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Light-emitting Diode–Induced Root Photomorphogenesis and Root Retention in *Populus*

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Abstract. **Light-emitting diodes (LEDs) are known to affect plant morphology. In this study, we examined the relationship between changes in stem and root morphology in *Populus sieboldii* × *Populus grandidentata* induced by irradiation with blue (450 nm), red (630 nm), and white (combination of red, blue, and green; 525 nm) LED lights. *Populus* samples were reared for 36–55 days in separate LED incubators, and changes in their appearance were observed. After rearing, the main stem of each seedling was cut, leaving a section of stem extending from the roots to ≈20 mm above the medium surface; this part was used for tensile testing. The tensile tests were performed to clarify the relationship between the tensile force and displacement until 100 mm. Irradiation with blue light produced the tallest seedlings. The highest dry weight (root and stem) and largest stem diameter were obtained under red light. The results of the tensile tests showed that the work required to displace seedlings 100 mm was highest in plants reared under red light, followed by white and blue light. Numerous root branches developed under red light, and taproots were longest in saplings reared under blue light. The observed differences in root system morphology that were induced by rearing under light of different wavelengths were reflected in the tensile force required to extract the trees from the medium. The morphological changes observed in roots are important given the role of roots in forests after landslides, earthquakes, and other disruptions.**

Since morphological changes in plants irradiated with light of different wavelengths were first reported (Olle and Viršile, 2013), numerous studies have investigated plant growth under different wavelengths of light (Hoenecke et al., 1992; H.H. Kim et al., 2004). For example, studies have been conducted to compare the growth of cucumbers (*Cucumis sativus* L.) reared under red or blue light-emitting diodes (LEDs). The findings showed that, compared with irradiation under white light, root elongation and growth were promoted under blue light and leaf thickening was promoted under red light (Hogewoning et al., 2010; Parkash et al., 2021). In previous studies on the effect of light wavelength on plant growth, combinations of red and blue light were used to compare the growth rates of plants (Brown et al., 1995; Hogewoning et al., 2010). Most of these studies examined plant photomorphogenesis after exposure to different wavelengths of light and how it could be applied to shorten the cultivation period of vegetables or to produce alternate generations (Ataka et al., 2014; Johkan et al., 2010; Nemhauser and Chory, 2002). However, relatively few studies have been conducted on photomorphogenesis in trees, likely because of the long generation times required.

Root growth and distribution have also been shown to be important for increasing crop production. For example, the root length and distribution under different soil moisture conditions were examined in grafted watermelon (Miller et al., 2013). Studying root growth in trees is also important, and we investigated the effect of light wavelength on the morphogenesis of tree seedlings, especially on root morphology. *Populus sieboldii* × *Populus grandidentata* was cultivated under LED lights of different wavelengths. *Populus* spp. were used as the model tree species because they grow quickly and because it is relatively easy to observe the changes in morphology.

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Materials and Methods

Plant materials and culture conditions. *Populus sieboldii* × *Populus grandidentata* was used to investigate root morphogenesis in this study. Figure 1 shows a photograph of plant specimens in an LED incubator. All of the samples were cultured under aseptic conditions in each experiment. Murashige–Skoog medium (Murashige and Skoog, 1962) was used as the growth medium, and the plant hormone indole-3-butyric acid, an auxin, was used at a final concentration of 0.05 mol·L^{-1}. Apical buds were planted in the Murashige–Skoog medium and incubated at 23 °C under white LED light until rooting occurred, and then the samples were placed in the experimental incubator (Plant Incubator V08; MRT Corp., Osaka, Japan). To synchronize the timing of rooting, we prepared several samples and used samples that sprouted roots on the same day. The day of sprouting was set as day 1.

After rooting, three seedlings were placed in each of the differently colored LED incubators in one test set, and three sets were tested. Due to the difference in growth rates, the culture period was 55 d for set I, 50 d for set II, and 36 d for set III. The seedlings were reared until their buds reached the top of the pot (≈25 cm). The plastic pots were 14 cm tall with a capacity of 1000 mL. The volume of culture medium in each pot was ≈500 mL. Because the amount of medium was more than the amount typically used for culturing (≈100 mL), the root morphology could be observed easily. One pot and one cover were usually used for culturing the seedlings. However, in these measurements, two pots were stacked to get enough headspace, which meant that a cover was not required (Fig. 1)

Tree growth measurements. After rearing, the main stem of each seedling was cut, leaving ≈20 mm of the stem extending above the surface of the medium; this part of the stem was used as the attachment site for the tensile testing device. This cut stem was used to measure the stem diameter, and the seedling height was measured from the point where the stem was cut to the tips of the buds, excluding leaves. After each measurement, seedlings were completely dried (48 h at 105 °C) (drying oven AT-S13; Isuzu Seisakusho Co., Niigata, Japan), and the dry weight of the roots and stems were recorded. The dry stem weight was measured without the leaves.

