the seedlings in the containers were brought to the laboratory in the evening of the day before the measurement and placed in the dark, covered with a plastic bag. The next day, a sample shoot from the seedlings was placed in a Scholander pressure chamber (model 3000, Soilmoisture Equipment Corp, Goleta, CA, USA), and a pressure-volume curve was determined using the expressed sap method, in which the water potential of the shoot and the weight of the expressed sap were measured repeatedly [44]. The osmotic potential at full turgor ($\Psi_{o.ft}$), leaf water potential at turgor loss point ($\Psi_{w.tlp}$), maximum bulk modulus of elasticity (ε_{max}), and relative water content at turgor loss point (RWC_{tlp}) were estimated using a pressure-volume curve-fitting routine program (v5.5, K. Tu, University of California Berkeley, Berkeley, CA, USA) based on the procedure of Schulte and Hinckley [47].

2.4. Application of Antitranspirant and Topping at Planting

On the day of planting, 7 July 2015, seedlings with control irrigation and fertilization treatment in Hiko-V-120 (HC) and JFA-150 (JC) containers were treated with two kinds of transpiration-reducing treatments: Application of antitranspirant (HC + Ant and JC + Ant) or cutting the upper part of the seedlings, i.e., topping (HC + Top and JC + Top). We diluted the liquid antitranspirant (Greenner, Greenner Co., LTD., Osaka, Japan) five times with tap water according to the user's manual and immersed the canopy of seedlings with HC treatment (n = 28) and JC treatment (n = 30) in the solution for a few seconds. According to the manual, both stomatal and cuticular transpiration are suppressed for three days, and cuticular transpiration is then suppressed for several weeks to a month by the antitranspirant. For topping, seedlings with HC treatment (n = 25) and JC treatment (n = 30) were cut with pruning scissors so that the crown was three-quarters of the length.

2.5. Summer Planting Experiment

Seedlings from each of the treatments (Table 1) were planted in summer on 7 July 2015, approximately 6 km from the greenhouse in the same experimental field as that of the previous study [27] in Mikasa City, Hokkaido, Japan (43°14′ N, 141°51′ E, 30 m a.s.l., flat). The field was divided into 10 plots, and 3 seedlings of each treatment were randomly planted in rows, with 1.5 m spacing between rows and 50 cm between plants within a row. However, because of the limited number of seedlings, some plots were planted with zero to two seedlings for each treatment grown in Hiko-V-120 containers. Seedling height and root collar diameter were measured at planting and at the end of the growing season on 5 October 2015. At planting, the heights of the 1-year-old stem, i.e., the heights after snow storage before hardening, were also measured. Seedlings with topping treatment were planted before topping, and the heights of the 1-year-old stems were measured. The topping treatment was then applied, and finally, the height of the seedlings with the treatment was measured. Survival was assessed at the end of the growing season. Weeding was conducted on the entire experimental field in mid-August when the planted seedlings were covered with weeds. Notably, compared with a previous study with a lower survival rate of seedlings planted in summer [27], precipitation was more frequent (Figure S1), and air temperatures were not high around the planting date (Figure S2).

Table 1. Details of each treatment used in the summer planting experiment.

Container Type	Watering	Fertilization	Antitranspirant	Topping	Abbreviation	No. of Seedlings
Hiko-V-120	Control	Control	No	No	HC	27
	Dry	Control	No	No	HD	28
	Control	High-K	No	No	HK	23
	Control	Control	Yes	No	HC + Ant	28
	Control	Control	No	Yes	HC + Top	27
JFA-150	Control	Control	No	No	JC	30
	Dry	Control	No	No	JD	30
	Control	Control	Yes	No	JC + Ant	30
	Control	Control	No	Yes	JC + Top	30

2.6. Leaf Gas Exchange

We measured the leaf gas exchange rate of planted seedlings for all Hiko-V-120 container treatments and the control treatment in JFA-150 containers to conduct a physiological evaluation after summer planting. Measurements took place on a sunny day, 9 September 2015, from 10:00 to 14:00 using a portable gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA) with an extended reach 1 cm chamber (6400-15, Li-Cor, Lincoln, NE, USA). We set the temperature of the measurement chamber to the same value as the ambient temperature measured using a thermometer (LR5001, Hioki E.E. Cor., Nagano, Japan) placed in the shade in the field every 30 min. Other conditions in the measurement chamber, such as irradiation, CO_2 concentration, and air humidity, were maintained at natural ambient conditions. Five plots were randomly selected from the 10 plots, and one seedling from each treatment was measured from each plot. Five to 10 needles from a fully sun-exposed shoot were placed in the chamber, and the photosynthetic rate and stomatal conductance were measured. Measurements with a low photosynthetic photon flux density (PPFD) were eliminated, resulting in four replications for the HC and HD treatments and five for the other treatments. Eventually, gas exchange was measured at a mean leaf temperature of 23.9 °C, a mean PPFD of 687 µmol m^{-2} s^{-1}, a mean CO_2 concentration inside the chamber of 378 µmol mol^{-1}, and a mean leaf-to-air vapor pressure deficit of 1.28 kPa. These conditions were not significantly different between treatments (one-way ANOVA, p = 0.45–0.98). The needles in the chambers were photographed using a digital camera to obtain the projected area of the measured needles using ImageJ software, and the photosynthetic rate based on leaf area (P_{mid}) and stomatal conductance ($g_{s.mid}$) at midday were determined. The leaves, including those with measured gas exchange, were collected, and leaf nitrogen content was determined using a CN analyzer (Vario MAX CN, Elementar, Langenselbold, Germany).

