

## Growth of Tsuru-rindo (*Tripterospermum japonicum*) Cultured *in Vitro* under Various Sources of Light-Emitting Diode (LED) Irradiation

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**We studied the effects of light generated by LEDs on the growth of Tsuru-rindo (*Tripterospermum japonicum*) shoots. Apical shoots (2-3 cm long) were cultured on MS basal media supplemented with 3% sucrose, and were maintained for four weeks under five different light qualities: F (fluorescent lamp), red LED (R), 70% red + 30% blue LED (R7B3), 50% red + 50% blue (R5B5), or blue LED (B). Rooting was promoted by red light (100%) but was inhibited by blue light. Plant growth, as defined by root number, fresh weight, and chlorophyll content, was generally healthier for cultures irradiated with mixed LEDs, and was the best under R7B3. Ventilation resulted in more rapid apical shoot growth and rooting compared with control plants, when both were treated with the R7B3 system. We demonstrated here that plant growth can be controlled by using LEDs to adjust for the most effective irradiation conditions, compared with the performance observed when conventional fluorescent lamps are utilized.**

*Keywords:* *in vitro*, LEDs, root growth, shoot growth, Tsuru-rindo

To increase the efficiency of *in vitro* techniques, culturing conditions, such as temperature, humidity, illumination, and ventilation, must be optimal. Cool-white fluorescent lamps are the most frequently used (90%) for plant tissue culture. However, the cost of their operation accounts for 65% of a total electricity bill, making them one of the highest non-labor expenses in a tissue culture laboratory (Dooley, 1991). The use of LEDs (light emitting diodes) as radiation sources has become considerably more attractive because of their vast potential for commercial application. One important advantage is their peak spectral output, which coincides closely with the red absorption peak of chlorophyll and the reported wavelengths for maximum photosynthetic efficiency (McCree, 1972). Other unique characteristics also demonstrate that LEDs are a promising electric light source for space-based plant growth chambers and bioregenerative advanced life support because of their small mass and volume, solid-state construction, safety, and longevity (Bula et al., 1991; Nhut et al., 2003).

*Tripterospermum japonicum* (Tsuru-rindo) is a rare and endangered species distributed in Ulnung and

Jeju Island, Korea. To facilitate its conservation, micro-propagation techniques could be applied, as is done with other such species (Mccartan and Staden, 2003; Liao et al., 2004; Malabadi et al., 2004), including those in the Gentianaceae family (Cho et al., 1992; Seong et al., 1993, 1995; Bang et al., 1994; Kim et al., 1999; Lim et al., 2000). Here, we investigated the *in vitro* development of Tsuru-rindo plantlets cultured under different LEDs, and compared their growth with those reared more conventionally, under cool-white fluorescent lamps.

### MATERIALS AND METHODS

Plantlets of Tsuru-rindo (*T. japonicum*) were derived from shoot tip cultures, and were then maintained at the Tissue Culture Laboratory of the Korea Forest Research Institute (KFRI) on MS (Murashige and Skoog, 1962) solid medium supplemented with 3% sucrose and 0.3% gelrite. Apical shoots (approx. 2- to 3-cm-long) with two to three attached leaves were excised from these stock plants and used as explants in all treatments. Seven explants each (in three replicates) were cultured on individual 4×10 cm Petri dishes that contained 80 mL of an MS medium supplemented with 3% sucrose and 0.3% gelrite.

Our light treatments included four types of LEDs—

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100% red (R), 100% blue (B), a mix of 70% red and 30% blue (R7B3), or a mix of 50% red and 50% blue (R5B5)—supplied from a GF-320 LED system (Good Feeling, Korea). Cool-white fluorescent lamps (F) served as the control. As spectral energy sources, the blue LED had a peak emission at 440 nm and red LED, at 650 nm. All cultures were maintained for four weeks in a growth chamber at  $24 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  relative humidity. The PPF (photosynthetic photon flux) was adjusted to  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  (measured at the bottom of the culture vessels), using a 16-h photoperiod.

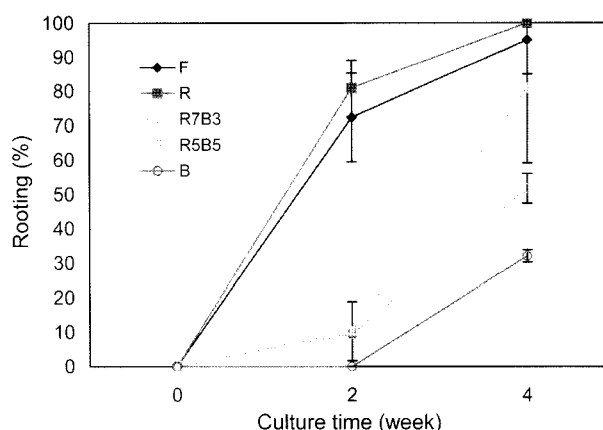
Rooting capacity was investigated after two and four weeks of culturing. Other parameters, i.e., node length, plant height, fresh weight, and chlorophyll content, were recorded after four weeks. Total leaf chlorophyll was measured by the method of Wellburn (1994). To examine the effect of air exchange, we attached  $0.5 \mu\text{m}$  gas-permeable microporous filters (Mill-Seal; Millipore, Japan) to the tops of the culture vessels under R7B3 LED lighting. For this comparison, air-tight vessels wrapped with Parafilm® were used under the same lighting. To observe the stomata, leaf abaxial side replicas were made via peeling, and stomatal lengths were determined by microscopic examination of five leaf-surface imprints with a light microscope (LM).

