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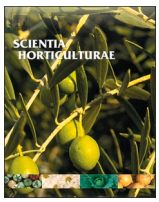

Effect of far-red and blue light on rooting in medicinal cannabis cuttings and related changes in endogenous auxin and carbohydrates

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ABSTRACT

Stem cuttings are used in the commercial cultivation of many crops, including medicinal cannabis, to produce large numbers of uniform and genetically identical plants. Light is an important environmental factor determining the success of the rooting of stem cuttings. The aim of this study was to investigate the influence of different fractions of far-red and blue during the adventitious rooting phase of medicinal cannabis stem cuttings on rooting and whether these effects are related to changes in endogenous auxin and/or carbohydrates. Two experiments were conducted in climate chambers with sole LED lighting (blue, red, far-red) using two cannabis cultivars. In Experiment 1, four light treatments were applied: 100 μmol $m^{-2}s^{-1}$ red:blue (88:12) with additional 0, 50 or 100 μmol $m^{-2}s^{-1}$ far-red and a fourth treatment with 100 μmol $m^{-2}s^{-1}$ sole red with additional 50 μmol $m^{-2}s^{-1}$ far-red. In Experiment 2, the following four light treatments were applied: 90 μmol $m^{-2}s^{-1}$ red:blue (45:45) with additional 0 or 45 μmol $m^{-2}s^{-1}$ far-red, a third treatment with 45 μmol $m^{-2}s^{-1}$ sole red with additional 45 μmol $m^{-2}s^{-1}$ far-red, and a fourth dynamic treatment which was 45 μmol $m^{-2}s^{-1}$ sole red with additional 45 μmol $m^{-2}s^{-1}$ far-red for 7 days followed by 90 μmol $m^{-2}s^{-1}$ red:blue (45:45) for 14 days. The effects on rooting in both experiments were measured after 21 days of light treatments. In Experiment 2, periodic measurements of auxin and carbohydrates were performed. Far-red improved adventitious rooting only in Experiment 2, where both cultivars responded similarly. Adding far-red only during the initial stage (7 days) of rooting was sufficient to improve rooting, while it did not result in excessive stem elongation. The presence or absence of blue did not significantly affect rooting. Although the positive effects of far-red on auxin and carbohydrate concentrations in stem cuttings are a likely explanation for the observed effects of far-red on rooting, we did not find a correlation between auxin or carbohydrates and rooting.

1. Introduction

Cannabis (*Cannabis sativa* L.) has a long history of usage as medicine, oil, fiber, and textile. This plant species contains a number of specialized metabolites, including cannabinoids, terpenoids, and flavonoids, with their contents depending on genotype (Andre et al., 2016). These specialized metabolites are synthesized and accumulated in glandular trichomes located densely on mature female inflorescences (Livingston et al., 2020). The dominant cannabinoids are delta-9-tetrahydrocannabinol (THC), which has psychoactive effects, and cannabidiol (CBD) which has therapeutic effects for a variety of chronic diseases, particularly those involving the neurological system (Andre et al., 2016; Richins et al., 2018). A synergistic effect of several cannabinoids and terpenes is known as the entourage effect, which has been hypothesized to lead to enhanced therapeutic benefits (Grotenhermen, 2003; Russo, 2011). Since its medical benefits have been demonstrated, the legalization of medicinal use cannabis occurs in a number of countries (Caulkins and Kilborn, 2019; Rehm and Fischer, 2015), resulting in rapidly growing demand. Hence, a high level of cultivation knowledge is needed to produce cannabis plants with uniform potency and stable production over time.

Abbreviations: THC, delta-9-tetrahydrocannabinol; CBD, cannabidiol; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid.

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In commercial cannabis cultivation, young plants are vegetatively propagated from stem cuttings to produce large numbers of uniform and genetically identical plants at relatively low cost (Potter, 2014). The rooting of stem cuttings is a crucial process. Endogenous auxin may induce adventitious roots (Pacurar et al., 2014). Indole-3-acetic acid (IAA) is the most abundant natural auxin (Kerr and Bennett, 2007); it is produced in the shoot apical meristem or young leaves and then polarly transported to the base by cell-to-cell transport or through phloem (Muday and DeLong, 2001; Swarup et al., 2001). The auxin accumulation at the root zone initiates cell division and adventitious root formation (Vanneste and Friml, 2009). In addition, rooting cofactors such as amino acids, vitamins, microelements, polyphenols, and phenolics may be required as auxin-synergists during root initiation (Foong and Barnes, 1981; Heuser, 1976; Jarvis, 1986). In practice, synthetic auxins such as indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are often applied to enhance root formation from cuttings in several horticultural plants, including cannabis (Blythe et al., 2007; Caplan et al., 2018; Chandra et al., 2017). Nevertheless, the use of synthetic chemicals may no longer be allowed in medicinal cannabis production due to food and drug safety (Lenton et al., 2018; Taylor and Birkett, 2020).