2.7. Statistical Analysis

Linear models were used to analyze the effects of treatments on dry mass parameters (DM_{root}, DM_{leaf}, DM_{stem}, the leaf:root ratio, and LMA), leaf water relation parameters ($\Psi_{o.ft}$, $\Psi_{w.tlp}$, ε_{max}, and RWC_{tlp}), and the REL of seedlings just before summer planting. Generalized linear mixed models (GLMMs) were used to analyze the effects of treatments on survival, growth parameters (height, root collar diameter, and height growth), gas exchange parameters (P_{mid} and $g_{s.mid}$), and leaf nitrogen. We assumed a binomial distribution for survival and a Gaussian distribution for the other parameters, and we incorporated plots as a random factor in GLMMs. The HC treatment, which showed a large decrease in survival of 22% in the summer planting from a previous study [27], was used as the reference category in the model analyses. A significant effect was assumed if the p-value of the estimated coefficient was <0.05. A marginal effect was assumed if the p-value of the estimated coefficient was >0.05, but the range of value of the coefficient did not cross zero when combined with the standard errors. All analyses were performed using R version 4.0.3 [48].

3. Results

3.1. Dry Mass and Relative Electrolyte Leakage from Fine Roots after the Two Month Nursery Treatment

The DM_{root} was not affected by the HD and HK treatments compared to the HC treatment, but it was significantly positively affected by the JC and JD treatments (Figure 1A). Seedlings with JC and JD treatments had 2.31 and 2.03 times higher DM_{root} than those with HC treatment. The DM_{leaf} was marginally negatively affected by the HD treatment (p = 0.11), marginally positively affected by the JD treatment (p = 0.19), and significantly positively affected by the JC treatment compared with the HC treatment, resulting in 0.55 times lower DM_{leaf} values in HD treatment and 1.67 and 1.36 times higher DM_{leaf} values in JC and JD treatments, respectively, than those in HC treatment (Figure 1B). DM_{stem} was marginally higher in the JC treatment (p = 0.12) than that in the HC treatment, and it was not affected by the other treatments (Figure 1C). The leaf:root ratio was significantly 0.53 and 0.67 times lower in HD ($p < 0.01$) and JD ($p < 0.05$) than that in HC treatment, and it was marginally 0.72 times lower in JC (p = 0.06) than that in HC treatment (Figure 1D). The LMA was not affected by the treatments (Figure 1E), and there were no treatments in which REL increased compared to that of HC seedlings (Figure 1F).