To study light-quality influences on acclimatization in soil, 80 plantlets each from ventilated and non-ventilated vessels under the R7B3 LEDs were transferred to a 1:1:1 (by volume) mixture of peat, vermiculite, and perlite. They were then placed in a controlled environment chamber at  $24 \pm 1^\circ\text{C}$  under fluorescent lamps ( $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Survival rates were recorded eight weeks after this transplantation.

Data were statistically analyzed using the SAS program (SAS Institute, USA); mean values were determined at the  $p \leq 0.05$  level, according to a Duncan's multiple range test.

## RESULTS AND DISCUSSION

Root growth responses were observed early on, with cultured explants (2- to 3-cm-long apical shoots) showing faster rates within two weeks under both R (80%) and F (70%) compared with other lighting conditions (Fig. 1). Those of plantlets under either B or mixed LEDs were more delayed, especially for the former, where plantlets had a rooting rate of only 32% after four weeks of culture. Although no significant differences in rooting were found between F and



**Figure 1.** *In vitro* rooting under different light qualities. Cultures were maintained on MS medium with 3% sucrose and 0.3% gelrite for 4 weeks.

R plantlets at week 4, their performances were significantly different from those grown under other lighting conditions. In contrast, more roots per explant (up to two-fold) were induced under F and mixed LEDs than under R and B LEDs (data not shown).

Light quality can profoundly affect rooting, sometimes as a function of plant species. For example, blue light (BL) can inhibit root development from *in vitro*-cultivated birch shoots (Pinker et al., 1989), as well as from *Prunus serotina* cultures (Fuernkranz et al., 1990). In contrast, the positive effect of BL, compared with red light (RL), in *Vitis* plants is manifested through a higher percentage of rooted shoots and longer roots (Chee, 1986). Nevertheless, on the same *Vitis* genotypes, when long-day applications of RL are used, root numbers double and root lengths increase five-fold, compared with results seen from BL-treated plants (Chee and Pool, 1989). Finally, the effectiveness of RL on rooting from shoots of *Prunus* GF 655/2 and plum Mr.S. 2/5 depends on genotype, the use and concentration of rooting substances, and light quality (Rossi et al., 1993; Casano, 1995). Therefore, light quality is critical to the goal of promoting faster rooting under a shorter culture period, thereby increasing efficiency while reducing cost.

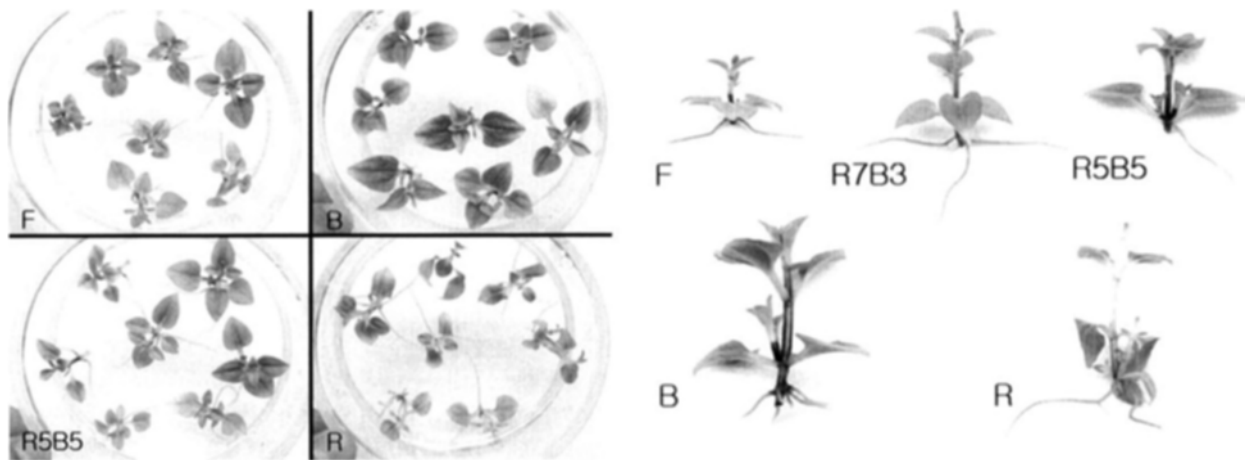
Light quality also influenced our plant morphology after four weeks of culture (Table 1; Fig. 2). For example, the longest nodes were observed from R-treated plants; the shortest, from the B treatment. Although plants were tallest under red light, their growth was fragile due to excessive internodal elongation that resulted from extending the culture period. Red light can significantly affect stem elongation, depending upon the species examined. Kim et al. (2004) have suggested that this elongation can be promoted or