Light is one of the environmental factors determining the success of rooting by being a source of energy in photosynthesis and a signal to control photomorphogenesis. Nevertheless, high radiation may cause auxin breakdown (Jarvis and Shaheed, 1987). Especially in some woody species, stem cuttings rooted better in the dark than in light (Druart et al., 1982; Fett-Neto et al., 2001). Far-red light (700–800 nm) increased endogenous auxin accumulation via phytochromes and triggered shade avoidance responses in *Arabidopsis* (Mroue et al., 2018; Tao et al., 2008). The higher expression of auxin synthesis genes was consistent with increased accumulation of auxin and stem elongation in far-red enriched light (Gommers et al., 2018). Hence, this increase in auxin by far-red could have a positive effect on rooting. In several species, such as *Chrysanthemum, Rhododendron*, Chinese *Thuja*, and *Leucothoe*, the rooting success rate of cuttings was greater under a lower red: far-red ratio (Christiaens et al., 2019, 2016; Park et al., 2022). Blue light (400–500 nm) generally inhibits plant elongation, mediated by cryptochromes (Ahmad et al., 2002; Pedmale et al., 2016), depending on the species and fraction of blue (Kong et al., 2018; Larsen et al., 2020). As the effects of blue on elongation might be opposite to those of far-red, blue might counteract the effects of far-red on rooting.

Besides auxin, carbohydrates also affect rooting. Carbohydrates are a substrate for root formation, and supply energy for roots (Corrêa et al., 2005). In some cases, a high accumulation of carbohydrates at the basal part improves the root formation of cuttings, as reviewed by da Costa et al. (2013). Far-red is reported to influence carbohydrate status in plants. Adding far-red to shorter-wavelength photons can enhance photosynthesis (Zhen and Bugbee, 2020). In soybean seedlings far-red increased photosynthetic rate, resulting in an increase in carbohydrate content (Yang et al., 2020). In tomato fruits and leaves, far-red increased soluble sugar content by regulating starch breakdown (Courbier et al., 2020; Ji et al., 2020). Thus, far-red may enhance rooting by altering the levels of carbohydrates.

Although it is known that light spectra influence the metabolism of auxin and carbohydrates, the consequences for rooting are less known and could be species-dependent. The role of light spectra in medicinal cannabis is still unrevealed. Therefore, this study aims to investigate the influence of different fractions of far-red and blue light during the adventitious rooting of medicinal cannabis stem cuttings. We investigated whether this potential influence is related to a change in auxin and/or carbohydrate accumulation, based on the hypothesis that presence of far-red and absence of blue results in accumulation of auxin and carbohydrates, leading to an enhancement of rooting.

2. Materials and methods

2.1. Cutting specification and rooting condition

Two experiments on the effects of light spectra on the rooting of stem cuttings of medicinal cannabis (*Cannabis sativa* L.) cultivars 'White Russian' ('WR') and 'Critical CBD' ('CCBD') were conducted. The 'WR' is a chemotype I (a high THC/CBD ratio; >1) while the 'CCDB' is a chemotype II (an intermediate THC/CBD ratio; 0.5–0.3) (de Meijer et al., 1992; Pacifico et al., 2006). Stem cuttings were cut from 3 to 6 months old mother plants grown in a glasshouse (Wageningen University and Research, Greenhouse Horticulture, Bleiswijk, The Netherlands). The cuttings were collected from different batches of mother plants, taken in May 2019 (replicate 1 of Exp 1), June 2019 (replicate 2 of Exp 1), July 2019 (replicate 3 of Exp 1), April 2020 (replicate 4 of Exp 1), June 2020 (replicate 5 of Exp 1), October 2020 (replicate 1 of Exp 2), and May 2021 (replicate 2 of Exp 2). The averages of percentage of rooted cuttings from all cultivars and treatments in each replicate were 53.6 %, 52.9 %, 66.1 %, 92.4 %, and 93.9 % in Exp 1 and 75.7 %, and 59.9 % in Exp 2. The climate conditions in the glasshouse were 24–25 °C, 65 %−70 % relative humidity (RH) and 550–750 μmol CO_2 mol^{-1} under a 16-h (replicate 4 of Exp 1) or an 18-h photoperiod (all other replicates) of solar light, supplemented with about 400 μmol $m^{-2}s^{-1}$ of either HPS lamps (SONT Philips, Eindhoven, The Netherlands) for Exp 1 or LED lamps, consisting of 86 % red, 6 % green and 8 % blue for Exp 2 (Green Power DRW LB, Philips, Eindhoven, The Netherlands). The cuttings were excised from the top shoots of the mother plants, 9 ± 1 cm long and 0.3 ± 0.1 cm stem diameter, with 2–3 fully expanded leaves. The cuttings were taken in the morning, placed in closed plastic bags, and transferred to a climate-controlled room where light treatments were applied from the same day onwards.

