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Initial growth and root system development of Cryptomeria japonica cuttings using different nursery methods

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Abstract

Using Japanese cedar (*Cryptomeria japonica*) cuttings developed through different methods, the effect of various initial underground root shapes on subsequent growth was evaluated by understanding various above- and belowground traits at the initial planting stage. The cuttings developed through four different techniques were used in this study: bare-root cuttings grown in an open field, BCC-containerized cuttings, biodegradable-containerized cuttings, and JFA150-containerized cuttings grown in greenhouses. After planting in December 2019, different parameters, namely, survival rate, seedling height, and ground diameter, were measured over three subsequent growth seasons. In addition, resistance to vertical pulling and digging out was determined to assess the development of the root system from each cutting. No statistically significant differences were observed in the survival rate and ground diameter of various cuttings after three growth seasons. Meanwhile, a statistically significant difference was noticed in the seedling length of the bare-root and containerized cuttings during the first growth season. After two growth seasons, the T/R ratio—a measure of root system development—reached a value of 4—a typical or benchmark value for healthy Japanese cedar growth—in all the cedar cuttings. Under dry conditions, the presence of a root ball reduced stress at the initial planting stage in all the containerized cuttings, making containerized cuttings an effective propagation method for Japanese cedar. The maximum resistance value in the root pull-out test showed a strong correlation with the root volume.

Introduction

Containerized seedlings are seedlings grown in a container, typically one made of hard plastic, in an enclosed or semi-enclosed environment. However, seedlings generated through conventional labor-intensive bare-root method are grown in open fields. Thus, the containerized seedling production method, a capital-intensive method, allows controlled fertilization and irrigation for optimal plant growth (Grossnickle and El-Kassaby, 2015). The performance of containerized technology's implementation has been evaluated post plantation (Donald et al., 1989; Javier et al., 1996; Thiffault et al., 2003; Vieira et al., 2019). For example, in Scots pine (*Pinus sylvestris*), no statistically significant difference was observed in the survival rate of containerized and bare-root seedlings three years after planting; however, the seedling height was significantly higher in the containerized seedlings than in the bare-root seedlings (Kïavìòa et al., 2013). In Norway spruce (*Picea abies*), the survival rate of containerized seedlings was significantly lower than that of bare-root seedlings three years after planting, and the containerized seedling height was significantly lower than that of the bare-root seedlings (Kïavìòa et al., 2013). In larch (*Larix kaempferi*), the survival rate was 48% for bare-root seedlings and 90% for containerized seedlings in the first year after planting, and the containerized seedlings exhibited higher seedling height than the bare-root seedlings (Yokoyama and Sasaki, 2013). However, the study found no significant differences in the survival rate or growth of Sakhalin fir (*Abies sachalinensis*), red glehnii (*Picea glehnii)*, and larch (*Larix gmelinii var. japonica*). Similarly, in Japanese cypress (*Chamaecyparis obtusa*), the survival rates were 89.5% and 56.5% for containerized and bare-root seedlings, respectively, when planted in August and evaluated in the October of the following year (Suwa et al., 2016). Furthermore, in red oak (*Quercus*

rubra L.), the survival rate was 75% for bare-root seedlings and 100% for containerized seedlings in the first year of planting (Wilson et al., 2007). Comparing the height and survival rate of bare-root and containerized seedlings has been the main focus in tree species research, with no universal superiority of either of the two methods (Grossnickle and El-Kassaby, 2015).

Containers have two effects: a root-pruning effect, in which the bottom is open and the roots are exposed to the air to stop root growth (air-pruning), and an anti-root curling effect, in which the ribs or solids on the sides prevent root curling. The ability of plants to limit their exposure to drought stress in the initial planting period is believed to depend on the root system, which is responsible for water absorption (Grossnickle, 2005; Sugihara and Tange, 2016). As the main morphological difference between containerized and bare-root seedlings lies in the roots, a comparative evaluation of the roots of these two types of cuttings is crucial (Hirata et al., 2014; Tsakaldimi et al., 2005). To date, several shapes of containers have been developed, such as BCC (FlexiFrame 77 High Model, BCC Plant the Planet Co. Ltd.)—a square pyramidal container; JFA150 (Forestry Agency)—a conical multicavity container; and an M-Star container—a multi-stage adjustable rolled container that uses single-sided corrugated sheets rolled up in a cylindrical shape. Besides, biodegradable seedling containers that decompose in the soil allows cuttings to be planted directly into the ground where the roots are entirely enclosed (Hirata et al., 2019; Kitahara et al., 2018; Muriuki et al., 2013; Silva et al., 2025). As the container type influences the seedling underground shape, comparative evaluations of the root shape corresponding to each container are necessary (Hirata et al., 2014; Tsakaldimi et al., 2005). However, belowground plant evaluation is more time-consuming and laborious (Reubens et al., 2007) than assessing aboveground parts, leading to fewer studies (Chapman and Colombo, 2007; Danjon et al., 2024; Ortega et al., 2006) on belowground analyses of containerized seedlings than those focused on aboveground analyses (Tobin et al., 2007).

Japanese cedar is a major forest tree species, covering 44% of the artificial forests in Japan. Presently, approximately 60% of the planted Japanese cedar trees are over 50 years old, which is a typical harvest age for the species (ForestryAgency, 2024). Containerized cedar seedlings are gaining popularity in Japan due to increased need for reforestation after harvesting (ForestryAgency, 2024). Containerized Japanese cedar seedlings have been in use for approximately 10 years, and the proportion of containerized cuttings planted in forests has been steadily increasing (ForestryAgency, 2024). Containerized cuttings demonstrated better growth than bare-root Japanese cedar cuttings planted in February(Yamagawa et al., 2013). Meanwhile, Hakamata et al. (2016) showed that bare-root Japanese cedar seedlings exhibited significantly better growth than containerized seedlings during the first four months after planting. Elsewhere, Yagihashi et al. (2016) showed that the growth of bare-root Japanese cedar seedlings was greater than that of containerized seedlings in the first and second growing seasons. In contrast, Hirata et al. (2014) found no significant initial growth advantage for containerized Japanese cedar seedlings or cuttings over bare-root Japanese cedar ones during the first two years after planting. Owing to the presence of a root ball, containerized seedlings are known to have a longer planting season than bare-root seedlings (Yamagawa et al., 2013). In fact, > 80% of bare-root Japanese cedar seedlings died, whereas containerized seedlings exhibited a mortality rate of < 10% when planted simultaneously during the dry season (Zushi, 2018). However, when these two types of seedlings were

planted in September and examined for the survival rate a year later, no significant difference was found between them (Shimbo et al., 2016). Likewise Grossnickle and El-Kassaby (2015), Kabeya et al. (2016) stated that scientific knowledge regarding the effects of container seedlings on Japanese cedar growth and establishment is still being accumulated.