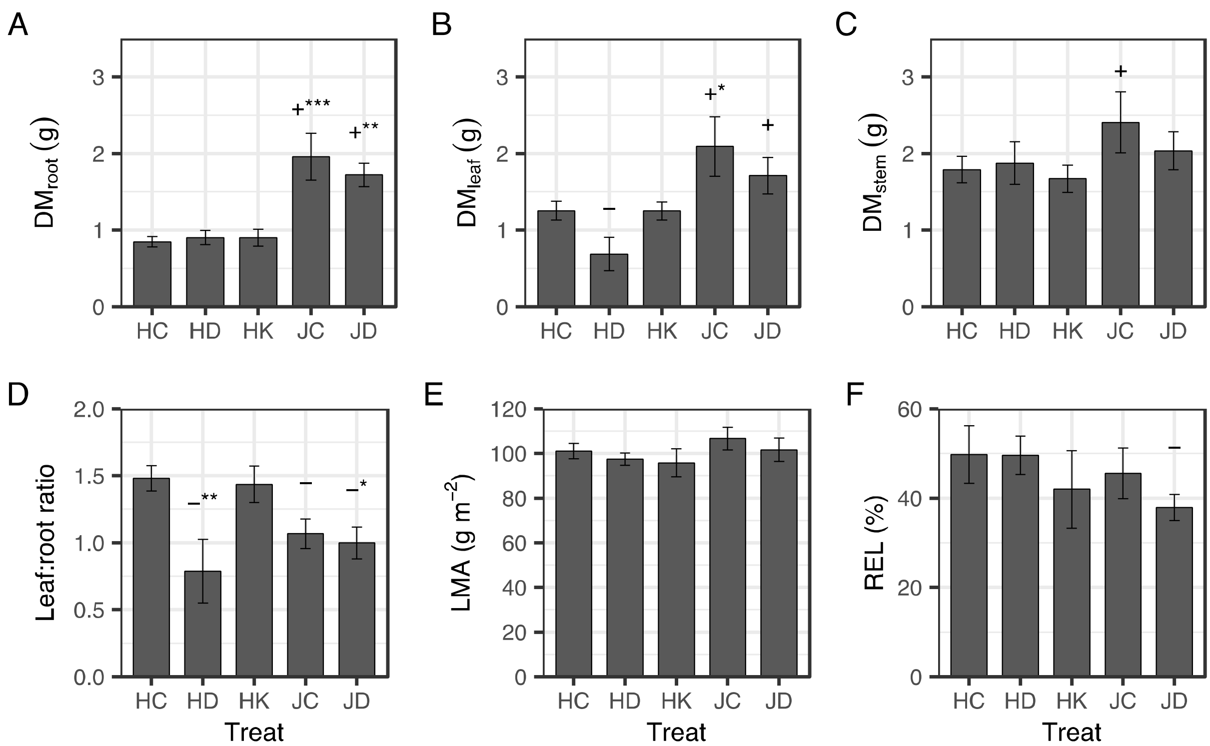

Figure 1. Mean values ± standard error (SE; n = 5) for dry mass parameters and relative electrolyte leakage from fine roots (REL) of container seedlings grown in Hiko-V-120 (H) and JFA-150 (J) containers with control irrigation and fertilization (HC and JC), dry irrigation (HD and JD), and high-potassium fertilization (HK). (**A**) DM_{root}, dry mass of a root; (**B**) DM_{leaf}, dry mass of a leaf; (**C**) DM_{stem}, dry mass of a stem with a branch; (**D**) leaf:root ratio; (**E**) LMA, leaf dry mass per area; (**F**) REL. The plus or minus signs indicate a positive or negative effect, respectively, compared with the HC treatment in the linear model. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.2. Leaf Water Relations after Two Months of Nursery Treatment

$\Psi_{o.ft}$ and $\Psi_{w.tlp}$ were marginally negatively affected by the JC (p = 0.22 for $\Psi_{o.ft}$ and p = 0.14 for $\Psi_{w.tlp}$) and JD (p = 0.15 for $\Psi_{o.ft}$ and p = 0.15 for $\Psi_{w.tlp}$) treatments and marginally positively affected by the HK treatment (p = 0.28 for $\Psi_{o.ft}$ and p = 0.30 for $\Psi_{w.tlp}$) compared with the HC treatment (Figure 2A,B); ε_{max} was marginally negatively affected by the HD treatment (p = 0.08) and positively affected by the JD treatment (p = 0.25)

(Figure 2C). RWC_{tlp} was significantly negatively affected by the HC, HK, JC, and JD treatments compared with the HC treatment (Figure 2D).

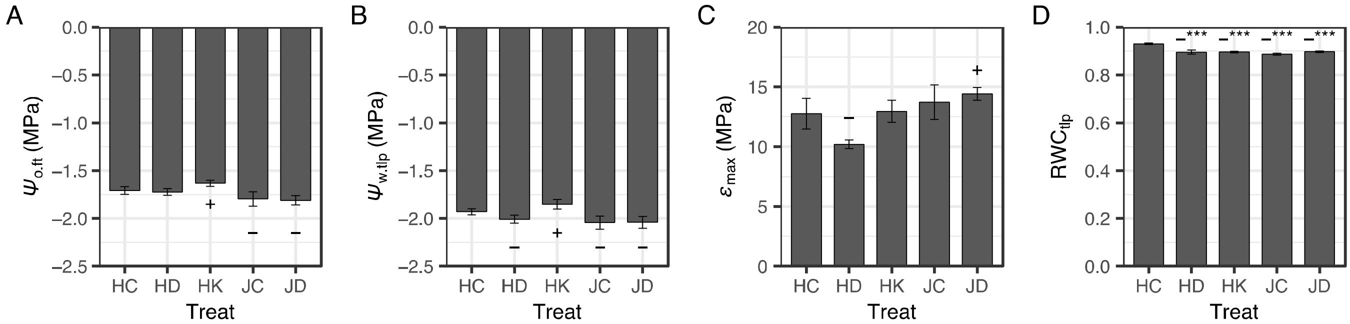

Figure 2. Mean values ± standard error (SE; n = 5) for leaf water relation parameters of container seedlings grown in Hiko-V-120 (H) and JFA-150 (J) containers with control irrigation and fertilization (HC and JC), dry irrigation (HD and JD), and high-potassium fertilization (HK). (**A**) $\Psi_{o.ft}$, osmotic potential at full turgor; (**B**) $\Psi_{w.tlp}$, leaf water potential at turgor loss point; (**C**) ε_{max}, bulk modulus of elasticity; (**D**) RWC_{tlp}, relative water content at turgor loss point. The plus and minus signs indicate positive and negative effects compared with the HC treatment in the linear model. *** $p < 0.001$.

