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baseLight: LED module for selective light application to the shoot base to study and improve adventitious root formation in plant *in vitro* cultures

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Abstract

In plant *in vitro* culture, shoot bases are typically exposed to light – unlike in *in vivo* cuttings – potentially inhibiting adventitious root (AR) formation. However, the specific impact of light at the shoot base in *in vitro* cultures remains poorly understood. To address this, a modular LED system called "baseLight" was developed, allowing controlled light application (blue, red, far-red, white) or darkness exclusively at the shoot base. The instruction given here enables the reproduction of "baseLight". Shoots of *Rosa × hybrida* genotypes with varying rooting abilities were tested under different light treatments in "baseLight". In the difficult-to-root genotype 'Nemo', far-red light and darkness increased root primordia and AR formation. Dark conditions enhanced root elongation across all genotypes. These results highlight the potential of light modulation to improve rooting, particularly for recalcitrant genotypes.

1. Introduction

In vitro root induction differs significantly from the rooting of cuttings in substrate-based systems. *In vitro* cultures are typically cultivated in transparent culture vessels and in semi-transparent growth media, exposing the entire shoot, including the basal region, to illumination. In contrast, *in vivo* cuttings are placed in substrates or hydroponic/aeroponic systems, where the basal cutting region is shielded from ambient light. According to Tester and Morris (1987), light penetration in soil is limited to the first 10 mm. Additionally, *in vitro* cultures are often placed on metal shelves, which reflect significant amounts of the applied radiation, further increasing light intensity at the shoot base in multi-directional ways.

Root morphogenesis can be affected by light, either indirectly via light perception of aerial parts and basipetal molecular signal transmission or directly via photoreceptors in the stem base or developing roots. The interaction between photoreceptors and plant hormones is well documented. With regard to the process of AR formation, for example, previous studies have shown that darkening the shoot base can enhance AR formation in difficult-to-root woody species grown *in vitro* (Rugini et al. 1993). Similarly, in *Eucalyptus* and *Prunus*, a temporary dark phase of whole shoots at the onset of rooting promoted AR development

(Fett-Neto et al. 2001; Quambusch et al. 2017). The regulation of auxin biosynthesis, transport, and signaling is strongly light-dependent, particularly in the context of AR formation (De Almeida et al. 2017). A low red:far-red light ratio applied to shoots has been suggested to promote AR formation through phytochrome-mediated auxin transport (Christiaens et al. 2019). However, until now, the effect on rooting of different light qualities and intensities applied exclusively to the shoot base has received only little attention in research and commercial micropropagation. Therefore, targeted light application to the shoot base – specifically modulating light intensity, wavelength distribution, or red:far-red ratios – could potentially enhance AR formation *in vitro* in general and in recalcitrant species in particular and optimize regeneration processes through light-mediated alteration of endogenous phytohormone concentrations. To test the influence of various illumination characteristics such as spectral composition to the AR formation of difficult-to-root *Rosa* × *hybrida* genotypes, the LED-system "baseLight" was designed and evaluated.

2. Data, Methods and Approach

LED module baseLight: corpus

A LED module called "baseLight" was designed to enable selective light application or light deprivation in the area of the shoot base only in *in vitro* shoot cultures. The corpus of the LED module was built of an aluminium base plate and the upper component of polypropylene. The 620 mm × 545 mm aluminium bottom was cut out of 4 mm thick material by LTO GmbH (D-33818 Leopoldshöhe-Greste, Germany) and the polypropylene parts by BMTEC B.V. (NL-7825 AG Emmen, Netherlands). The LED module offers space for 25 round polypropylene culture vessels with 500 mL volume (Plastikbecher.de, D-89537 Giengen, Germany) divided into five rows. The circular cut-outs for the cups were 77 mm in diameter so that the vessels fitted into the module without touching the base plate. In addition, the module offers space at one side for the electronic control unit and the required power supply unit. All cut files of the "baseLight" corpus are freely accessible (Chluba et al. 2025).