For the statistical analysis, Tukey's test was used to perform multiple comparisons among LED treatments from significant one-way ANOVA ($P < 0.05$) using Microsoft Excel 2019 (Hogewoning et al., 2010).

LED characteristics. The photosynthetic photon flux density density was used to measure the light intensity inside the incubator. The photosynthetic photon flux density value at the top of the incubator was ≈120 μmol·m^{-2}·s^{-1} in this incubator. The inner dimensions of the incubator measured 27 cm wide, 33 cm deep, and 35 cm high. Room temperature was 23 °C. The irradiance was measured at the center of incubator floor using a light meter (Light analyzer LA-105; Nippon Medical and Chemical Instruments Co., Osaka, Japan). The characteristics of each LED are summarized in Table 1. Figure 2 shows the relationship between the light irradiance and the wavelength of the three LEDs. The wavelength of the blue light peaked at 450 nm; the red light peaked at 630 nm; and the white light, which was a combination of red, blue, and green, peaked at 525 nm.

Tensile test measurements. Figure 3A shows the tensile test setup used for the

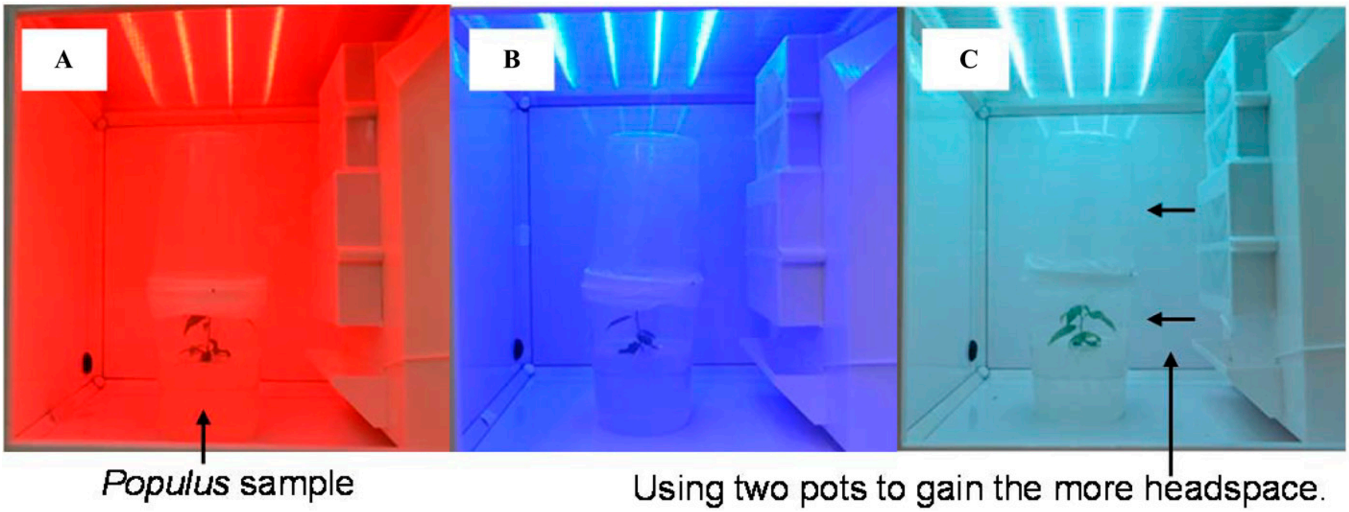

Fig. 1. Photographs of representative experimental plants inside the light-emitting diode (LED) incubator; (**A**) red LED, (**B**) blue LED, (**C**) white LED.

Table 1. Optical characteristics of light-emitting diodes measured at the bottom of the incubator.