3.3. Survival after Summer Planting

The survival rate after summer planting was 74% for seedlings with HC treatment (Figure 3). The HD treatment had a marginally positive effect on survival (93%, $p = 0.07$), but the HK treatment had no effect compared with the HC treatment. The JC treatment had a marginal positive effect on survival (87%, $p = 0.22$), and the JD treatment had a significant positive effect, with the highest survival rate at 97% ($p < 0.05$). Additionally, the HC + Ant and JC + Ant treatments marginally positively affected survival, with rates of 89% ($p = 0.15$) and 93% ($p = 0.05$), respectively. However, the HC + Top and JC + Top treatments did not affect survival compared to the HC treatment.

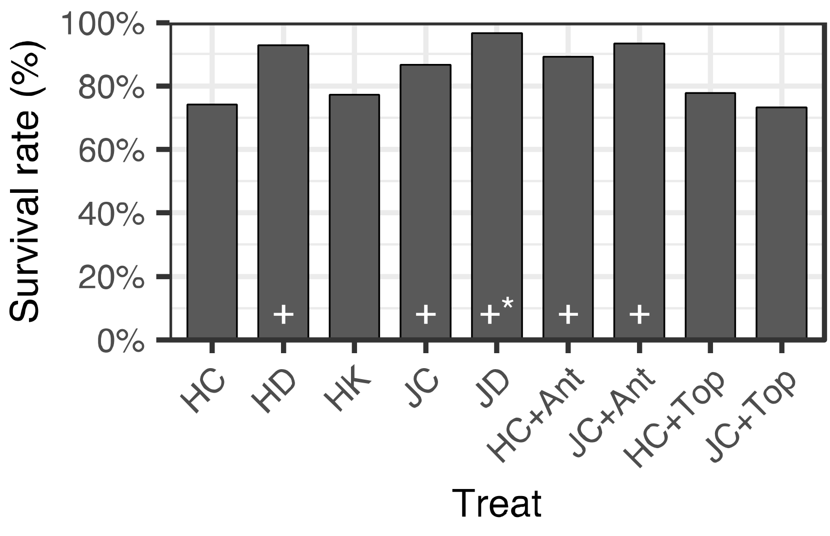

Figure 3. Survival of container seedlings (n = 23–30) grown in Hiko-V-120 (H) and JFA-150 (J) containers with control irrigation and fertilization (HC and JC), dry irrigation (HD and JD), high-potassium fertilization (HK), application of antitranspirant (+Ant), and topping (+Top). The plus sign indicates a positive effect compared with the HC treatment in the generalized linear mixed model. * $p < 0.05$.

3.4. Height and Root Collar Diameter of Seedlings before and after Summer Planting

The height of seedlings after a year of growth in the containers after sowing, just before drought hardening and high-K fertilization treatments, was significantly higher in the Hiko-V-120 containers than in the JFA-150 containers (Figure S3). After two months of drought hardening or high-K treatment, the HK treatment had a marginally negative effect on height (p = 0.28) compared with the HC treatment, and the height of seedlings growing in JFA-150 containers (JC, JD, and JC + Ant) remained significantly lower than that of seedlings with HC treatment (Figure 4A). The topping treatment (+Top) had a significant negative effect on seedling height, and seedlings were the shortest in the JC + Top treatment (Figure 4A). In October, three months after summer planting, topping also had a significant negative effect on seedling height compared with the HC treatment. The JC + Top treatment still had the shortest seedlings among treatments (Figure 4B). The JC and JD treatments significantly negatively affected height, as shown in May and July. By contrast, the JC + Ant treatment had a marginal negative effect (p = 0.06), which was no longer significant.