LED module baseLight: hardware and software

For control of the LED module "baseLight", microcontrollers were integrated. The hardware and circuitry were adapted from Bethge (2018). A primary power supply (Meanwell LPV-150-24) delivered a stable 24 V output, while DC-DC converters (LM2596HVS) reduced voltage to 5 V for the microcontrollers. The control unit was a Wemos D1 mini with ESP8266 processor and Wi-Fi, managing LED intensity, photoperiod, and frequency. Data were transmitted to a Raspberry Pi web server. LED control was achieved via pulse width modulation (PWM) using PCA9685 modules (12-bit, 40-1000 Hz, 16 channels). A real-time clock (DS3231) ensured accurate timing. Constant current drivers (Meanwell LDD-H 350) supplied 350 mA at 2-52 V, modulated via PWM (40-1000 kHz). Communication between microcontrollers and user interface was handled by a Raspberry Pi 3 Model B, transmitting JSON-based light recipes. The electrical components were soldered onto custom double-sided printed circuit boards (PCBs) (produced by PCBWay.com Limited, Hong Kong). The software setup was adopted and modified with necessary adaptations for the specific experimental requirements of this project from Bethge (2018). The Raspberry Pi was configured with as a webserver to host a web-based control interface, allowing to define light spectra, photoperiod, frequency and intensity profiles separately for every of the five rows. Configuration data were transmitted to the microcontrollers via a wireless connection and stored in JSON format in the microcontroller's EEPROM (non-volatile memory).

Beneath the 25 culture vessel slots, a single-layer aluminium PCB (PCBWay.com Limited, Hong Kong) was installed, onto which four types of LEDs were soldered (LED-PCB). This setup enables illumination from below, targeting the shoot base. Blue (Osram OSOLON® SSL120 Deep Blue, 455 nm, $\theta = 120^\circ$), Red (Osram OSOLON® SSL120 Red, 657 nm, $\theta = 120^\circ$), and far-red (Osram OSOLON® SSL150 Far Red, 727 nm, $\theta = 150^\circ$) LEDs were selected based on the absorption maxima of key plant photoreceptors (Bethge et al. 2018), while white LEDs (Osram OSOLON® SSL150 White, 6500 K, $\theta = 150^\circ$) served as a control to simulate conventional fluorescent lighting used in plant *in vitro* culture. All files for reproducing the "baseLight" LED module are provided at Chluba et al. (2025).

Adventitious root formation experiments in Rosa × hybrida

Shoots of *Rosa × hybrida* cultivars 'Herzogen Friederike' (HF), 'Nemo' (NE) and 'Mariatheresia' (MT) were rooted in the LED module "baseLight". Culture media for shoot proliferation and rooting were used as described in Wamhoff et al. (2024a), but 4 g L⁻¹ Gelrite instead of Plant Agar and 0.98 μ M IBA were added. For rooting 100 mL medium was poured in 500 mL vessels. The medium surface was covered with dry heat sterilized (180 °C for 3 h) 2 mm thick polypropylene discs with five wholes of 2 mm in diameter to hold the shoots. Shoots were rooted in five different treatments, i.e. different light regimes applied to the shoot bases (Table 1) and under 16 h photoperiod (40 μ mol m⁻² s⁻¹ PPFD), provided from above by tubular fluorescents tubes. The photoperiod for the shoot base light treatments were synchronized with the overhead lightning. In experimental repetition 2, one vessel per treatment with five shoots was evaluated after 7 d and shoots were fixed, embedded, microsectioned and stained as previously described by Wamhoff et al. (2024a) to determine the number of root primordia. After 21 d, rooting (% rooted shoots), the number of roots, root fresh mass, and the length of the longest root (repetition 2) were evaluated.

Table 1. Light treatments applied to shoot bases in rooting experiments with three selected *Rosa × hybrida* cultivars *in vitro*. Treatments 2 and 3: PFD_{350-800 nm} = 10 μ mol m⁻² s⁻¹.

