Title:

Rooting of sugi cuttings in closed and semi-closed conditions under mist irrigation

Author information:

Satoshi Ito[1,*], Manabu Kurita[2], Ryoko Hirata[1], Kiwamu Yamagishi[3]

[1] Faculty of Agriculture, University of Miyazaki, Japan

[2] Forest Tree Breeding Center, Forestry and Forest Products Research Institute, Hitachi, Japan

[3] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Japan

Present address: Kyusyu Research Center, Forestry and Forest Products Research Institute, Kumamoto, Japan

* Corresponding author:

Satoshi Ito

Faculty of Agriculture, University of Miyazaki

1-1 Gakuen-kibanadai-nishi, Miyazaki 889-2192, Japan

E-mail: s.ito@cc.miyazaki-u.ac.jp

ORCID: 0000-0003-2198-5729

Abstract

We examined whether 100% atmospheric humidity had a positive or negative effect on rooting rate and root development in sugi shoot cuttings. To assess the effectiveness of producing shoot cuttings using the ‘aerial cutting’ method, we compared the rooting rate, root mass and shoot growth of sugi shoot cuttings reared in a closed environment with nearly 100% atmospheric humidity (vapor pressure deficit (VPD) = 0; closed aerial cuttings (CL)) with individuals grown in an open environment (VPD > 0; open aerial cuttings (OP) and open aquaculture cuttings (AQ)). The results showed that an atmospheric humidity of 100% had a negative effect on rooting. The CL shoot cuttings tended to have a lower rooting rate and smaller root dry mass compared to cuttings grown under open conditions (OP and AQ), suggesting a possibility that the rooting of sugi cuttings requires moderate water stress so that the water-absorbing organs can develop and compensate for water loss due to transpiration. Shoot elongation was highest in CL shoot cuttings with frequent mist irrigation, and the lowest in AQ shoot cuttings with no foliar water uptake (FWU) above the water level. The findings suggest that FWU promoted stem elongation in CL shoot cuttings without roots.

Keyword: aerial cuttings, atmospheric humidity, foliar water uptake (FWU), rooting rate, water stress

Introduction

'Aerial cutting' is newly developed method that is used to promote root development in shoot cuttings supported in the atmosphere and irrigated using mist irrigation without any soil medium (Kurita et al. 2020). It is expected that this method will increase the production efficiency of container-grown cuttings because the development of roots by shoots that are suitable for transplanting into growth containers can be assessed visually, unlike conventional rooting methods in nurseries.

Depending on the irrigation regime and/or atmospheric humidity, shoot cuttings exposed to the atmosphere by aerial cutting have a high risk of severe shoot tissue desiccation. In such cases, the water balance of exposed shoot cuttings is basically determined by the relationship between water uptake via living tissue, such as by foliage through foliar water uptake (FWU) (Burgess and Dawson 2004; Ishii et al. 2014; Barry et al. 2019; Schreel and Steppe 2020; Kagawa 2022), water storage, and water loss through transpiration. In the conventional methods used to rear shoots in soil medium, maintaining the water balance of shoot cuttings is relatively straightforward because the belowground parts (e.g., foliage buried in the soil medium) can take up water from the soil stably without losing water by transpiration. In aerial cuttings, however, all of the foliage is exposed to the atmosphere, and the loss of water by transpiration is high when the vapor pressure deficit (VPD) of atmosphere is high, i.e., when the air is dry. Therefore, in order to minimize water loss and maintain the water balance of the whole shoot, it may be more important to ensure FWU for the aerial cuttings and keep VPD low.

As an irrigation regime for aerial cuttings, Kurita et al. (2020) suggested that frequent irrigation is extremely important for the successful rooting of sugi (*Cryptomeria japonica*) aerial cuttings. Although they suggested that maintaining a high atmospheric humidity could improve the rooting rate of aerial cuttings, an appropriate atmospheric humidity for this new cutting method has not yet been fully investigated. Regarding the effect of atmospheric humidity on the rooting and growth of shoot cuttings, the following two contrasting hypotheses can be proposed: (1) An atmospheric humidity of 100% (i.e., VPD = 0) can theoretically be used to avoid water stress in the