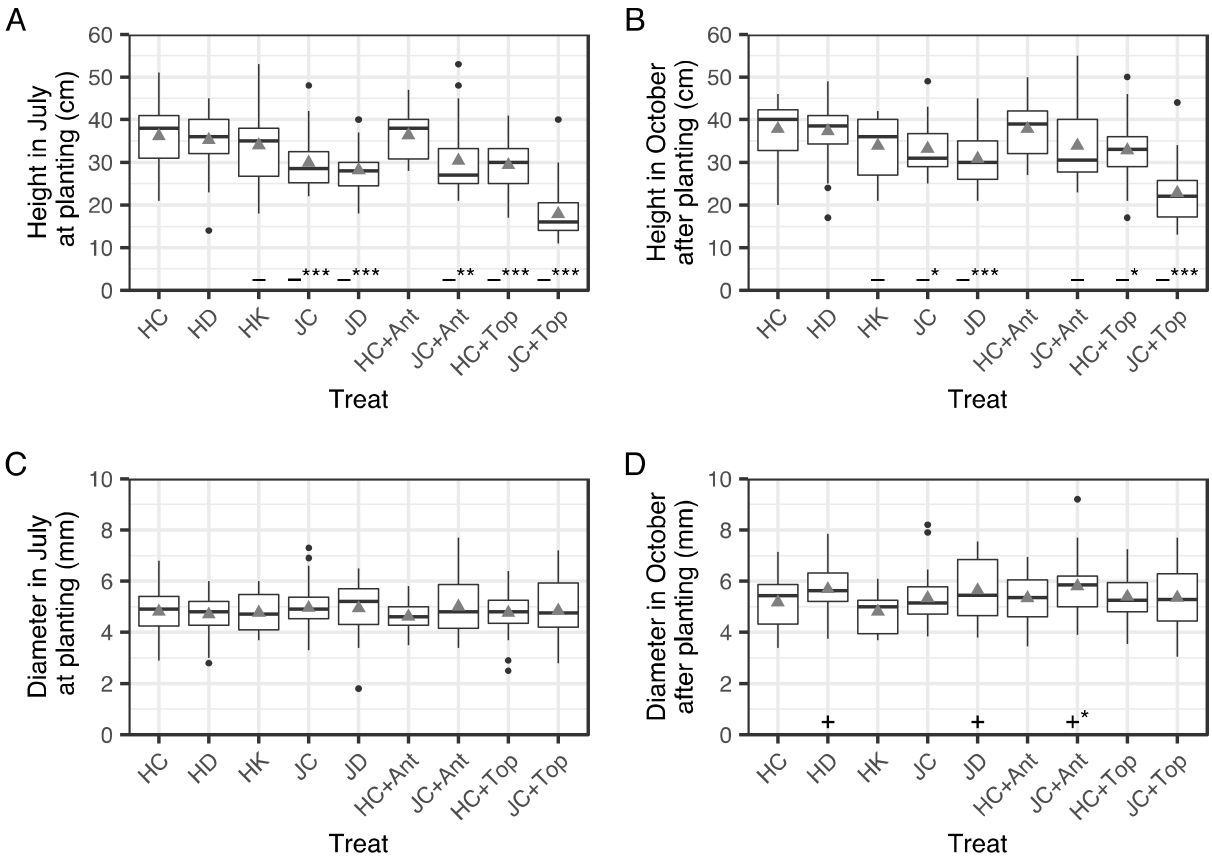

Figure 4. Boxplots with mean values (gray triangles, n = 17–30) of height and root collar diameters of surviving container seedlings of Japanese larch grown in Hiko-V-120 (H) and JFA-150 (J) containers with control irrigation and fertilization (HC and JC), dry irrigation (HD and JD), high-potassium fertilization (HK), application of antitranspirant (+Ant), and topping (+Top) measured at planting (**A**,**C**) and three months after planting (**B**,**D**). The dots indicate outliers. The plus or minus sign indicates a positive or a negative effect, respectively, compared with the HC treatment in the generalized linear mixed model. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Treatments had no effect on root collar diameter at planting in July (Figure 4C). In October, three months after summer planting, the JC + Ant treatment had a significant positive effect. The HD and JD treatments had marginal positive effects (p = 0.10 and p = 0.13, respectively) on diameter compared to the HC treatment (Figure 4D).

The increase in height in the three months after summer planting averaged 2 cm and ranged from −11 to 8 cm with the HC treatment (Figure 5). The JC and JC + Top treatments marginally positively affected height growth (p = 0.18 and p = 0.08, respectively) compared with the HC treatment, but the difference in the average height growth between the HC treatment and these two treatments was less than 2 cm. The other treatments did not affect height growth in the three months after planting.

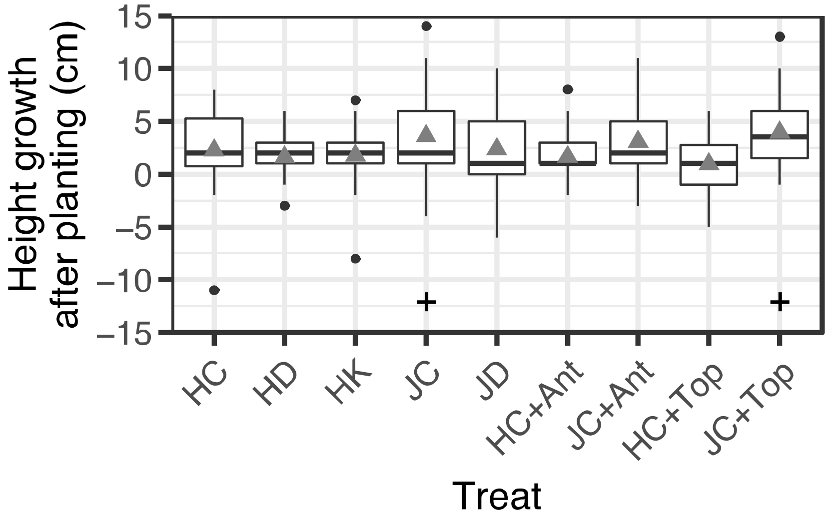

Figure 5. Boxplot with the mean value (gray triangle, n = 17–29) of height growth during three months after summer planting of surviving Japanese larch container seedlings in Hiko-V-120 (H) and JFA-150 (J) containers with control irrigation and fertilization (HC and JC), dry irrigation (HD and JD), high-potassium fertilization (HK), application of antitranspirant (+Ant), and topping (+Top). The dots indicate outliers. The plus sign indicates a positive effect compared with the HC treatment in the generalized linear mixed model.