**Table 1.** Effect of light quality on growth of Tsuru-rindo plantlets.

Light <sup>γ</sup>	Plant height (cm)	Internodal length (mm)	No. of roots	Fresh weight (mg)	Chlorophyll content (ug g <sup>-1</sup> FW)
F	3.3 b <sup>z</sup>	3.3 bc	3.8 ab	154.3 b	20.3 ab
R	4.6 a	6.8 a	2.8 bc	157.3 b	12.5 c
R7B3	3.5 b	3.0 bc	4.7 a	181.0 a	19.0 b
R5B5	3.4 b	4.2 b	4.2 ab	168.7 a	21.7 a
B	2.5 c	2.2 c	2.3 c	115.0 c	14.2 c

<sup>γ</sup>, F-fluorescent lamp; R7B3-70% red + 30% blue; R5B5-50% red + 50% blue; B-100% blue; R-100% red.

<sup>z</sup>, Values followed by the same letter within a column are not significantly different, as indicated by Duncan's multiple range test ( $P = 0.05$ ).



**Figure 2.** Shoot and root growth under different light qualities (F-fluorescent lamp; R7B3-70% red + 30% blue LED; R5B5-50% red + 50% blue LED; B-100% blue LED; R-100% red LED).

inhibited by different synergistic interactions between blue/red light receptors and phytochrome.

Plant heights were similar under F and mixed LEDs, but were significantly taller than those treated with B. This negative effect of blue light has been reported previously, although it has an important role in chlorophyll formation and chloroplast development (Schuerger et al., 1997; Kim et al., 2004). In contrast, red light promoted both internodal elongation and height growth, but general plant development appeared to be inhibited, with chlorophyll content being the lowest among all four treatments. Levels of Chl did not differ among F and mixed LED plants, but were significantly lower for those exposed to either red or blue light (Table 1). Tibbitts et al. (1983) and Sabo et al. (1995) have also observed that chlorophyll contents are lowest under red-light illumination. Although our plants seemed to have normal photosynthetic functioning under F and mixed LED conditions, their general characteristics, e.g., height, root number, and fresh weight, were better with the mixed LEDs than the flu-

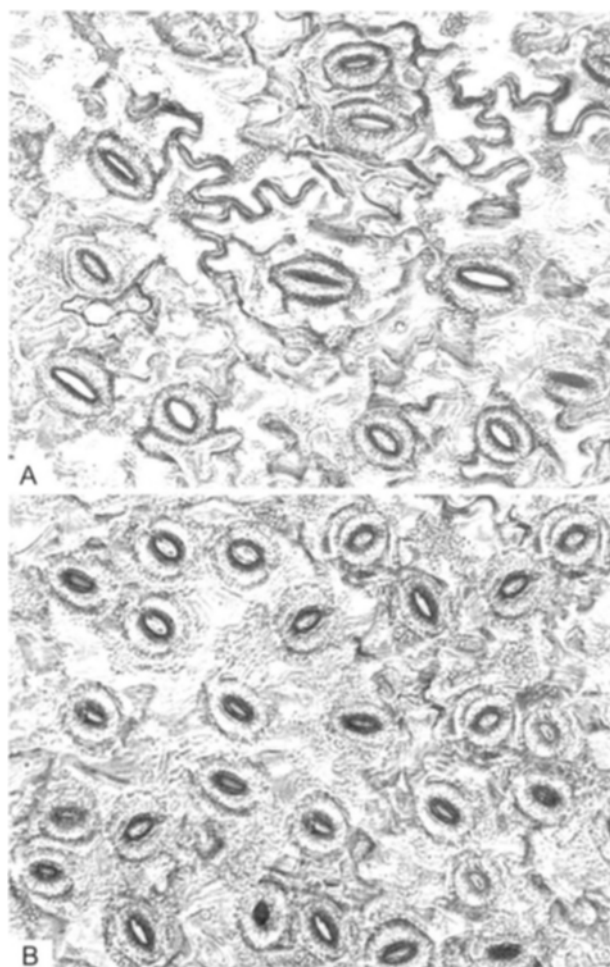
orescent lighting. Therefore, we conclude that normal growth is induced under mixed LEDs, especially R7B3.