For the first 7 days, the cuttings were under a transparent plastic to maintain high humidity. The light transmission of the transparent plastic without condensation was about 95 %. The temperature and RH in the growth chamber were set at 25 °C and 80 % (Exp 1) or 28 °C and 85 % (Exp 2), without supplemental carbon dioxide. The realized temperature was 25.0 ± 0.1 °C and RH was 78.7 ± 1.9 % (Exp 1), or 27.8 ± 0.8 °C and 84±0.6 % (Exp 2), measured at plant level. The cuttings were irrigated with a nutrient solution with EC 1.5 dS m^{-1}, pH 5.0, 1.25 mM NH_4^+; 6.2 mM K^+; 1.9 mM Ca^{2+}; 0.9 mM Mg^{2+}; 10.5 mM NO_3^-; 0.85 mM SO_4^{2-}; 0.85 mM PO_4^{3-}; 60 μM Fe^{2+}; 20 μM Mn^{2+}; 3 μM Zn^{2+}; 20 μM B^{2+}; 0.5 μM Cu^{2+}; 0.5 μM Mo^{2+}, at 2–4 days interval depending on the wetness of the substrate. Light was provided during an 18-h photoperiod by LED with adjustable spectrum (Green Power Dynamic 2.0 LED research modules, with a GrowWise Control System, Philips, Eindhoven, The Netherlands). The room was divided into 4 compartments separated by white plastic sheets to arrange 4 light treatments (Table 1). The light spectrum and intensity were measured using a spectroradiometer (Specbos 1211, JETI, Jena, Germany) for 9 points m^{-2} without the transparent plastic covering, at 15 cm height from the table (approximately at plant height); the distance from the plant height to the lamps was 165 cm, and the results are shown in Table S1.

2.1.1. Experiment 1

Cuttings were inserted into fine river sand in black plastic pots (8 × 8 × 13 cm, width × length × height) with one cutting per pot. The sand allowed for the observation of the roots at the end of the experiment. Before insertion into the sand, for half of the cuttings, the lower end of the stem was dipped in a synthetic auxin powder, 0.25 % indole-3-butyric acid (IBA) (Rhizopon, The Netherlands). Four light treatments were applied, consisting of (1) 100 μmol $m^{-2}s^{-1}$ red:blue (88:12) without far-red, R88B12; (2) with 50 μmol $m^{-2}s^{-1}$ far-red, R88B12+FR50; (3) with 100 μmol $m^{-2}s^{-1}$ far-red, R88B12+FR100; or (4) 100 μmol $m^{-2}s^{-1}$ sole red with 50 μmol $m^{-2}s^{-1}$ far-red, R100+FR50 (Table 1, Table S1).

Final rooting assessment, after washing out the sand, was performed

Table 1
Overview of light treatments in Exp 1 and 2 with their spectral distribution. R is red light (600–700 nm), B is blue light (400–500 nm), and FR is far-red light (700–800 nm). Phytochrome stationary state (PSS) is calculated according to Sager et al. (1988). PPFD means photosynthetic photon flux density (400-700 nm). PFD means photon flux density (400-800 nm).

Light treatments		PFD (μmol $m^{-2}s^{-1}$)				PSS	PPFD (μmol $m^{-2}s^{-1}$)
		R	B	FR	Total		
Exp 1	R88B12	88	12	0	100	0.88	100
	R88B12+FR50	88	12	50	150	0.77	100
	R88B12+FR100	88	12	100	200	0.68	100
	R100+FR50	100	0	50	150	0.78	100
Exp 2	R45B45	45	45	0	90	0.86	90
	R45B45+FR45	45	45	45	135	0.69	90
	R45+FR45	45	0	45	90	0.70	45
	Dynamic						
	-Day 1–7 (R45+FR45)	45	0	45	90	0.70	45
	-Day 8–21 (R45B45)	45	45	0	90	0.86	90

twenty-one days after start of treatments. Cuttings with at least one root longer than 0.5 cm were considered as rooted cuttings, when calculating the percentage of rooted cuttings. The roots were dried in a ventilated oven at 105 °C for 48 h to obtain the dry weight. The height of rooted cuttings was measured from top internode to stem end.