shoot cuttings because the absence of water loss due to transpiration can encourage or compensate vigorous root development without water stress. On the other hand; (2) the absence of transpiration in a 100% atmospheric humidity environment with sufficient FWU can maintain the water balance of whole shoot cuttings, and could promote shoot growth without the need for rooting, i.e., such conditions can result in unsuccessful rooting. The previous study on loblolly pine stem cuttings has suggested that moderate water stress achieve optimum rooting (Lebude et al. 2004). Kagawa (2022) recently reported that a significant part of water uptake in well-watered sugi could be attributed to FWU. In this way, both positive and negative effects of high atmospheric humidity on rooting and root development are expected, but these hypotheses have not yet been fully explored.

We therefore tested the above two hypotheses of the effects of atmospheric humidity on rooting rate and root development in sugi shoot cuttings. We experimentally created a closed environment with nearly 100% atmospheric humidity (VPD = 0) for the aerial cuttings, and compared the rooting rate, root mass and shoot growth of sugi shoot cuttings with aerial cuttings maintained under open (VPD > 0) conditions.

Method

Plant materials

A sugi cultivar “Ken-Kisima-1”, one of the plus trees selected from Saga Prefecture, southwestern Japan, was used as the plant material. Thirty green shoots (35 cm in length) were collected on March 29, 2021 from the clonal garden of the Kyushu Regional Office of the Forest Tree Breeding Center in Koshi, Kumamoto Prefecture (130° 44′ E, 32° 53′ N). After being soaked in a benomyl fungicide (Sumitomo Chemical Co. Ltd., Japan) and rooting promoter containing indole-3-butyric acid (IBA) with acetone and ethylene glycol (OXYBERON SL, Bayer Crop Science Co. Ltd., Japan) for one night, the shoot cuttings were transferred under moist, cool conditions to a laboratory at the University of Miyazaki (ca. 200 km distant from the scion garden) and prepared for the start of the

88 experiment on March 31, 2021.

89

90 ***Experimental design***

91 Ten shoot cuttings were separated into three groups and subjected to the following three treatments:
92 (1) Closed aerial cutting (CL): a steel frame (50 cm width × 31 cm depth × 65 cm height) covered with
93 a transparent plastic bag was prepared, and the shoot cuttings were suspended at the center of the frame
94 using a wire mesh (Fig. 1A). A tray filled with water heated by an electric heater was placed at the
95 bottom of the container to keep the air temperature at 26–28°C and to supply water vapor to the air in
96 the plastic bag. Mist irrigation was applied for 5 minutes at 6-hour intervals by using four mist nozzles
97 (G703, Takagi Ltd., Japan) installed at the top frame to ensure all the shoot cuttings irrigated equally
98 and sufficiently. (2) Open aerial cutting (OP): the same apparatus that was used for the CL treatment
99 was prepared, but a window (5 cm × 25 cm) was opened on the upper surface of the plastic enclosure
100 to allow water vapor escape the plastic enclosure (Fig. 1B). (3) Open aquaculture cutting (AQ): a 60
101 L acrylic aquarium tank was prepared and the shoot cuttings were set such that 10 cm of the shoot
102 base was submerged in the water (Fig. 1C). As in the CL and OP containers, the water in the tank was
103 heated to maintain temperature 26-28 ˚C. All of the treatments were illuminated continuously (24
104 hours per day) by LED light (HPGL1000, Horiuchi Electro-Design Corporation, Japan) at a
105 photosynthetic photon flux density (PPFD) of 400–800 μmol/m^2/s.

106 Rooting from the shoot cuttings was observed every day from the beginning of the
107 experiment (March 31, day 0) until the time of first rooting in all of the treatments (April 19, day 19),
108 and then every 2–7 days thereafter until the end of week 12 (June 23, day 84).