		Light intensity (μmol·m^{-2}·s^{-1})		
Photon index	Wavelength (nm)	Red	Blue	White
PPFD	400–700	23	23	23
Ultraviolet	380–400	0.024	0.026	0.026
Blue	400–500	0.30	23	7.3
Green	500–600	0.52	0.34	8.5
Red	600–700	22	0.15	7.3
Far-red	700–780	0.29	0.15	0.15

PPFD = photosynthetic photon flux density.

universal testing machine (AG-X plus 50 kN; Shimadzu, Tokyo, Japan). In this study, the tensile force and displacement for the roots in the growth medium were measured. The tensile speed was 10 mm·min^{-1}. Measurements were ended once the main stem of the seedling had been extracted to a distance of 100 mm from the medium. Figure 3B shows the relationship between the displacement and tensile force. The maximum tensile force and tensile work were calculated and are compared for each color treatment in Fig. 3B (Takahashi et al., 2021). The tensile work is the area indicated in gray in the figure. The maximum tensile force was taken as the strength of the taproot, which corresponds to the root pull-out resistance. The tensile work was thus used as a proxy for the difficulty of root removal and mainly represented the resistance conferred by the lateral roots. The maximum tensile strength was used to evaluate the contribution of the tree roots to preventing landslides. However, measurement of tensile work in the field is typically not possible because of difficulties conducting measurements in a natural environment (Abe and Ziemer, 2013). Therefore, the tensile work is considered to be well suited for evaluating the resistance conferred by the lateral roots of root systems.

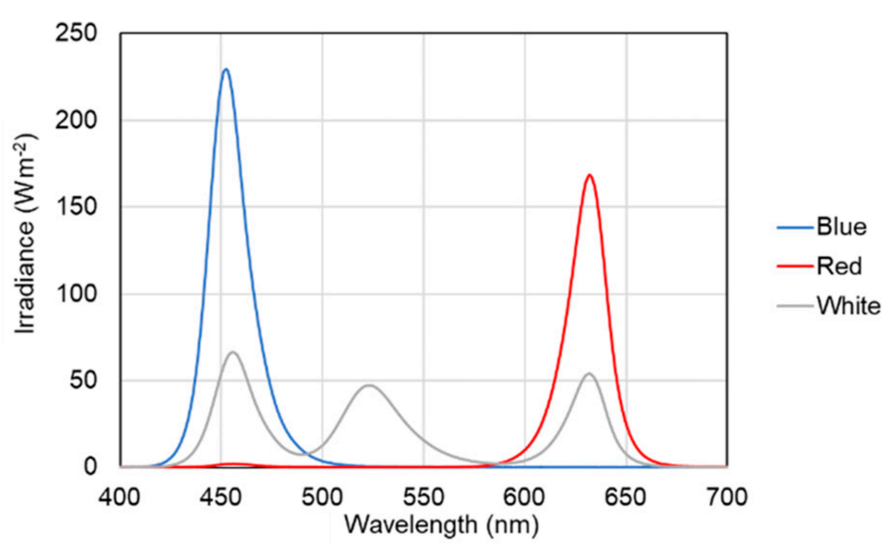

Fig. 2. Relationship between irradiance and wavelength for different light-emitting diodes.

Results and Discussion

Differences in seedling growth and morphology. Figure 4 shows the difference in seedling growth after cultivation under each LED. Saplings reared under blue light grew taller than those reared under red and white lights. The leaves and stems on the main stem of the seedlings grown under blue light were vivid green, and the stems were thin and long. Compared with the blue light treatment, the seedlings grown under red light had yellowish-green leaves and stems, and some of the leaves were yellow-colored and exhibited withering, with the tips of the leaves turning black. These changes were observed mainly from day 29 to day 36 after rooting. S.J. Kim et al. (2004) reported that the chlorophyll content of chrysanthemums cultured under a red LED was lower than that of chrysanthemums cultured under a blue LED. The yellow color might have been due to etiolation caused by a relatively high amount of carotenoids and less chlorophyll. The seedlings grown under white light had the same vivid green stems as the seedlings cultured under blue light.