3.5. Gas Exchange after Summer Planting

Two months after summer planting in July, only the HC + Top treatment had a significant positive effect on P_{mid} compared with the HC treatment. By contrast, the other treatments, including HD, HK, JC, and HC + Ant treatments, had no effect on P_{mid} (Figure 6A). The JC and HC + Top treatments had marginal positive effects on $g_{s.mid}$ (p = 0.28 and p = 0.11, respectively) compared with the HC treatment (Figure 6B). The JC treatment had a significant positive effect, and the HC + Top treatment had a marginally positive effect (p = 0.25) on leaf nitrogen compared with the HC treatment. By contrast, the HD, HK, and HC + Ant treatments did not affect leaf nitrogen (Figure 6C).

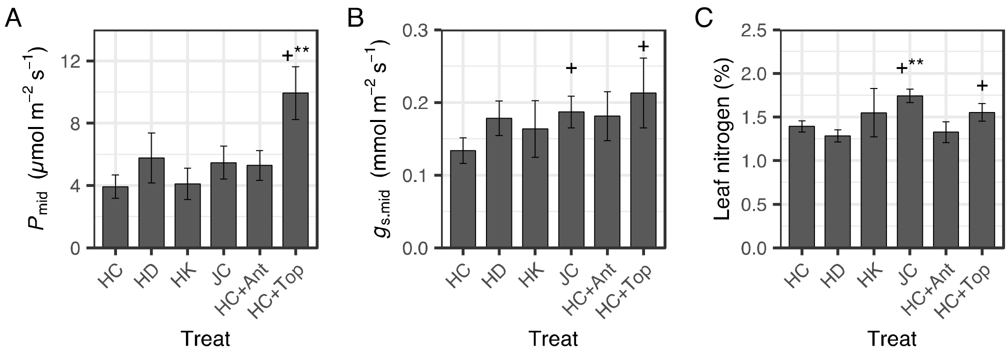

Figure 6. Mean values ± standard error (SE; n = 4 or 5) for photosynthetic rate (P_{mid}, (**A**)) and stomatal conductance at midday ($g_{s.mid}$, (**B**)) and leaf nitrogen (**C**) of container seedlings grown in Hiko-V-120 (H) and JFA-150 (J) containers with control irrigation and fertilization (HC and JC), dry irrigation (HD), high-potassium fertilization (HK), application of antitranspirant (+Ant), and topping (+Top). Measurements were taken at >300 μmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) from sunlight and 22.8 °C–25.7 °C leaf temperature on a sunny day in September, two months after summer planting. The plus sign indicates positive effects compared with the HC treatment in the generalized linear mixed model. ** $p < 0.01$.

4. Discussion

The survival of container seedlings of Japanese larch after summer planting was enhanced through drought hardening treatment by reducing the frequency of irrigation in the nursery for two months before planting, the application of an antitranspirant to the crown of seedlings at planting, and using JFA-150 containers with a larger cell capacity and a lower growing density than those of Hiko-V-120 containers (Figure 3). However, seedling survival was not increased by high-K fertilization or topping. Notably, even when the same growing protocols were compared, the survival with HC treatment in this study was 74%, which is considerably higher than the 22% in a previous study conducted at the same study site in a previous year [27]. The difference in survival could have been caused by differences in precipitation around the planting date, with less precipitation and more days of no precipitation in the previous study than in the present study (Figure S1), and high air temperature after planting in the previous study (Figure S2); the sum of precipitation for a month, including the planting date, the number of consecutive no precipitation days before planting date, and mean daily maximum air temperature during the two weeks after planting were 20.5 mm, 20 days, and 27.8 °C in the previous study and 48 mm, 8 days, and 25.9 °C in the current study, respectively. Therefore, further research is necessary on whether the seedling growing methods that have increased survival in summer planting in this study will also be effective under more severe drought stress, as in the previous study [27].