109 Even if rooting was not observed in CL under this setting, it cannot be denied that the shoot
110 cuttings subjected to CL did not originally have rooting ability for some reason. That is, in order to
111 test properly the hypothesis (2) (inhibition of rooting by 100% humidity), it is necessary to confirm
112 whether the shoot cuttings in CL had rooting ability. This confirmation should be done after a certain

period of time, at least when sufficient rooting is observed in OP and AQ. Thus, seven weeks after the beginning of the experiment (May 19, day 49) when all the shoot cuttings in OP and AQ had rooted, a window with the same dimensions as that used in the OP treatment was opened in the CL treatment. From May 15 (day 45) to June 23 (day 84, at the end of the experiment), air temperature (T, °C) and relative humidity (RH, %) were monitored in the CL treatment to calculate VPD by using a thermo-hygrometer (HOBO Pro v2 U23-002A, ONSET, MA, USA). VPD (kPa) was calculated as the following equation:

$$VPD = Es \times (1\text{-}RH/100),$$

where Es was vaper pressure approximated by the following equation:

$$Es = 0.61087 \times 10^{(7.5\times T / (T+237.3))}.$$

The T, RH and VPD in the OP treatment were assumed to be similar to that in the CL treatment after opening window.

On June 23 (i.e., after 12 weeks, day 84), shoot elongation over the 12-week period was measured at the three largest shoot apexes (the main stem and the two dominant primary branches) of each shoot cutting. Shoot elongation was determined by comparing the distance from the branching position to the apexes at the beginning and the end of the experiments. All of the roots were then detached from each shoot cutting, oven-dried for 48 hours at 65°C, and the dry mass of the roots from each shoot cutting was measured. Two of the shoot cuttings from the CL treatment, which had not rooted at the end of week 12, were left under the same experimental conditions until week 15 (day 105) for observation of their rooting ability.

Data analysis

The rooting rate (number of rooted shoot cuttings/ total shoot cutting) was compared between each treatment for each measurement date using Fisher's exact test. Shoot elongation and root mass at the end of the experiment were compared between treatments by the Steel-Dwass multiple comparison

test.

Results

Rooting rate

The first rooting was observed on April 18 (18 days after the beginning of experiment) in two shoot cuttings in the CL treatment, followed by two and three newly rooted shoots in the OP and AQ treatments, respectively, on the next day (April 19, day 19) (Fig. 2). On April 28 (day 28), four weeks after the beginning of the experiment, the number of rooted shoot cuttings was eight each for the OP and AQ treatments. The number of rooted shoot cuttings in the CL treatment increased to five on April 31, but stopped increasing for the two weeks thereafter. On May 10 (day 40), six weeks after the beginning of the experiment, all of the shoot cuttings in the OP and AQ treatments were rooted, and the rooting rate of these treatments (100%) was significantly higher than that of the CL treatment (50%) until May 16 ($p<0.05$). On May 19 (day 49), an additional rooted shoot cutting was observed in the CL treatment.

On the same day (May 19, day 49), seven weeks after the beginning of the experiment, the VPD in the CL treatment increased abruptly from 0 kPa under the closed condition to 0.5–1.0 kPa under the newly opened condition (Fig. 3). The number of rooted shoot cuttings in the CL treatment started to increase again on June 16 (day 77), four weeks after opening the window of the CL treatment; all together, eight shoot cuttings had developed roots in the CL treatment by the end of week 12 after the beginning of the experiment (day 84). The two unrooted shoot cuttings, which were left under the same experimental conditions in the CL treatment, rooted by the end of week 15 (day 105) after the beginning of the experiment (data not shown).

Shoot growth and root mass

Mean shoot elongation over 12 weeks was the highest in the CL treatment, followed by the OP and

AQ treatments, with a significant difference observed between CL and AQ ($p<0.05$) (Fig. 4A). The mean root dry mass at the end of week 12 (day 84) was lower in the CL treatment than in the OP and AQ treatments ($p<0.05$) (Fig. 4B).

Discussion

This study examined the influence of atmospheric humidity on rooting and growth of sugi shoot cuttings. The results showed that an atmospheric humidity of 100% had a negative effect on rooting in sugi shoot cuttings. In the CL treatment (i.e., the closed condition) before opening window on May 19 (day 49), it was assumed that FWU was sufficient if maintained by frequent mist irrigation (Barry et al. 2019; Schreel and Steppe 2020; Kagawa 2022), and that there was no water loss by transpiration under the completely humid conditions (VPD=0) until the window was opened on May 19 (day 49) (Fig. 3). It is considered that these conditions ensured that the shoot cuttings did not experience tissue desiccation. However, under these conditions, there is no requirement for roots as the water-absorbing organ for the shoot cuttings, possibly explaining the low rooting rate (Fig. 2). The small root dry mass (Fig. 4B) observed in the CL treatment could also be explained by closed environment at the first seven weeks (before opening window) though the last five weeks must have had similar condition with the OP treatment. Similar results of low rooting late of sugi shoot cuttings was reported under a well-watered (sprayed) condition (Suzuki 1973).