Differences in root appearance could be observed for different light treatments from day 8 after rooting. As in the white LED treatment, the roots of seedlings irradiated with red light tended to extend in the horizontal direction, and numerous finely branched roots could be observed. Conversely, the roots of seedlings irradiated with blue light tended to extend in the vertical direction. A similar tendency in root morphology was observed in Japanese cedar [*Cryptomeria japonica* (L. f.) D. Don] saplings grown in

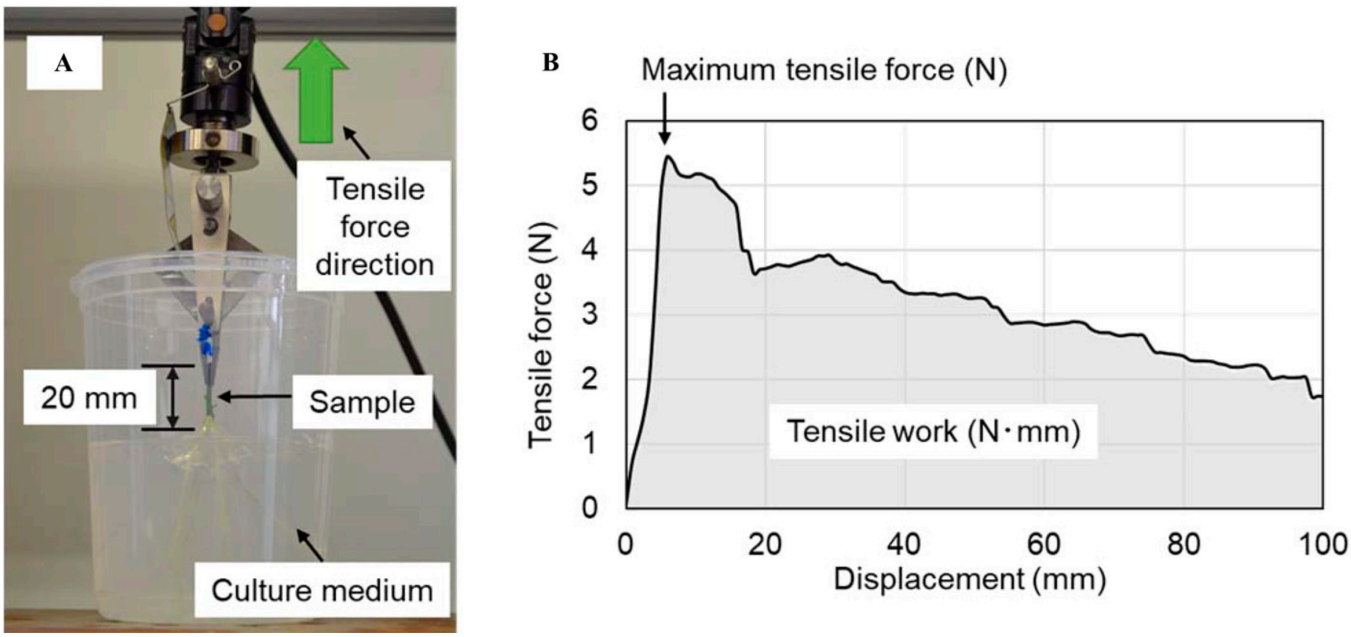

Fig. 3. (**A**) Photograph of sample tensile test and (**B**) relationship between the displacement and tensile force.

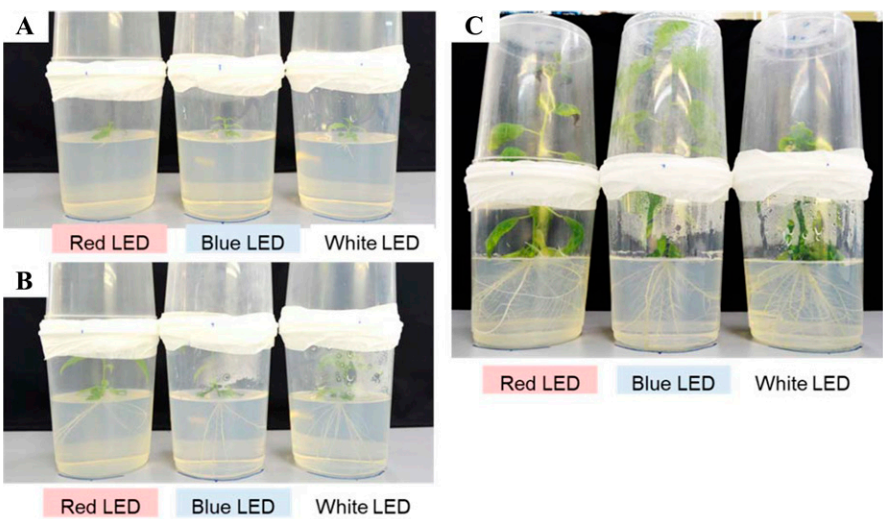

Fig. 4. Photograph of *Populus* specimens after rearing for (**A**) 1 d, (**B**) 15 d, and (**C**) 36 d in set III. LED = light-emitting diode.