	Treatment	Disc on medium	Inside/outside LED module
1	dark	yes	inside
2	far-red	yes	inside
3	white	yes	inside
4	control +disc	yes	outside
5	control	no	outside

The bias-reduced generalized linear model (BRGLM) was fitted using the brglmFit method from the brglm2 package in R (Kosmidis 2023), assuming a binomial (rooting) or Poisson (root number, root primordium number) distribution of the data. Metric parameters were analyzed in linear models. Root primordia were modeled using light treatment, genotype, and their interaction as predictors. For rooting traits after 21 d, light treatment, genotype, experimental repetition, and their interactions were included. In case of significant results for deviance/variance analyses, pairwise comparisons (Tukey, $p < 0.05$) were performed by using the R package emmeans (Lenth 2024).

3. Results and Discussion

A modular LED-based illumination system, "baseLight", was successfully constructed to enable quantitatively and qualitatively selective light control at the shoot base in plant *in vitro* cultures. As shown in Figure 1, the system integrates programmable LED units beneath each culture vessel and a polypropylene disc to block ambient light from above. The system comprises a PCB with a real time clock (RTC) controller, constant current sources, and PWM-regulated LED channels, offering adjustable light quality (blue, red, far-red, white) or darkness at the shoot base (Fig. 1 A, B).

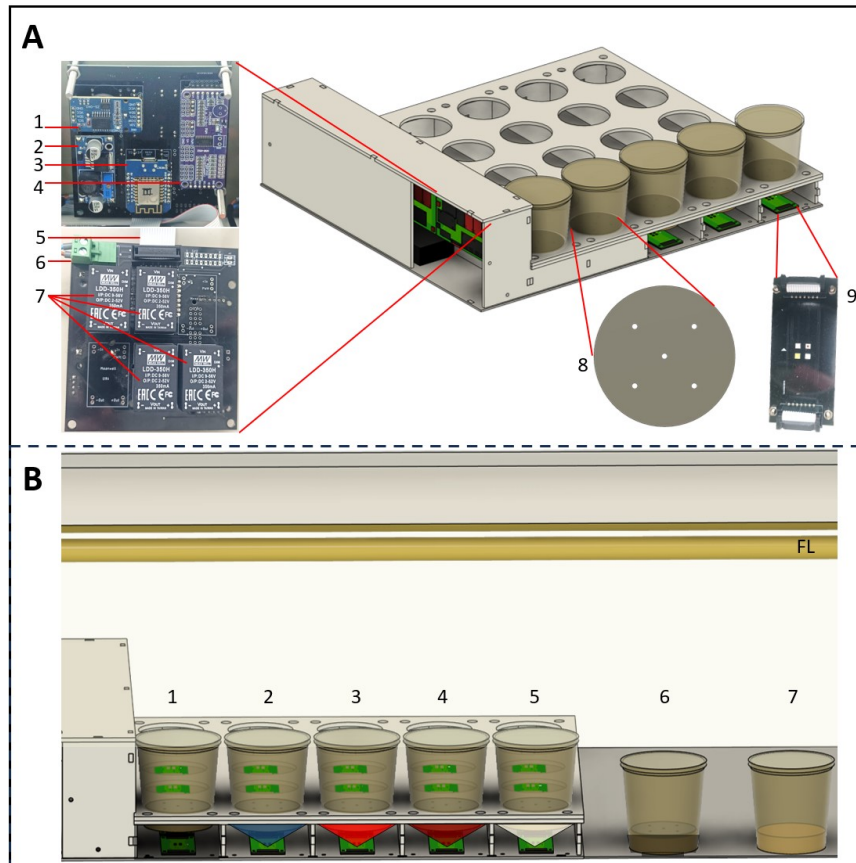


Figure 1. **(A)** Scheme of the LED module "baseLight" with PCB unit components, RTC controller (1), step-down module (2), microcontroller (3) and PWM controller (4) at the front side. Power connector (5), a 7 × 2 pin socket for connecting the LED elements via flat ribbon cables (6) and four constant current drivers for the four different LEDs within one row (7) mounted on the back side. The polypropylene disc excludes the ambient light from the overhead lighting in the culture room from the shoot bases (8) and the single-layer aluminium PCB with the four LEDs (9). **(B)** Possible light programmes in LED module "baseLight": Dark (1), blue (2), red (3), far-red (4) and white (5) from left to right. In rooting experiments, controls were included with the conventional in-shelve cultivation under fluorescent light (FL) with "control +disc" (6) and without disk "control" (7).