Many studies have compared single-matched containerized and bare-root seedling. However, few studies have comprehensively evaluated the differences between containerized and bare-root seedlings using multiple containerized seedling configurations. In this study, bare-root seedlings and different containerized seedling configurations—BCC containers, biodegradable containers, and JFA150 containers—were planted in the same location and their rooting and growth processes were evaluated over three growing periods. Furthermore, the development of the root system of all the containerized seedlings and bare-root seedling was evaluated. Thus, the study aimed to clarify how different root configurations at the planting time affect survival rate, aboveground growth, and root development.

Materials and Methods

Site and materials

This study was conducted in Fukuoka Experimental Forest (33˚66'N, 130˚55'E, ca. 350 m a.s.l), Faculty of Agriculture, Kyushu University, Fukuoka Prefecture, Japan. The slope was oriented nearly toward north with an obliquity of 0°–20°. The study area was a metamorphic pumiceous zone with annual mean temperature of 17.8°C and annual mean precipitation of 1,781 mm over the last 10 years. In addition to bare-root cuttings, three containerized cuttings developed in a BCC container (SideSlit Cell 150 with FlexiFrame 77 High Model: https://bccab.com/products-planting/growing-systems/), a JFA150 container (https://www.rinya.maff.go.jp/j/kanbatu/houkokusho/attach/pdf/syubyou-1.pdf), and a biodegradable container (composed of wood fiber and pulp, Daiken Corporation and Japan Conservation Engineers and Co. Ltd.) were used in this study. Grossnickle and Ivetić (2022) classified containers with smooth interior wall surfaces as Category A, those with vertical ribs on the interior walls as Category B, and those with vertical side slits or open mesh/paper containers as Category C. According to this classification, BCC containers fall into Category C, whereas biodegradable seedling containers and JFA150 containers fall into Category B. In October 2019, an approximately 30 m × 20 m (length × breadth) test plot was established along the slope of the study site. After preparing the ground in November 2019, 30 cuttings per row were planted at a distance of 1 m × 1 m from top to bottom of the slope in December 2019. A total of five rows, one each of bare-root, BCC-containerized, and JFA150-containerized cuttings, and two rows of biodegradable containerized cuttings, were planted. Thereafter, 12 sensors, four at each of the three locations at the top, middle, and bottom of the slope, were installed to measure the soil matric potential (Watch dog, 6450WD, ESPEC MIC Corp.). The precipitation data during the test period was obtained from the the Japan Meteorological Agency. The planted test area was enclosed with netting to prevent deer browsing, and protective tree shelters (PSS, Phytoculture Co., Ltd.) were placed on all the cuttings. Weeding treatment was conducted twice a year in June and October.

Methods

Survival, height, and diameter

The survival rate, height, and diameter at the cutting base were measured at four time points: March 2020 (before the start of the first growing season), November 2020, December 2021, and October 2022. To calculate the survival rate, individuals with green leaves were considered alive. Meanwhile, individuals with dry brown leaves that fall off easily were considered dead. Individuals subjected to destructive testing of root system development were excluded from survival rate calculations. The seedling height was measured from the top of the slope using an aluminum staff (MTR 22, Myzox Co. Ltd.) as a measuring tool to visually align with the top of the translucent tree shelter from the outside. The ground-level diameter was measured by elevating the tree shelter a few centimeters and inserting a digital caliper (GDCS-100, Niigata Seiki Co. Ltd.) into the gap created.

Root system development

Three individuals in each row were dug up in November 2020 after the first growth season, and two individuals in each row were dug up in December 2021 after the second growth season. The root systems were dug up using a hoe or grafting trowel while avoiding any root damage. The dug root systems and aboveground parts were brought back to the laboratory, dried in a greenhouse for at least 6 months, and then dried in a dry oven at 68°C for at least 48 h to a constant weight. The belowground volume was then measured using the underwater displacement method. The aboveground part was dried at 68°C for at least 48 h in a dry oven to a constant weight. Four individuals per row, including their root system, were pulled out in November 2020 after the first growth season, two individuals per row were pulled out in December 2021 after the second growth season, and three individuals per row were pulled out in October 2022 after the third growth season. The pull-out test is generally conducted by wrapping a rope around the tree and pulling it horizontally with a winch (Nonoda et al., 1996; Todo et al., 2022), and the resistance value is indicative of root length, branching, and rooting angle (Abe, 1991). This provides an indirect assessment of root system development. As preliminary tests revealed that horizontal pulling was not suitable for the soft young cuttings in this study, it was decided to instead use a vertical pulling technique. In this study, a tripod was set up with its center directly above the cuttings, a chain block was hooked to the top of the tripod, and the cuttings were pulled up by applying a constant vertical force to one end with the other end fixed. The pulling load data were obtained using strain gauges (HJ-FDS-X20K, Nextech Co. Ltd.), and the maximum load was used in the subsequent analysis (Supplementary Fig. 1).