In contrast, moderate water stress is expected to have occurred in the shoot cuttings in the OP treatment; the escape of water vapor through the window of the plastic enclosure in the OP treatment may have increased the water loss by transpiration, and then increased the need for roots to recover the water balance of the whole shoot, which in turn, may have promoted rooting as suggested in stem cuttings of loblolly pine reported by Lebude et al. (2004). Suzuki (1973) also reported relatively high rooting rate of sugi shoot cuttings in less-watered conditions. Thus, the results suggest a possibility that the rooting of sugi cuttings requires moderate water stress to stimulate the need for a

188 water absorbing organ to compensate for the loss of water through transpiration. This finding was
189 corroborated by the vigorous rooting observed in the AQ treatment (open aquaculture with VPD > 0)
190 (Fig. 2, Fig. 4B), and by the increase in rooted shoot cuttings in the CL treatment after the window
191 was opened (Fig. 2). The increased shoots rooted in the CL treatment under the increased VPD
192 indicated that all the shoot cuttings in CL treatment had rooting ability, and that the rooting might have
193 inhibited under the completely humid conditions (VPD=0) before opening window.

194 Further, shoot elongation was largest in the CL treatment, likely because the shoots
195 maintained FWU (at least during the first seven weeks before opening window), and smallest in the
196 AQ treatment, as there was no foliar water uptake above the water level (Fig. 4A). The large shoot
197 elongation with less or no roots in the shoots of the CL treatment (Fig. 4B) suggests that FWU during
198 the first seven weeks directly supported shoot elongation (Schreel and Steppe 2020; Kagawa 2022).

199 The rooting of the two shoots in the CL treatment, which were unrooted at the end of week
200 12, demonstrated that all of the shoot cuttings in the experiment possessed rooting ability.
201 Consequently, the delayed rooting in the CL treatment can be assumed to have occurred due to rooting
202 being inhibited by the lack of water stress in these cuttings.

203 Water stress in shoot cuttings could stimulate rooting in the following two ways: (1)
204 continuous moderate tissue desiccation is needed to stimulate rooting for a certain period required for
205 differentiation root tissue, or (2) rooting is promoted (even under 100% humidity) if shoot cuttings
206 have once experienced tissue desiccation exceeding a threshold. In the present study, early rooting was
207 observed in weeks 3–5 in the CL treatment. This could be attributed to the accidental desiccation of
208 parts of the shoot cuttings during transport from the clonal garden to the laboratory including the
209 processes of collecting shoots and soaking the shoot cuttings in rooting promoter. This observation
210 may suggest the possibility of the latter (desiccation exceeding threshold can stimulate rooting).
211 However, the detailed mechanism should be examined in the further study.
212

Acknowledgements

We thank Masahiro Kubota and Yuki Fukuda from the Kyushu Regional Office of the Forest Tree Breeding Center for their kind assistance with the present study. This study was supported in part by a Grant-in-Aid from JSPS KAKENHI (Grant No. 16H14945), and grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (Research Program on the Development of Innovative Technology) (30028C).

References

Barry ZC, Emery NC, Gotsh SG, Goldsmith GR (2019) Foliar water uptake: Processes, pathways, and integration into plant water budgets. Plant Cell Environ. 42:410–423.

Burgess SSO, Dawson TE (2004) The contribution of fog to the water relations of Sequoia sempervirens (D. Don): foliar uptake and prevention of dehydration. Plant Cell Environ. 27:1023-1034.

Ishii HR, Azuma W, Kuroda K, Sillett SC (2014) Pushing the limits to tree height: could foliar water storage compensate for hydraulic constraints in *Sequoia sempervirens*? Funct Ecol. 28:1087–1093.