In plant *in vitro* cultures, shoot bases are typically exposed to ambient light, which can negatively affect AR formation (Rugini et al. 1993). In this study, the LED module "baseLight" was applied to modulate light exposure specifically at the shoot base during rooting of three *Rosa × hybrida* genotypes (HF, MT, NE) with known differences in rooting ability (Wamhoff et al. 2024a). While no significant differences in rooting rates were observed in repetition 1,

the difficult-to-root genotype NE showed significantly improved rooting in repetition 2 under far-red light (70% rooted shoots) compared to white light (19% rooted shoots) or the "control +disc" treatment (30% rooted shoots) (Fig. 2). A tendency for improved rooting under dark conditions was also observed in NE (60% rooted shoots). Light-induced inhibition of AR formation has been reported for other species such as *Prunus serotina* and *Pisum sativum*, often linked to increased ROS levels and reduced photoreceptive pigments when light was applied to whole shoots (Fürnkranz et al. 1990; Hansen 1975; Han et al. 2025). These findings highlight the potential of targeted light regulation at the shoot base to improve rooting efficiency, particularly in recalcitrant genotypes.

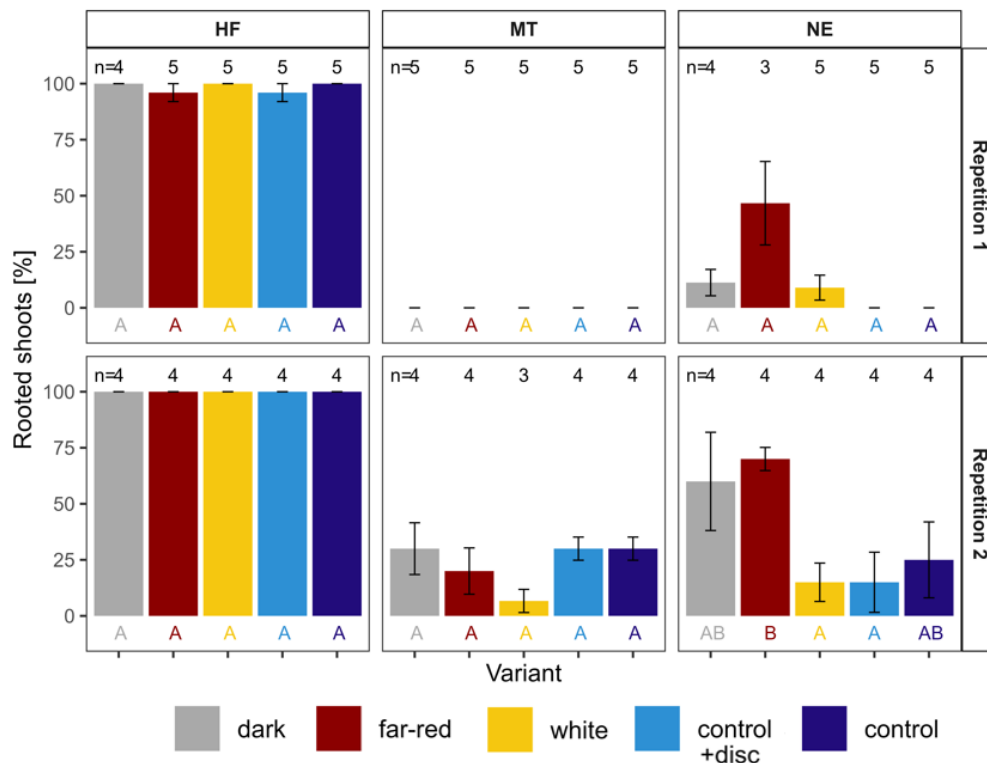






Figure 2. Rooting rates of the genotypes HF, MT, and NE depending on light treatments of the shoot base. Mean \pm standard error, significance letters indicate differences between the variants within a genotype, Tukey test, $\alpha = 0.05$, n = number of vessels.