Statistical analysis

All analyses were performed with the statistical package R (4.2.1). Various parameters were measured in all the cuttings. The survival rates were calculated by dividing the total number of surviving individuals by the number of seedlings planted, excluding the ones used in the destructive test. A generalized linear model was used to compare survival rates. The dependent variable was a binary data set (survival

(1)/death (0)), and a binomial distribution with a logit link function was assumed. To detect differences among different cuttings, cutting varieties was included as a fixed effect in the statistical model. Tukey-Kramer multiple comparison tests were performed to compare seedling length, ground level diameter, height-to-diameter ratio (HDR: seedling length/base diameter), and T/R ratio (aboveground dry weight/underground dry weight) across different cutting varieties. A linear model was used for the analysis, incorporating cutting varieties as a fixed effect. The stat_poly_eq function found within the misc package in R was used to generate a regression line to determine the relationship between the subsurface volume and pull-out resistance. The pairs.panels function from the psych package in R was used to compare the trait values. Welch's t-test was used to compare dig-out and pull-out test findings. The seedling occupancy rate in the container (root bound index) was calculated using the following formula.

$$Root\ bound\ index\ =\ Root\ collar\ diameter\,(mm)\ /\ Cell\ diameter\,(mm)$$

Results and Discussion

Differences in survival rate, height, and ground level diameter among cutting varieties

South et al. (2005) evaluated the survival rates of several containerized seedlings and bare-root seedlings of longleaf pine (*Pinus palustris*). They found that the root diameter affected survival rates more than the cutting varieties, and that survival rates dropped sharply when the root bound index (root collar diameter / cell diameter) exceeded 27%. Furthermore, they noted that unhealthy container seedling cultivation results in poor growth. The seedlings used in this study were transported to the study location within the standard growth period of 12–18 months. Immediately after planting, the root bound index was 15% for BCC-containerized seedlings, 11% for biodegradable-containerized seedlings, and 14% for JFA150-containerized seedlings (Appendix 1), and no poor growth was observed during containerized seedling cultivation. Apart from the cuttings subjected to destructive tests (pull-out and dig-out tests), only two cuttings died—one in the BCC container during the first growth season and the other one in the biodegradable container during the second growth season (Table 1). There were no statistically significant differences ($p > 0.05$) in the survival rates of the cutting varieties at any stage. This may be due to the study being initiated in December—no high temperature stress—and the sheltered environment—reduced cold and wind stress. All cutting varieties showed survival rates of 97%–100%, and even bare-root cuttings successfully survived at the appropriate planting timing. In this study, the containerized cuttings were not found to be superior to bare-root cuttings in terms of the survival rate.

Immediately after planting, the height was 45.0 ± 8.2 cm (± SD) for the bare-root cuttings, 38.9 ± 5.5 cm for the BCC-containerized cuttings, 38.7 ± 5.4 cm for the biodegradable-containerized cuttings, and 36.9 ± 6.6 cm for the JFA150-containerized cuttings, with only bare-root cuttings being significantly taller than the other cutting varieties (Fig. 1a; $p < 0.05$). After the third growing season, the seedling heights were 169.3 ± 39.3 cm, 191.2 ± 21.5 cm, 193.9 ± 21.8 cm, and 190.8 ± 21.7 cm for the bare-root cuttings, BCC-

containerized cuttings, biodegradable-containerized cuttings, and JFA150-containerized cuttings, respectively (Fig. 1a; $p < 0.05$). During the study period, the increase in height was 124.2 ± 38.1 cm for the bare-root cuttings, 152.5 ± 22.3 cm for the BCC-containerized cuttings, 155.2 ± 22.9 cm for the biodegradable-containerized cuttings, and 153.4 ± 23.5 cm for the JFA150-containerized cuttings (Fig. 1d).

After planting, the ground level diameters were 6.6 ± 1.2 mm for the bare-root cuttings, 6.2 ± 1.0 mm for the BCC-containerized cuttings, 5.8 ± 1.0 mm for the biodegradable-containerized cuttings, and 6.1 ± 1.0 mm for JFA150-containerized cuttings, indicating no significant difference among the cutting varieties (Fig. 1b: $p > 0.05$). After the third growing season, the ground level diameters were 15.5 ± 4.4 mm, 17.7 ± 5.0 mm, 18.2 ± 6.9 mm, and 17.7 ± 6.4 mm for the bare-root cuttings, BCC-containerized cuttings, biodegradable-containerized cuttings, and JFA150-containerized cuttings, respectively (Fig. 1b; $p > 0.05$). During the study period, the increase in the ground level diameters were 8.8 ± 4.4 mm for the bare-root cuttings, 11.7 ± 5.0 mm for the BCC-containerized cuttings, 12.4 ± 6.7 mm for the biodegradable-containerized cuttings, and 11.7 ± 6.1 mm for the JFA150-containerized cuttings (Fig. 1e).

After planting, the HDR values, calculated by dividing the seedling height by the ground level diameter, were 68.9 ± 11.0 for the bare-root cuttings, 64.6 ± 14.1 for the BCC-containerized cuttings, 68.6 ± 14.0 for the biodegradable-containerized cuttings, and 61.1 ± 12.3 for the JFA150-containerized cuttings. After the third growth season, the HDR values were 111.8 ± 20.6, 113.0 ± 21.7, 113.8 ± 21.6, and 115.1 ± 23.2, for the bare-root cuttings, BCC-containerized cuttings, biodegradable-containerized cuttings, and JFA150-containerized cuttings, respectively, indicating no significant difference among the cutting varieties (Fig. 1c; $p > 0.05$).

The HDR increased markedly until the second growth season in all the cutting varieties, which may have been due to the beneficial micro-environment created by the tree shelter. When tree shelters are used, the lack of wind stress allows the plant to focus energy on the upward growth (Abe, 2021; Costello et al., 1996) rather than on the radial growth at the ground level for wind resistance (Coutand et al., 2008). This trend was confirmed in the present study for the first two growth seasons until the trees grew beyond the height of the tree shelter (140 cm). The HDR, which averaged 130 in the second growth season, decreased to an average of 113 in the third growth season. This HDR value was still high compared to the values shown in previous studies on containerized seedlings or cuttings (Hirata et al., 2019; Hirata et al., 2014; Yagihashi et al., 2016). However, it began to decrease from the third growth season when trees grew beyond the tree shelter height, suggesting that the HDR can be optimized over time.