Kagawa A (2022) Foliar water uptake as a source of hydrogen and oxygen in plant biomass. Tree Physiology. tpac055, https://doi.org/10.1093/treephys/tpac055.

Kurita M, Kuramoto N, Kubota M, Fukuyama T, Takeda N, Kurahara Y, Matsunaga K, Otsuka J, Sato S, Watanabe A (2020) Examination of new cuttings method for Sugi (*Cryptomeria japonica*) without using soil: Toward development of production technology for Sugi cuttings using plant factory. J Kyushu For Soc, 73: 57-61. (in Japanese).

Lebude AV, Goldfarb B, Blazich FA, Wise FC and Frampton J (2004) Mist, substrate water potential and cutting water potential influence rooting of stem cuttings of loblolly pine. Tree Physiology.

24:823-831.

Schreel JDM, Steppe K (2020) Foliar water uptake in trees: negligible or necessary? Trends in Plant Science. 25(6), https://doi.org/10.1016/j.tplants.2020.01.003.

Suzuki T (1973) Studies on the method make use of Automatic mist spray installation to raise cultivated cuttings (III) The relations between amounts of water spray and soils in cuttings bed, and rooting of the cuttings from sugi (*Cryptomeria japonica* D. DON) clone in the best conditions. J Jpn For Soc, 55:112-115. (in Japanese).

246 Captions

247

248 Fig. 1. Illustration of the experimental settings of (A) closed aerial cutting (CL); (B) open aerial cutting

249 (OP); and (C) open aquaculture cutting (AQ). See the text for details of the experimental settings.

250

251 Fig. 2. Number of rooted shoot cuttings from each of the three treatments over the 12 weeks of the

252 study. CL: closed aerial cutting, OP: open aerial cutting, and AQ: open aquaculture cutting. Asterisks

253 indicate significantly higher rooting rate in OP and AQ than in CL (Fisher's exact test, $p<0.05$). The

254 arrow in the graph indicates the date when the window was opened in the CL treatment (May 19, day

255 49).

256

257 Fig. 3. Change in the vapor pressure deficit (VPD) before and after opening the window in the CL

258 treatment. The arrow in the graph indicates the date when the window was opened (May 19, day 49).

259 The VPD was zero for the duration of the closed condition, then increased with marked fluctuations

260 in response to weather conditions after opening window.

261

262 Fig. 4. Comparisons of shoot elongation over 12 weeks (A) and root dry mass at the end of the week

263 12 (B). CL: closed aerial cutting, OP: open aerial cutting, and AQ: open aquaculture cutting. Different

264 letters indicate significant differences between the treatments (Steel-Dwass test, $p<0.05$).

265

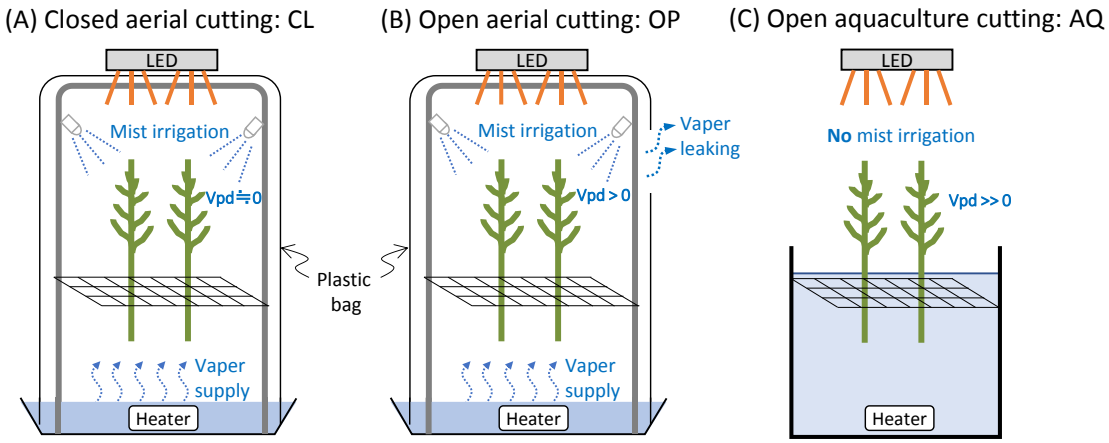
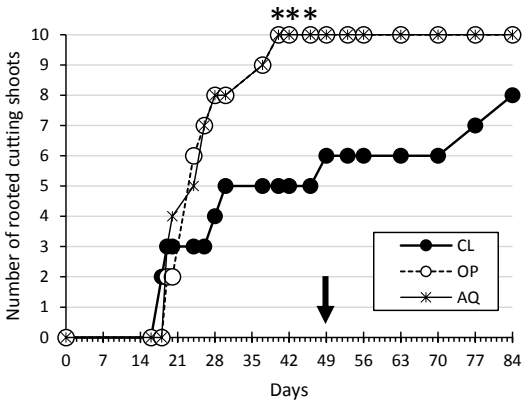