Further rooting parameters observed in experimental repetition 2 are presented in Table 2. While the genotype MT did not show any variation in quantitative rooting characteristics across treatments, the easy-to-root genotype HF exhibited its highest values for both the number of roots per rooted shoot and root fresh mass in the control treatments. In genotype NE, the highest number of roots per shoot was observed in the dark treatment (5.2), although statistical significance could not be reached due to limited sample size. Interestingly, all three genotypes showed the longest roots in the dark treatment. This dark-induced root elongation is consistent with previous findings, where darkness at the root zone promoted root growth dynamics in *Arabidopsis thaliana* seedlings (Silva-Navas et al. 2015).

Previous studies have already demonstrated that genotype's MT poor rooting performance is not caused by the absence of root primordia formation capability (Wamhoff et al. 2024b). In the present study, a clear increase in the number of root primordia per shoot was observed for genotype NE in both the dark (5) and far-red (3.4) compared to the other three

treatments (0 to 1.6). However, this effect was not observed in genotypes HF or MT. The results in this study show that certain treatments (the selective darkening or light exposure of shoot bases) enabled by the "baseLight" module improved root primordium formation and thus rooting performance *in vitro*. However, the reaction varied between genotypes. The observed light effects have to be investigated for further genotypes and species and in absence of exogenously applied auxins in future studies. Furthermore, it has to be proven, whether only the exclusion of light or also the selective application of specific light qualities can impact the AR formation: The application of far-red showed a positive effect on AR formation of NE in repetition 2. Regarding further traits such as root number and or root fresh mass the improvement through the application of far-red light was not as clear as it was for the dark treatment. As the light intensities and associated electrical power input applied in this experiment were very low ($\text{PFD}_{350-800 \text{ nm}} = 10 \mu\text{mol m}^{-2} \text{s}^{-1}$), temperature differences caused by the light treatments were expected negligible and unlikely to have contributed to the observed rooting effects. However, in future experiments – particularly when higher light intensities are applied – temperature in the rooting compartments and/or the rooting medium should be monitored. These first experiments should be understood as a proof of concept of the functionality of the LED module "baseLight" and the physiological observations have to be investigated and proven in further experiments.

Table 2. Rooting parameters depending on light treatments after 7 (root primordia) and 21 d for repetition 2. Mean \pm standard error, significance letters indicate differences between the variants within genotypes, Tukey test, $\alpha = 0.05$, if $n < 3$, no letters are indicated. Cell filling from white to dark-green with white for values of zero and dark green for maximum value of each parameter. For n, please see Supplementary Table S1.

		 dark	 far-red	 white	 control +disc	 control
Roots per rooted shoot	HF	7.5 \pm 0.5 AB	5.9 \pm 0.4 A	6.9 \pm 0.7 AB	8.5 \pm 0.6 B	7.9 \pm 0.5 AB
	MT	1.6 \pm 0.2 A	2.5 \pm 0.3 A	1	1.3 \pm 0.1 A	2.2 \pm 0.2 A
	NE	5.2 \pm 0.9 A	3.8 \pm 0.6 A	2 \pm 0.3 A	2 \pm 0 A	2.2 \pm 0.4 A
Root fresh mass [mg] per rooted shoot	HF	22 \pm 2.2	21 \pm 3.3	27 \pm 2.1	23 \pm 2.7	35 \pm 4.6
	MT	1.2 \pm 0.6	2.1 \pm 0.2	0.8	0.7	4.1 \pm 1.5
	NE	15 \pm 6	7.1 \pm 1.1	5.4 \pm 2.8	0.7	3.9 \pm 2.8
Length of longest root per rooted shoot [cm]	HF	2.9 \pm 0.1 B	2.7 \pm 0.1 B	2.7 \pm 0.1 B	2.2 \pm 0.1 A	2.1 \pm 0 A
	MT	0.5 \pm 0.2 A	0.2 \pm 0 A	0.2	0.1 \pm 0 A	0.1 \pm 0 A
	NE	1.5 \pm 0.6 B	0.5 \pm 0.1 AB	0.3 \pm 3 AB	0.1 \pm 0 A	0.2 \pm 0 A
Root primordia per shoot base	HF	4.8 \pm 1 A	4.2 \pm 0.7 A	5 \pm 1.5 A	4.8 \pm 1.9 A	4 \pm 1.1 A
	MT	1 \pm 0.7 A	1.3 \pm 0.3 A	2.4 \pm 0.8 A	1 \pm 1 A	1.8 \pm 0.5 A
	NE	5 \pm 2 B	3.4 \pm 0.9 AB	1.6 \pm 5 A	0 \pm 0 A	1.4 \pm 1 A