Soil moisture is an important factor influencing the plant growth (Haase and Rose, 1993). Soil moisture sensors installed at the top, middle, and bottom of the slope showed that the top of the slope was the driest, the bottom was moderately moist, and the middle of the slope was the wettest (Fig. 4a). Thirty individuals in each row were divided into three equal groups of ten: upper, middle, and lower groups positioned along the slope; the growth of each cutting variety was then compared within these groups over the study period (Fig. 4b). No statistically significant difference was observed in the cutting

varieties in the wettest middle part of the slope; however, the bare-root cuttings grew inferiorly in the drier upper and lower parts of the slope. There were no significant differences in the survival rate or growth of all the containerized varieties, suggesting that the presence of a root ball in the containerized Japanese cedar cuttings reduced planting stress (Hirata et al., 2014; Sugihara and Tange, 2016), regardless of the container shape. Rose and Haase (2005) showed that containerized seedlings have better root system development than bare-root seedlings in the early growth stages and the survival rate of containerized seedlings is significantly higher than that of bare-root seedlings, especially in poor soil conditions. In Japanese cedar, the containerized seedling growth was less affected by the dry conditions on the upper and lower slopes than that of the bare-root seedlings, suggesting that containerized seedlings may be an effective approach in unfavorable growth environments.

Differences in root system development among cutting varieties

The root systems of 15 seedlings, 3 in each row, were dug up in November 2020 after the first growth season, whereas ten seedlings, 2 in each row, were dug up in December 2021 after the second growth season. The T/R ratio (aboveground dry weight to belowground dry weight)—a representative indicator of root system development—was 9.8 ± 4.9 and 4.8 ± 0.9 after the first and second growth seasons, respectively. The results showed that there were no clear differences among the cutting varieties (Fig. 2a). After the first growth season, the belowground volumes were 24.0 ± 3.5 cm^3 for the bare-root cuttings, 26.0 ± 11.1 cm^3 for the BCC-containerized cuttings, 19.3 ± 4.5 cm^3 for the biodegradable-containerized cuttings, and 20.7 ± 4.2 cm^3 for the JFA150-containerized cuttings. After the second growth season, the belowground volumes were 35.0 ± 4.2 cm^3, 28.0 ± 8.5 cm^3, 25.5 ± 5.5 cm^3, and 30.0 ± 19.8 cm^3, for the bare-root, BCC-containerized, biodegradable-containerized, and JFA150-containerized cuttings, respectively (Fig. 2b). The belowground volumes showed an overall trend of a slight increase, with no distinct differences among the cutting varieties. This suggests that restricted root system shapes, such as truncated cones and square pyramids, do not significantly affect root system development after planting.

Besides, pull-out tests were conducted on four individuals in each row in November 2020 after the first growth season, two individuals in each row in December 2021 after the second growth season, and three individuals in each row in October 2022 after the third growth season. The maximum pull-out resistance was 38.7 ± 21.2 kgf for the bare-root cuttings, 41.8 ± 9.6 kgf for the BCC-containerized cuttings, 40.2 ± 13.7 kgf for the biodegradable-containerized cuttings, and 63.0 ± 13.7 kgf for the JFA150-containerized cuttings after the first growth season, showing no statistically significant difference among all the cutting varieties (Fig. 2c; $p > 0.05$). The maximum pull-out resistance values for the third growth season were 126.0 ± 86.6 kgf, 196.0 ± 75.1 kgf, 173.2 ± 56.2 kgf, and 149.3 ± 110.7 kgf, for the bare-root, BCC-containerized, biodegradable-containerized, and JFA150-containerized cuttings, respectively. These values did not differ significantly among all the cutting varieties (Fig. 2c; $p > 0.05$).

In different cutting varieties, the comparison of the relationship between the belowground volume and maximum pull-out resistance revealed that the correlation coefficient was 0.13 after the first growth season, 0.65 after the second growth season, and 0.9 after the third growth season (Supplementary Fig. 2). This indicates that these two factors became increasingly correlated with the passing of each growth season. In different cutting varieties, the comparison of the relationship between the belowground volume and maximum pull-out resistance showed that the coefficient of determination of the regression line was in the range of 0.76–0.95 for all the cutting varieties except the biodegradable-containerized cuttings, which presented an R^2 value of 0.51 (Fig. 3). Even though the container depths were largely the same, it was observed that the roots of the biodegradable-containerized cuttings were less abundant in the lower part of the root pot than in the other containers (Fig. 2d). Hirata et al. (2019) showed that Japanese cedar cuttings grown in a biodegradable paper pot and other containers differed in the positioning of root development. Wilson et al. (2007) found that containerized red oak (*Quercus rubra L.*) seedlings exhibited a greater number of primary lateral roots and more comprehensively developed fine roots than bare-rooted seedlings. Rose and Haase (2005) showed the extensively developed root system in containerized seedlings to not be a problem in the long term; however, the number of new roots differed between containerized seedlings and bare-root seedlings depending on the planting season. In this study, the correlation between the root volume and uprooting resistance was particularly low in the biodegradable containerized seedlings compared to the other cutting varieties, suggesting that the detailed root system structure or the root ball breakdown process may differ among the cutting varieties.

After the third growth season, the maximum pull-out resistance among the cutting varieties showed correlation coefficient values of 0.84 and 0.87 for height and ground level diameter, respectively (Fig. 5), suggesting its clear relationship with the aboveground growth. The coefficient of the regression line increased with the passing of each growth season (Supplementary Fig. 2). This was supposed to be due to the simultaneous development of new roots and the collapse of roots making up the root ball (Supplementary Fig. 3), which increased the proportion of roots contributing strongly to the pull-out resistance relative to the entire root system. Meanwhile, the correlation coefficient value for the number of primary roots was 0.55, and the correlation coefficient values for mean and maximum primary root diameters were 0.33 and 0.27, respectively (Fig. 5). Thus, it is difficult to evaluate the contributions of individual root system traits, such as the number and thickness of primary roots to maximum pull-out resistance. As shown by Abe (1991), maximum pull-out resistance reflects the development of the entire belowground root system as the sum of individual root system traits. Therefore, to understand the influence of the root system structure on uprooting resistance, it is desirable to improve the estimation accuracy, by conducting tests, such as pull-out tests using simulated root systems that are characterized in terms of branching, root length, and rooting angle.