4.1. Effects of Irrigation and K Fertilization for Two Months after Winter Storage

The increase in the survival rate of seedlings exposed to drought hardening treatment with a reduced frequency of irrigation for two months in the nursery before summer planting (Figure 3) was primarily attributable to the change in morphology; that is, there was a decrease in the leaf:root ratio (Figure 1D) caused by the lower leaf dry mass (Figure 1B), rather than acclimation of leaf water relations (Figure 2). By contrast, changes in the physiological properties of holm oak seedlings, such as osmotic adjustment rather than morphological characteristics, contributed to increased drought tolerance, due to drought hardening in the nursery [49]. Moderate drought stress from a controlled wetting and drying cycle often enhances the drought tolerance of seedlings associated with osmotic adjustment involving the active accumulation of solutes in leaves in many tree species but not in other species [50]. For example, when irrigation was suppressed for 8 weeks in 5-month-old *Pinus halepensis* Mills seedlings, there was a significant effect on morphology but no effect on leaf water relations [30]; this is a finding similar to that of the present study. To the best of our knowledge, no studies have reported osmotic adjustment in response to drought in seedlings or saplings of the genus *Larix*. Current-year long shoots of the genus *Larix* typically continue to elongate during the growing season, so part of the shoots contains newly expanded leaves. Thus, as newly expanded leaves generally have a higher osmotic potential [46,51–53], osmotic adjustment by drought stress might be less likely to occur in shoots of the genus *Larix*. The characteristic of larch to continue extending shoots throughout the growing season would enhance drought tolerance, due to drought hardening by reducing the leaf:root ratio through the regulation of leaf production. Severe drought stress can increase the REL [54,55], but this was not observed in this study, indicating that the drought hardening treatment in this study would be moderate drought stress, even for Hiko-V-120 containers with lower soil moisture content.

An adequate supply of K can inhibit reduced photosynthesis and promote high growth and longevity of roots during drought [35]. K can act as an osmoticum, energetically cheaper than sugar alcohols or amino acids for plants. Thus, the K supply can also support osmotic adjustment, resulting in the maintenance of cell turgor and consequent cell expansion in dry soil [34–56], thereby alleviating drought stress. However, two months of high-K fertilization (1.5 times higher than the control) had no effect on the morphological (Figure 1) and physiological (Figure 2) traits that enhance the drought tolerance of Japanese larch seedlings. Consequently, it did not improve the survival and growth of summer-planted seedlings (Figures 3–5). The failure of high-K fertilization in this study to increase drought tolerance of seedlings can be attributed to the fact that it was applied to the seedlings in the well-irrigated treatment but not seedlings under the drought hardening irrigation treatment in the nursery. An insufficient amount and duration of K fertilizer application may also be responsible. Additionally, the results suggest that a high application of K in the form of a liquid fertilizer before outplanting in the nursery will not increase the drought tolerance of seedlings when subject to drought stress after outplanting in the field, potentially because K might not be retained in the seedlings long enough to provide drought tolerance after outplanting. It might be effective to apply an additional controlled-release fertilizer, rather than a liquid fertilizer, before or at outplanting [11,57] with a high-K content during summer planting with drought stress.

The effects of treatments on seedlings are potentially attributable to the intensity and duration of treatments [2]. Future research is needed to clarify whether K fertilization, as well as drought hardening or a combination of K fertilization and drought hardening, with greater intensity and duration than those employed in this study, will result in drought acclimation of physiological and morphological traits of larch container seedlings, leading to better performance after summer planting.

4.2. Effects of Antitranspirant and Topping at Summer Planting

The application of an antitranspirant at planting to container-grown Japanese larch seedlings improved survival after summer planting (Figure 3). The high $g_{s.mid}$ with the HC + Ant and JC + Ant treatments (Figure 6B) suggests that the effect of the antitranspirant disappeared within two months after treatment. It also suggests that the leaves of seedlings with antitranspirant application had a better water status two months after planting because the antitranspirant reduced water loss by transpiration after planting, and thus, reduced planting shock under dry soil [37,38,58]. The fact that the diameter was significantly higher after the end of the growth period with the JC + Ant treatment (Figure 4D) implies that the root growth would have been better in the treatment, which might have also contributed to the higher survival rate. The application of an antitranspirant increases the labor and cost requirements of container seedling production. Nevertheless, it is a very simple method for summer planting, as nursery producers can determine the required application of the antitranspirant according to the soil dry conditions of the plantation and use seedlings grown under standard nursery practices. The application of an antitranspirant, which has been attempted in the distant past [59,60], is not commonly used in seedlings for afforestation at present compared with agricultural crops [61]. However, it can be an effective technique for ensuring better performance of leafy container-grown seedlings of Japanese larch for dry summer planting.