Ventilation affected growth and stomatal characteristics under R7B3 LEDs (Table 2). When air was introduced to the plant vessels, rooting was faster and more successful (i.e., 100%), although such treatment did not significantly influence the total number of roots produced. In contrast, shoot numbers were significantly different between ventilated and non-ventilated plants. We suspect that stored gases, especially ethylene, in the culture vessels may have favorably influenced axillary bud initiation from the explants, as has been observed with *Pharbitis nil* (Prasad and Cline, 1985a, b). Nevertheless, this ethylene effect on organogenesis and shoot production varies by species (Gonzalez et al., 1997; Fal et al., 1999). Under ventilated conditions, our plant leaves appeared darker green and showed stronger apical growth on thicker stems, such that their fresh weights were significantly greater compared with the non-ventilated

**Table 2.** Effect of ventilation on plantlet growth under R7B3 LED lighting.

Vent.	Rooting (%)	No. of roots	No. of shoots	Fresh weight (mg)	No. of stomata (mm <sup>2</sup> )	Stomatal length (μm)
No	85.7 b <sup>z</sup>	4.7 a	4.0 a	181.0 b	162.8 a	29.7 b
Yes	100.0 a	4.7 a	2.8 b	257.7 a	135.0 b	32.8 a

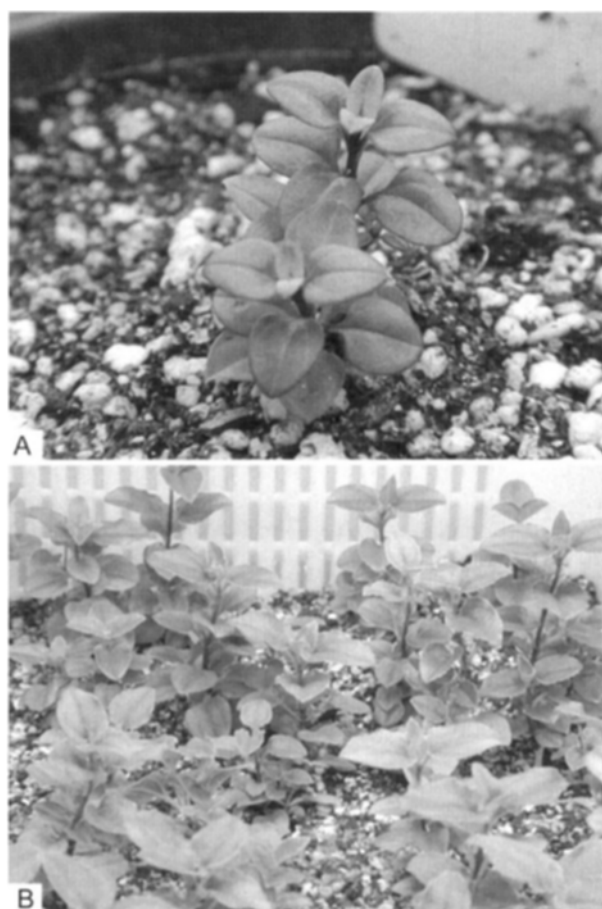
<sup>z</sup>, Values followed by the same letter within a column are not significantly different, as indicated by Duncan's multiple range test ( $P = 0.05$ ).



**Figure 3.** Stomata structure of the vented leaf (A) and non-vented leaf (B) under R7B3 LEDs (Bar = 100 μm).

plants (Table 2). This contradicted our previous assumption that axillary shoot growth might be suppressed under such aerated conditions.

The addition of ventilation also affected the density and morphological characteristics of the stomata (Fig. 3). Leaves from ventilated plants showed lower densities and larger stomata (Table 2) in contrast to the greater densities and smaller sizes found with our non-ventilated plants. The stomata were frequently elliptical under ventilated conditions, but manifested



**Figure 4.** Acclimatized plantlets in artificial soil mixture (A, 3-month-old; B, 6-month-old plantlets).

a more circular shape when not ventilated (Fig. 3). Paek and Hahn (2000) also have reported a higher density of stomata from *in vitro*-grown plantlets than from those produced in the field. Similar results have been noted with rose (Capellades et al., 1990) and apple (Blanke and Belcher, 1989).

Healthy, *in vitro* plant production is an important factor if one is to achieve efficient acclimatization. Therefore, we suggest that ventilation be applied during development, and that media selection and PCR treatments be optimized. Here, we have demonstrated that the *in vitro* production of Tsuru-rindo

plants is enhanced through the use of mixed LED systems under ventilated conditions. Although not statistically different, our results showed that this type of lighting promoted slightly better growth performance and soil acclimatization (Fig. 4). Therefore, further research is necessary to confirm whether *in vitro* growth of Tsuru-rindo plants can be controlled by adjusting the effective irradiation from LEDs when compared with conventional fluorescent lamps.

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