Comparing the dry weight of the belowground part and the number of primary roots in the dug-out and pulled-out cuttings showed that the dry weight of the belowground part of all the cuttings was 5.7 ± 2.5 g for the dug-out cuttings and 3.5 ± 2.4 g for the pulled-out ones after the first growth season. After the second growth season, the dry weight values of the belowground part of all the cuttings were 19.8 ± 3.7

g and 17.1 ± 5.8 g for the dug-out and pulled-out cuttings, respectively. A statistically significant difference was observed between the dug-out and pulled-out cuttings in terms of dry weight of the belowground part after the first growth season (t-test; $p < 0.05$); however, this significance was lost after the second growth season (t-test; $p > 0.05$, Supplementary Fig. 4a). In the pull-out test, root system tips were observed to be lost, with an average dry weight loss rate of 38.6% and 13.6% after the first and second growth seasons, respectively. Similarly, the number of primary roots in all the cuttings were 17.6 ± 6.6 and 11.4 ± 4.8 in the dug-out and pulled-out individuals, respectively, after the first growth season. After the second growth season, the numbers of primary roots were 18.1 ± 4.2 and 16.2 ± 6.2 in the dug-out and pulled-out individuals, respectively. A statistically significant difference was observed in this variable between the dug-out and pulled-out cuttings after the first growth season (t-test; $p < 0.05$); however, this significance was lost after the second growth season (t-test; $p > 0.05$, Supplementary Fig. 4b). Yamada and Shidei (1968) used two methods to obtain intact root systems: a full washing method, which required substantial water and time; and a pulling method, which was simple but resulted in the loss of fine roots at the tips. The amount of roots obtained via the pulling method was 1.5–3.0 times greater than that obtained by the full washing method in 3-year–old plants. However, this difference between the two methods decreased to approximately 3% after 20 years of age. The difference between the two methods lies in the differences in the ratio of fine roots to the total root mass. Addo-Danso et al. (2016) stated that direct root digging by excavation is more precise than other indirect estimation methods as long as lost and broken root issues are resolved. In the present study, the difference in the root volume between the dug-out and pulled-out individuals decreased over time. Therefore, even if the pull-out resistance of the cuttings was evaluated several years after planting, there would be no significant gaps in their shape or developmental process compared to that of the dug-out cuttings.

Conclusion

This study showed that Japanese cedar cuttings were under low environmental stress when planted in December, and there were no significant differences in the survival rate of the cutting varieties. However, the containerized cuttings with a root ball exhibited better growth in terms of aboveground height than those without a root ball (bare-root cuttings), especially under dry conditions. This suggests that Japanese cedar cuttings with a root ball, regardless of the root ball's shape, could escape physiological stress after planting. No significant difference in the underground volume was observed among the cutting varieties. A correlation was observed in terms of aboveground growth among the cutting varieties. However, the coefficient of determination varied, suggesting differences in the detailed root system structure among the cutting varieties. However, the root system is remarkably developed after three growing seasons, and digging out undamaged seedlings is highly laborious and time-consuming. The pulling method was more efficient than the digging method, and it was considered potentially effective for evaluating root system development. In this study, the initial evaluation was conducted for the first three years. It is known that initial differences in the influence of seedling characteristics

diminish between the second and fourth year (Kamo et al., 2005; Pinto et al., 2011). Therefore, it is unlikely that new distinct seedling differences will emerge among these cutting varieties in the future.

Declarations

Author Contribution

Conceptualization:TY, SK, andAWProject administration: SK, AWMeasurement: TY, AWAnalysis and methodology: TYWriting - original draft preparation: TYWriting – review and editing: SK, AW

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Data Availability

Meteorological data used for the analysis: https://www.data.jma.go.jp/gmd/risk/obsdl/index.phpThe datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1 Survial data for each growth season

	Number at the beginning of the season	Number for dig-out test or pull-out test	Dead	Number at the end of the season
After Planting				
bare-root	30	0	0	30
BCC	30	0	1	29
biodegradable	30	0	0	30
JFA 150	30	0	0	30
1st Growth Season				
bare-root	30	7	0	23
BCC	30	7	0	22
biodegradable	30	7	1	22
JFA 150	30	7	0	23
2nd Growth Seasson				
bare-root	23	4	0	19
BCC	22	4	0	19
biodegradable	22	4	0	19
JFA 150	23	4	0	19
3rd Growth Seasson				
bare-root	19	3	0	16
BCC	19	3	0	16
biodegradable	19	3	0	16
JFA 150	19	3	0	16