plant soil under LEDs with different colors (white, red, and blue) for 52 weeks (Kobayashi and Kurata, 2022). It is also possible that plant hormones, such as the auxins that are biosynthesized at the apical meristems, affect root morphology.

Statistical analysis. Table 2 shows a summary of different parameters for each LED lighting condition. Different letters indicate significant differences ($P < 0.05$). Significant differences in tree height and tensile work parameter were observed by Tukey's test. In addition, a difference in the morphological appearance of stems and roots was observed for different lighting conditions; however, the LED color did not affect parameters such as the stem diameter, dry weight, and maximum tensile force.

Plants irradiated with a blue LED were the tallest. Dougher and Bugbee (2001) studied the differences in the response of wheat (*Triticum aestivum* L.), soybean [*Glycine max* (L.) Merr.], and lettuce (*Lactuca sativa* L.) to blue light. Their findings showed species-specific differences in dry mass, stem length, and leaf area. For such parameters, wheat was not changed, and lettuce was highly sensitive to blue light. In this study, blue light had a clear effect on stem elongation in *Populus* apps.

Tensile tests of seedlings grown under different light treatments. Figure 5 shows the relationship between the displacement and tensile force for set I (Fig. 5A), set II (Fig. 5B), and set III (Fig. 5C). The maximum tensile force in sets I and II was recorded for plants grown under red light. The tensile work for plants grown under red light was the highest among all sets. The plants grown under red light had a high tensile force even when the displacement was high. Figure 6A shows the root morphology under each light treatment in set III at 36 d, and Fig. 6B shows the relationship between the root pattern and the growth medium during tensile testing. It is considered that the root's ability to retain the culture medium varied depending on the morphology of the root system. The roots of the seedlings grown under red light were

Table 2. Summary of average value and significant differences among parameters estimated using Tukey's test ($P < 0.05$). Different letters indicate significant differences. The standard deviation is given in parentheses.

	Light color		
Factor	Red	Blue	White
Root dry weight (g)	0.11 a (0.02)	0.07 a (0.04)	0.10 a (0.05)
Stem dry weight (g)	0.16 a (0.05)	0.09 a (0.03)	0.10 a (0.01)
Stem diameter before drying (mm)	2.41 a (0.46)	1.88 a (0.20)	2.07 a (0.25)
Tree height before drying (cm)	20.67 a (0.59)	21.87 a (2.28)	14.90 b (1.65)
Maximum tensile force (N)	4.63 a (1.16)	3.23 a (0.59)	3.60 a (0.35)
Tensile work (N·mm^{-1})	260.15 a (83.10)	85.79 b (22.77)	143.58 ab (33.66)

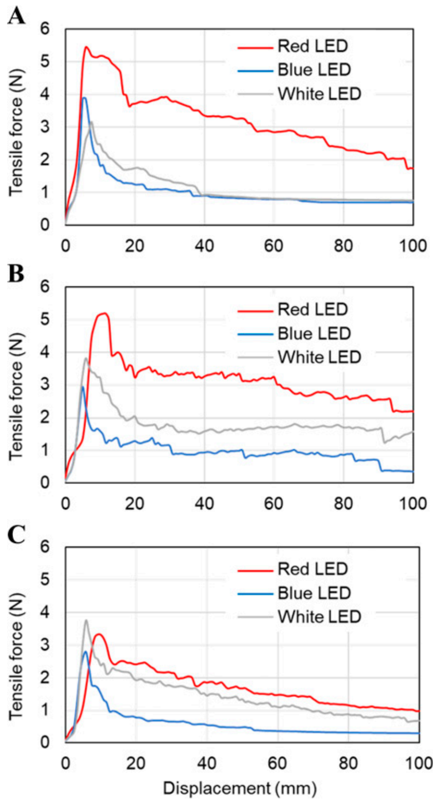

Fig. 5. Relationship between the displacement and tensile force in (**A**) set I, (**B**) set II, and (**C**) set III. LED = light-emitting diode.