Fig. 1. Illustration of the experimental settings of (A) closed aerial cutting (CL); (B) open aerial cutting (OP); and (C) open aquaculture cutting (AQ). See the text for details of the experimental settings.

272



273

274

275 Fig. 2. Number of rooted shoot cuttings from each of the three treatments over the 12 weeks of the
276 study. CL: closed aerial cutting, OP: open aerial cutting, and AQ: open aquaculture cutting. Asterisks
277 indicate significantly higher rooting rate in OP and AQ than in CL (Fisher's exact test, $p<0.05$). The
278 arrow in the graph indicates the date when the window was opened in the CL treatment (May 19, day
279 49).

280

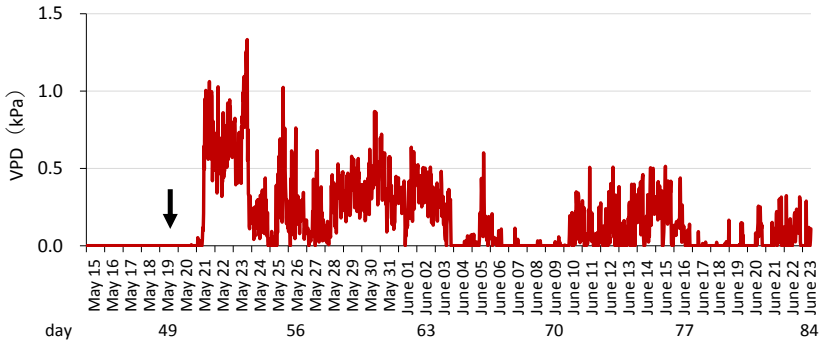
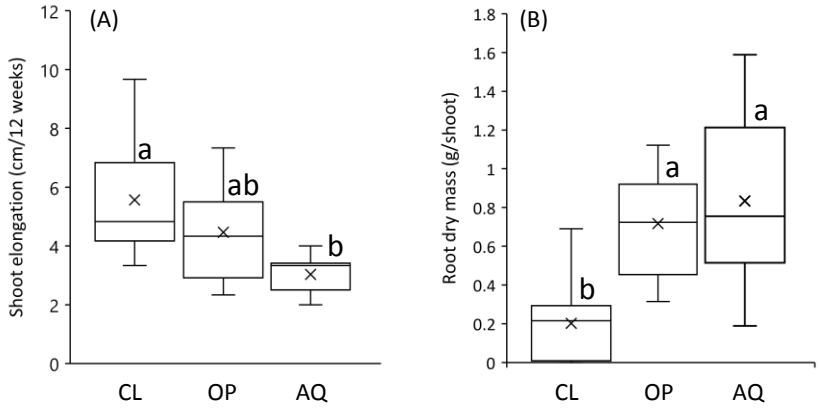


Fig. 3. Change in the vapor pressure deficit (VPD) before and after opening the window in the CL treatment. The arrow in the graph indicates the date when the window was opened (May 19, day 49). The VPD was zero for the duration of the closed condition, then increased with marked fluctuations in response to weather conditions after opening window.

288



289

290

291 Fig. 4. Comparisons of shoot elongation over 12 weeks (A) and root dry mass at the end of the week

292 12 (B). CL: closed aerial cutting, OP: open aerial cutting, and AQ: open aquaculture cutting. Different

293 letters indicate significant differences between the treatments (Steel-Dwass test, $p<0.05$).