Figures

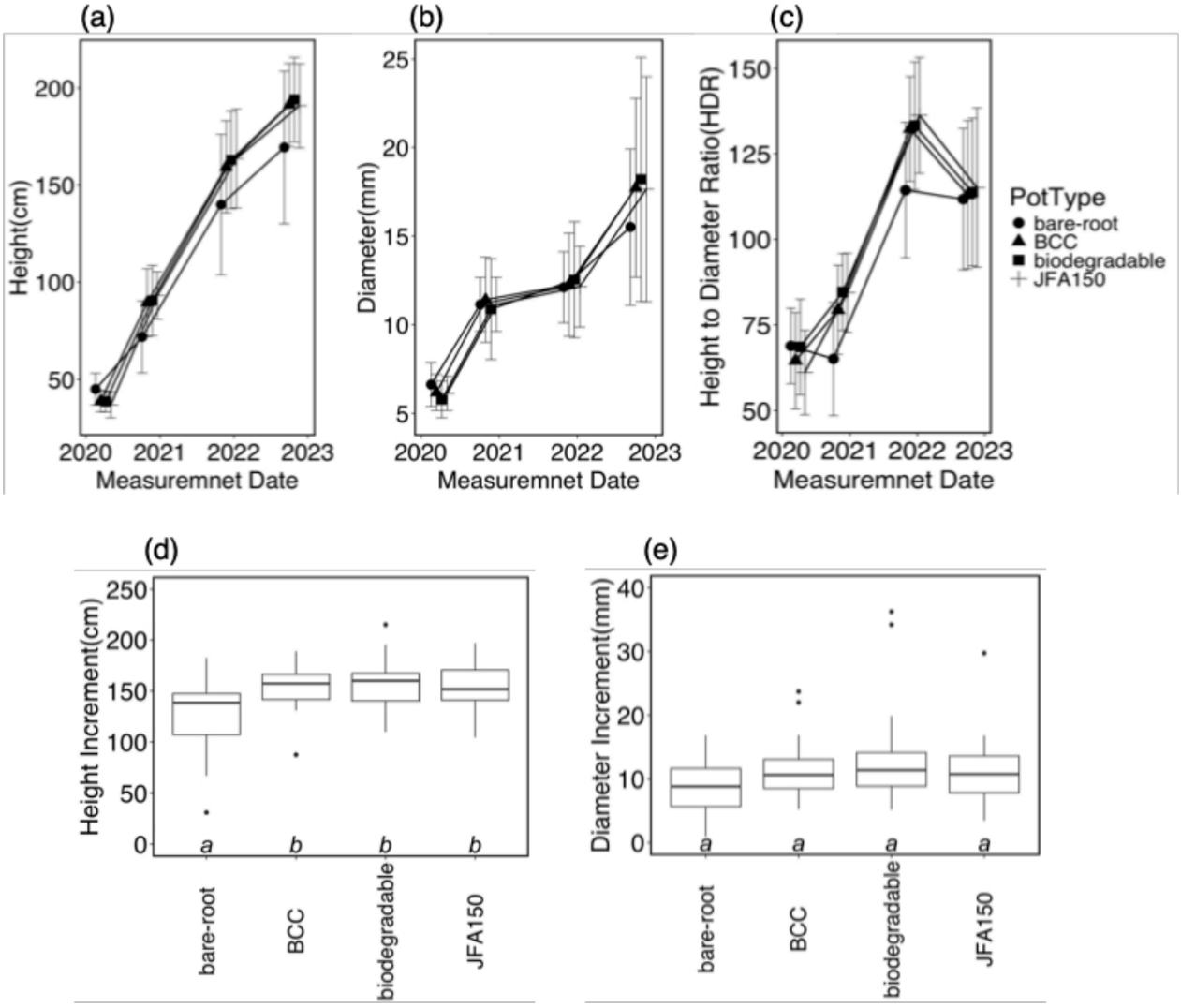


Figure 1

Time course of the growth data among the cutting varieties

(a) Height (b)Diameter (c)Height to Diameter Ratio (d) Height increment between after planting and 3rd growth season (e) Diameter increment between after planting and 3rd growth season.

Error bars mean standard deviation.

Different letters indicate significant differences according to Tukey's test at 5% error probability