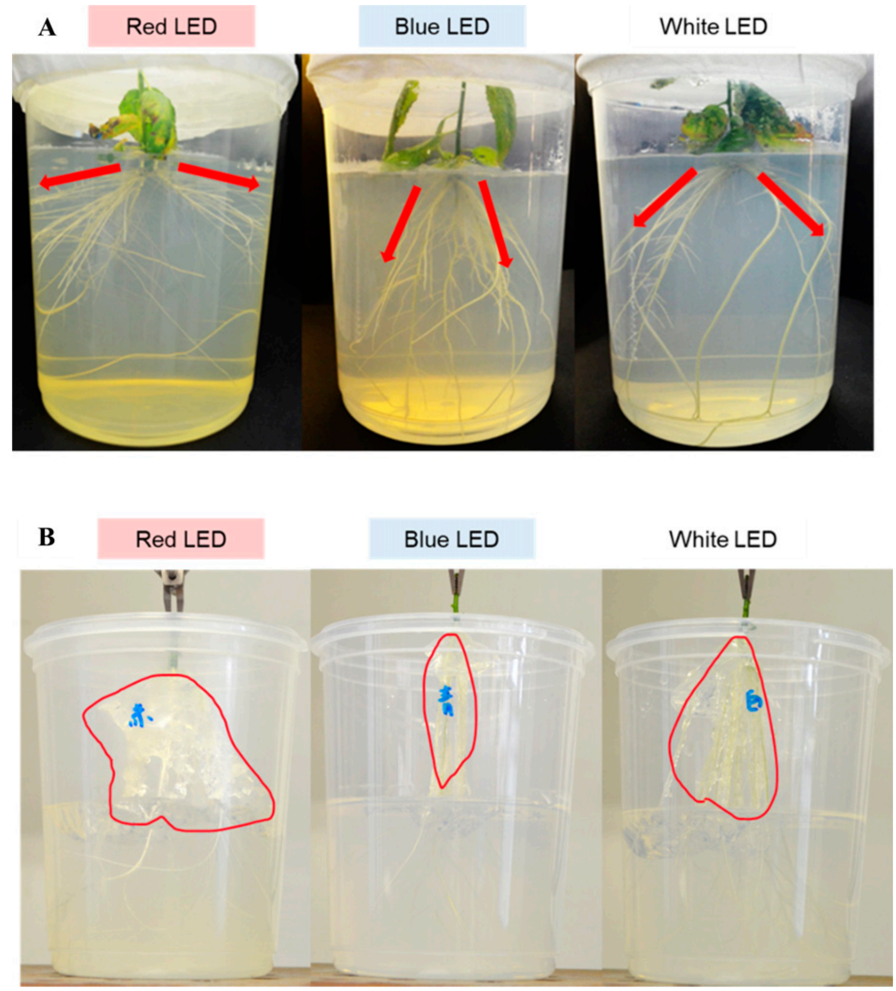

Fig. 6. Difference in root system morphology in *Populus sieboldii* × *Populus grandidentata* reared under different light-emitting diode (LED) wavelengths in set III at 36 d: (**A**) root growth pattern, (**B**) under tensile test. Red arrows in (**A**) indicate the root growth direction tendency. Red polygons in (**B**) indicate the root and culture medium complex.

more incorporated into the medium than those of the seedlings grown under blue light (see red circle in Fig. 6B). The root–medium complex for plants grown under a red LED might have contributed to the high tensile test values.

Conclusions

The effect of LED lighting on the morphogenesis of *Populus sieboldii* × *Populus grandidentata* was examined in this study. For seedlings grown under blue light, the growth rate was the highest of the three light wavelengths tested. Under red light, the main stem diameter and the dry weight of aboveground biomass were the largest. The roots of seedlings grown under red light tended to extend in the horizontal direction, and numerous branched roots could be observed. Under blue light, the roots tended to extend in the vertical direction. The tensile work required to displace the plants grown under red light was the highest. This suggests that the morphology of stems and roots of the same *Populus sieboldii* × *Populus grandidentata* differ depending on the light wavelength, which might affect the retention of the root medium. The moderate root growth observed under white light might be better suited to producing seedlings.

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