The topping treatment at planting, in which a quarter of the canopy length was cut from the top, should have reduced the leaf–root ratio. However, it did not improve the survival of seedlings planted in summer (Figure 3). By contrast, seedlings with a low leaf:root ratio grown using other methods, such as drought hardening irrigation and JFA-150 containers (Figure 1D), had higher survival rates. The high P_{mid}, $g_{s.mid}$, and leaf nitrogen content of HC + Top seedlings (Figure 6) indicates that drought stress after summer planting was reduced at needle or shoot level with the topping treatment. Therefore, the lack of an increase in survival of the seedlings with topping treatment could be attributed to increased competition from weeds, due to reduced seedling height rather than to drought tolerance because larches are highly susceptible to weed competition [62–64]. In fact, although weeding was conducted one-and-a-half months after planting in this study, it was observed that some seedlings, especially small ones, were entirely covered by competing vegetation. Topping is one of the old and easy ways of balancing root and leaf at transplanting [65], but in areas where weeds thrive, including Japan, topping treatments can have a negative impact on the performance of seedlings, especially for less shade-tolerant species, after planting.

4.3. Effect of Container Type

For seedlings with control irrigation and fertilizer treatment, the increase in the survival rate in JFA-150 containers relative to Hiko-V-120 containers (Figure 3) was primarily, due to a change in morphology, i.e., higher root mass (Figure 1A) and a consequently lower leaf:root ratio (Figure 1D). Although the cell volume of JFA-150 is only 1.25 times that of Hiko-V-120, the root mass of seedlings grown in JFA-150 was almost twice that of those in Hiko-V-120 (Figure 1A), suggesting that root growth in the Hiko-V-120 containers in the nursery could be inhibited by their small cell capacity [12,66–68], resulting in lower survival after summer planting. Additionally, the high growing density in Hiko-V-120 containers could have contributed to the inhibition of root growth in the nursery [66,69], although seedling densities were reduced by half in each container two months prior to planting. The higher g_s and leaf N with the JC treatment compared to the HC treatment after planting (Figure 6B,C) indicates that the greater root mass with JC treatment could actively absorb water and nutrients in the field after summer planting. As the cell volume increased, leaf water relation parameters, namely, $\Psi_{o.ft}$, $\Psi_{w.tlp}$, and RWC_{tlp}, were changed slightly to increase drought tolerance. However, these changes were minimal, suggesting that the changes in leaf water relations were complementary factors to the increase in survival after summer planting. The Hiko-V-120 containers had a side slit wall that prevents root spiraling better than the ribbed wall [70] in the JFA-150 containers, which did not seem to improve summer planting survival. However, further research is needed because we did not test the JFA-150 containers with a side slit wall.

Reduced survival in containers with small cell volume and high growing density has also been reported for other tree species, such as hybrid poplars [71], eastern white cedar [72], and silver birch [69]. However, in other studies, there was no relationship between containers with different cell volumes and growing densities and survival after outplanting [12,68]. This discrepancy could be that favorable conditions with sufficient precipitation and extremely unfavorable conditions with lower precipitation can dilute survival differences. In Japan, where most nurseries are small, and forestry workers are aging, the production of seedlings in containers with small cell volume and high growing density tends to be favored by nurseries because of the low cost of seedling production and small growing area and by forest workers because of the small size and light weight of seedlings that make them easier to transport. However, when planting container-grown seedlings in areas or seasons where the soil tends to dry, it is beneficial to grow the seedlings in a container with a large cell volume to avoid restricting root growth in the nursery and to improve survival after outplanting.

5. Conclusions

We investigated whether drought hardening and increasing K fertilization during the two month period before planting, the application of the antitranspirant, topping at planting, and changing containers from the Hiko-V-120 to the JFA-150 with high cell capacity and low cell density in the nursery can increase the survival of 1-year-old container-grown seedlings of Japanese larch planted in the dry summer. Drought hardening, by reducing the irrigation frequency from every day to twice a week and using JFA-150 containers, increased survival after summer planting. This increase was primarily due to the lower leaf:root ratio caused by decreased leaf mass production by drought hardening treatment and greater root mass using the JFA-150 container. Additionally, the application of the antitranspirants can also increase survival. The topping treatment reduced the leaf:root ratio, resulting in enhanced drought tolerance, but it did not increase survival, due to increased competition from weeds for the less shade-tolerant Japanese larch. An effect of high-K fertilization was not observed on the morphology or physiology of the seedlings, and thus, their survival in this study. A restrained irrigation regime using JFA-150 containers before planting appears to ensure better survival of container-grown Japanese larch seedlings after dry summer planting. The application of the antitranspirant is also effective but costly.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12081115/s1: Figure S1: Daily precipitation during the growing season in a previous study (2014), this study (2015), and over the period of 1981–2010. Figure S2: Daily maximum air temperatures during the growing season in a previous study (red, 2014), the current study (blue, 2015), and over the period of 1981–2010 (gray). Figure S3: Boxplot with mean value (gray triangle, n = 23–30) of the height in May before each treatment for seedlings of Japanese larch grown in Hiko-V-120 (H) and JFA-150 (J) containers with control irrigation and fertilization (HC and JC), dry irrigation (HD and JD), high-potassium fertilization (HK), and application of the antitranspirant (+Ant) and topping (+Top).

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