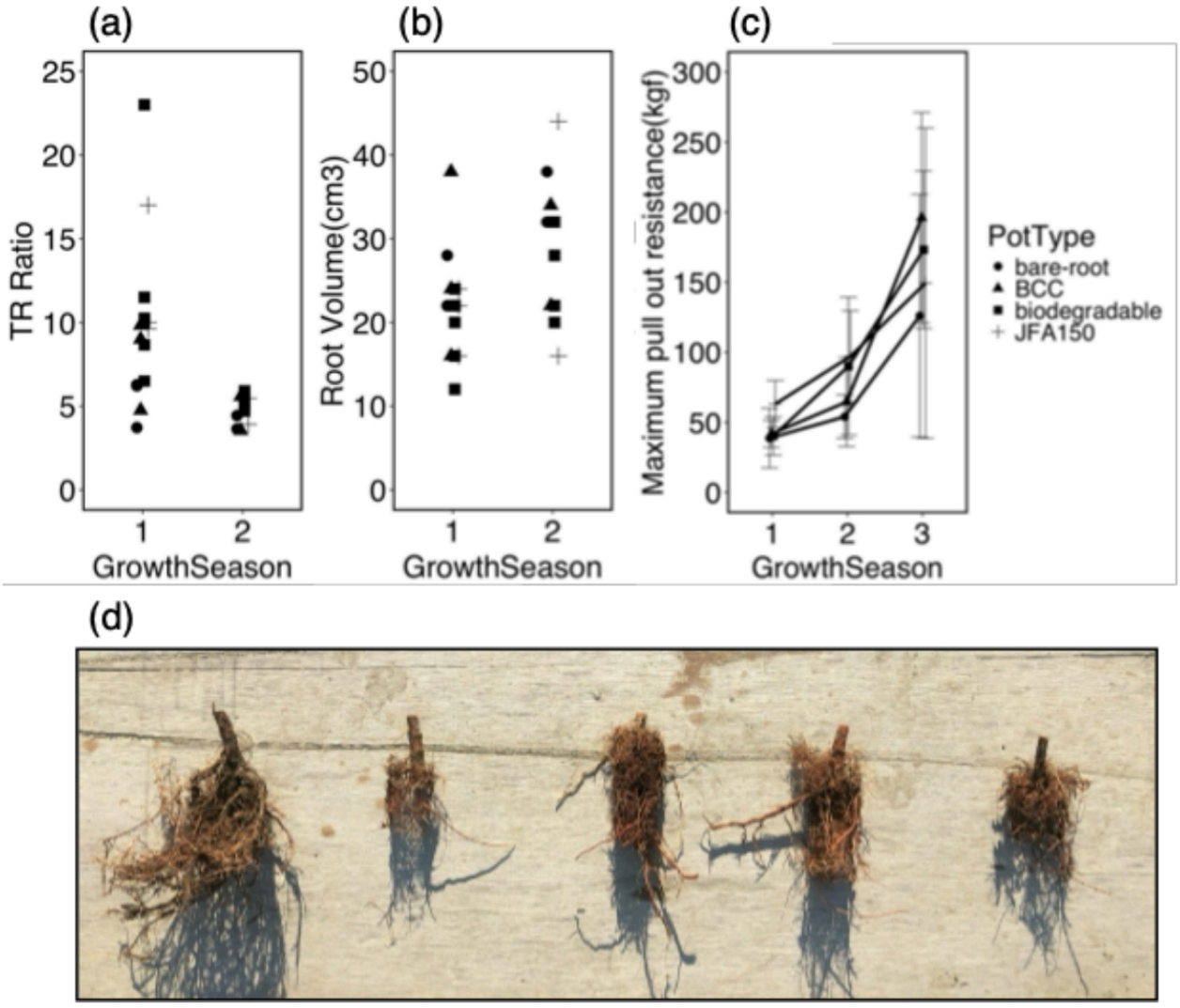


Figure 2

Comparison about root development among the cutting varieties

(a) T(Aboveground dry weight)/R(Belowground dry weight) Ratio (b)Root volume

(b) Time-series variation of maximum pull out resistance (d)Photograph of the roots

after 2nd growth season.

From left to right, “bare-root”,” biodegradable”, ”JFA150”, ”BCC”, ” biodegradable”

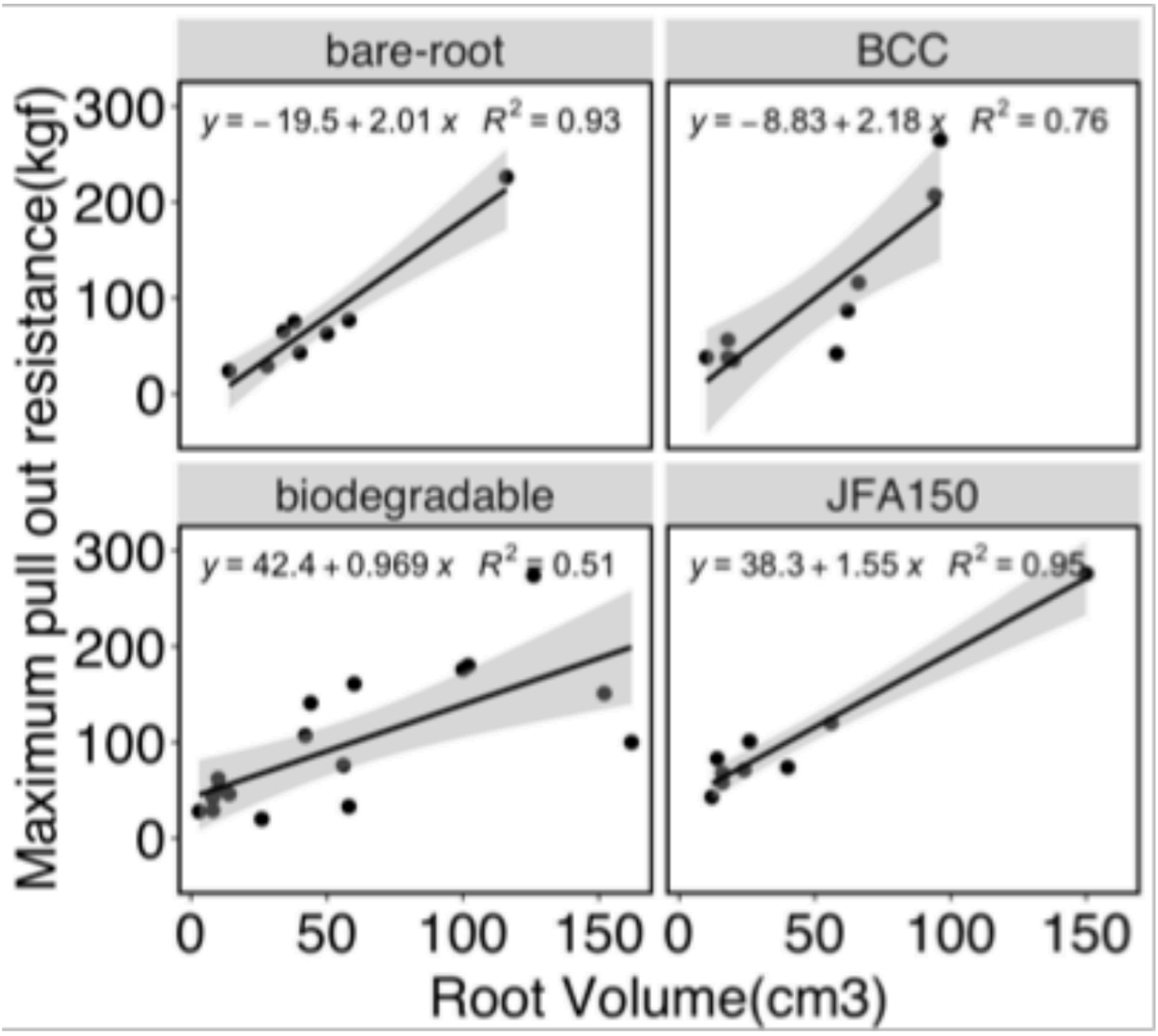


Figure 3

Correlation between Root Volume and maximum pull out resistance among the cutting varieties