4. Conclusions

"BaseLight" represents a powerful tool for manipulating basal light environments *in vitro* and thus can improve various AR formation parameters through selective light application. This enables genotype-specific rooting optimization for commercially and scientifically relevant plant species and novel research opportunities. Although in commercial *in vitro* production the selective light treatment of shoot bases is not relevant by now, the modular design of "baseLight" makes it suitable for integration into commercial micropropagation workflows.

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Supplementary Material

Supplementary Table S1. Results and replicate numbers for additionally investigated adventitious root formation traits and not presented in Figure 2 or Table 2.

Genotype	Treatment	Repetition	Number of vessels	Number of rooted shoots	Roots per rooted shoot		Root fresh mass per rooted shoot [mg]		number of shoots RP analysis
					mean	Standard error	mean	Standard error	
HF	dark	1	4	15	8,5	0,6	15,9	2,0	NA
HF	dark	2	4	19	7,5	0,5	22,3	2,2	5
HF	far-red	1	5	24	6,2	0,5	12,3	1,3	NA
HF	far-red	2	4	20	5,9	0,4	21,2	3,3	5
HF	white	1	5	25	7,4	0,7	17,5	2,7	NA
HF	white	2	4	19	6,9	0,7	26,6	2,1	5
HF	control	1	5	25	7,3	0,6	28,5	1,2	NA
HF	control	2	4	20	7,9	0,5	35,2	4,6	5
HF	control +disc	1	5	24	7,3	0,6	15,5	2,3	NA
HF	control +disc	2	4	20	8,5	0,6	23,1	2,7	4
MT	dark	1	5	0	NA	NA	NA	NA	NA
MT	dark	2	4	6	1,7	0,2	1,2	0,6	4
MT	far-red	1	5	0	NA	NA	NA	NA	NA
MT	far-red	2	4	4	2,5	0,3	2,1	0,2	3
MT	white	1	5	0	NA	NA	NA	NA	NA
MT	white	2	3	1	1,0	NA	0,8	NA	5
MT	control	1	5	0	NA	NA	NA	NA	NA
MT	control	2	4	6	2,2	0,2	4,1	1,5	4
MT	control +disc	2	4	6	1,3	0,1	0,7	NA	3
MT	control +disc	1	5	0	NA	NA	NA	NA	NA
NE	dark	1	4	2	2,0	0,3	7,1	2,9	NA
NE	dark	2	4	12	5,2	0,9	15,4	6,0	5
NE	far-red	1	3	7	2,1	0,1	4,4	0,5	NA
NE	far-red	2	4	14	3,8	0,6	7,1	1,1	5
NE	white	1	5	2	2,5	0,1	7,0	1,0	NA
NE	white	2	4	3	2,0	0,3	5,4	2,8	5
NE	control	1	5	0	NA	NA	NA	NA	NA
NE	control	2	4	5	2,2	0,4	3,9	2,8	5
NE	control +disc	1	5	0	NA	NA	NA	NA	NA
NE	control +disc	2	4	3	2,0	0,0	0,7	NA	5