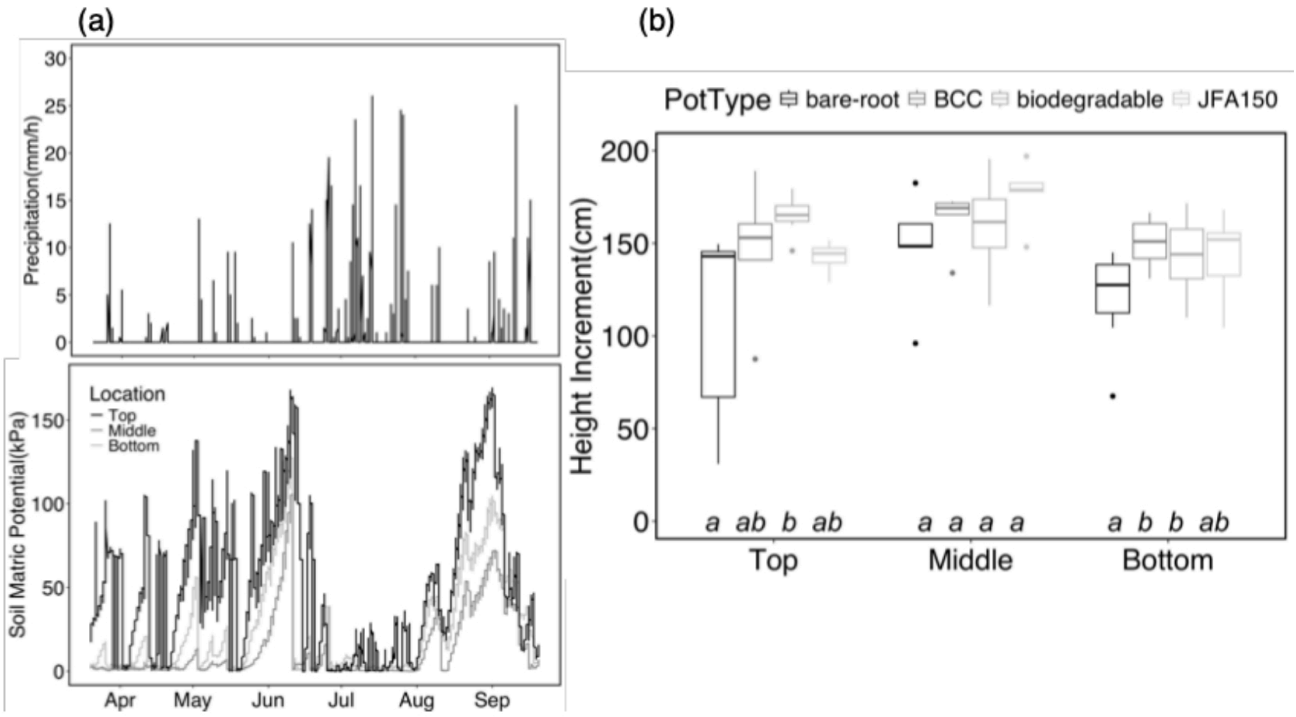


Figure 4

Correlation between soil environment and growth

(a)above: Precipitation data in Hakata, Fukuoka prefecture in 2020

(a)below: Soil moisture data (soil matric potential) in top, middle, and bottom positions in test site slope in 2020.

(b) Data on the growth of 10 individuals grouped by slope site (top, middle, and bottom positions) and compared the amount of growth increment among the cutting varieties

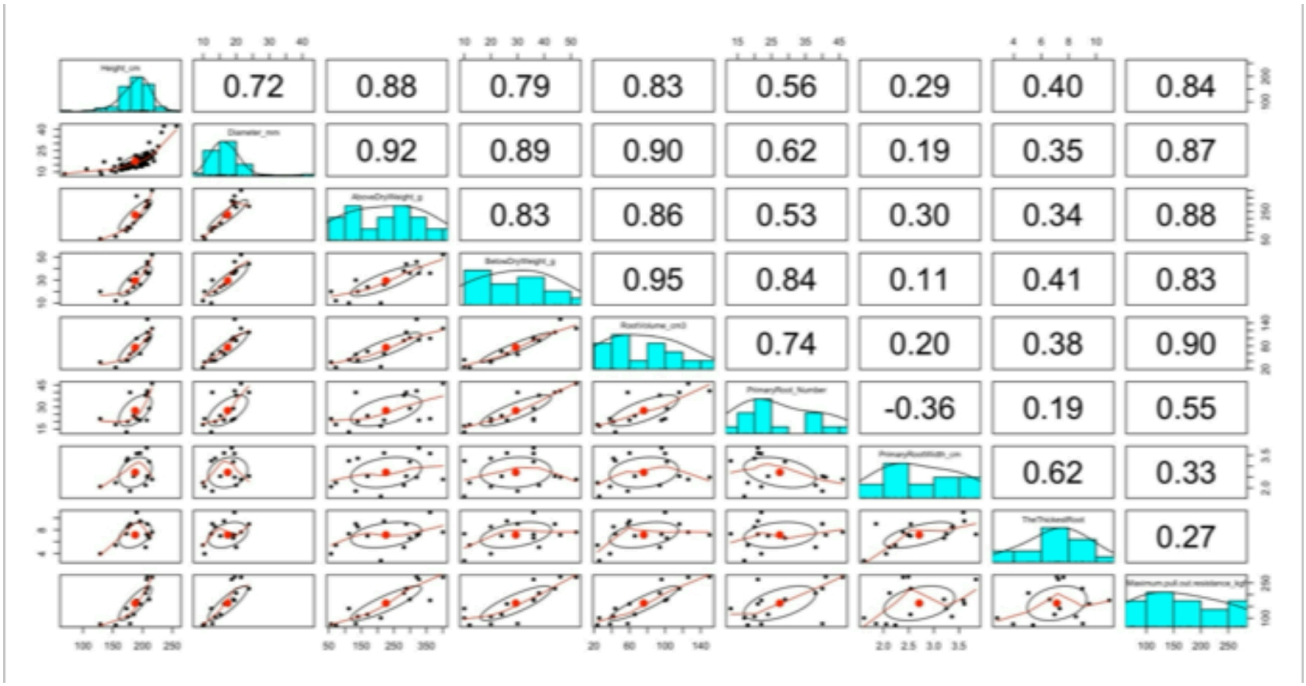


Figure 5

Correlation between the data that is obtained after 3rd growth season in all cutting varieties

From top to bottom, height, diameter, aboveground dry weight, belowgroung dry weight, root volume, number of the primary root, average diameter of primary root, the thickest diameter of the primary root, and maximum pull out resistance. Numbers indicate correlation coefficients between data

Supplementary Files

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