2.1.2. Experiment 2

Cuttings were inserted into 3.6 × 3.6 × 4 cm stonewool plugs (Grodan, The Netherlands), placed in a 7 × 11-hole plastic tray. Four light treatments were applied, consisting of (1) 90 μmol $m^{-2}s^{-1}$ red:blue (45:45) without far-red, R45B45; (2) with 45 μmol $m^{-2}s^{-1}$ far-red, R45B45+FR45; (3) 45 μmol $m^{-2}s^{-1}$ sole red with 45 μmol $m^{-2}s^{-1}$ far-red, R45+FR45; and (4) a dynamic treatment in which the cuttings were under R45FR45 for 7 days followed by R45B45 for 14 days (Table 1, Table S1). No external synthetic auxin was applied.

Final rooting assessment was performed twenty-one days after start of treatments, same as for Experiment 1. Rooting was scored after vertically cutting the stonewool plug into two halves using the following criteria: 1= no browning, no thickening, no callus, and no rooting, 2= browning and thickening stem, 3= callus (larger than 1 mm^3), 4= root tips, 5= few roots longer than 0.5 cm inside plug, 6= few roots outside plug, 7= roots covered half of the stonewool plug, 8= roots fully covering the stonewool plug. The cuttings with a score of 4 or higher were considered as rooted cuttings. The height of rooted cuttings was measured as explained for Experiment 1.

2.2. IAA extraction and analysis

In Experiment 2, the youngest fully expanded leaf per cutting on day 5, 10 and 21 (final assessment) after start of treatments was collected to analyze the IAA content. The leaflet tissue was immediately frozen using liquid nitrogen and stored at −80 °C. Three leaflets from 3 different cuttings were pooled into one sample, in total there were 6 samples of each light treatment. The samples were ground by a ball mill at 80 Hz for 20 s, after which samples of 0.01 g FW were extracted with 1 mL of ice-cold methanol (MeOH) containing [phenyl $^{13}C_6$]-IAA (0.1 nmol mL^{-1}) as an internal standard in a 2-mL Eppendorf tube. The tubes were vortexed and sonicated for 10 min in an ultrasonic water bath (Branson 3510, Branson Ultrasonics, Eemnes, The Netherlands) and placed overnight in an orbital shaker at 4 °C. Next, samples were centrifuged for 10 min at 11,500 rpm in a centrifuge (Heraeus Fresco 17, Thermo Fisher Scientific, Waltham, USA) at 4 °C, after which the organic phase was loaded on a 100 mg 1.5 mL^{-1} Extra-Clean SPE Amino cartridge (S*Pure Pte. Ltd., Singapore). The cartridge was equilibrated prior to sample loading, and it was subsequently washed and eluted as previously described (Ruyter-Spira et al., 2011). The MeOH was evaporated in a speed vacuum system (SPD121P, Thermo Savant, Hastings, UK) at room temperature and the residue was resuspended in 100 μL acetonitrile: water:formic acid (20:80:0.1, v/v/v). The samples were filtered through a 0.45 μm filter (Minisart SRP4, Sartorius, Goettingen, Germany) and measured on the same day. IAA was analysed using a Waters Xevo TQs tandem quadruple mass spectrometer as previously described (Schiessl et al., 2019).

2.3. Carbohydrate analysis

In Experiment 2, carbohydrates were measured according to Larsen et al. (2022). The youngest fully expanded leaf and lower 3 cm from the base of stem tissue were sampled on day 10 and 21 after start of treatments, freeze dried, and ground in a ball mill. 0.015 g DW of tissue powder was extracted with 5 mL of 80 % ethanol at 80 °C for 20 min in a shaking water bath. Then the extracts were centrifuged for 5 min at 8500 rcf (Universal 320R, Hettich). 1 mL of supernatant was transferred to a 2-mL Eppendorf tube and dried in a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific, Waltham, USA) at a setting of 50 °C and 5.1 mbar for 120 min. The pellet with the remaining supernatant was stored for starch measurement at −20 °C. The dried samples in Eppendorf tube were resuspended in 1 mL Milli-Q water and sonicated in an ultrasonic water bath (Branson 2800, Branson Ultrasonics, Eemnes, The Netherlands) for 10 min. The solutions were centrifuged at 21,100 rcf for 10 min (Sorvall Legend Micro 21R, Thermo Fisher Scientific). After 10 times of dilution with Milli-Q water, glucose, fructose and sucrose were quantified using a High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD; Dionex ICS500. Thermo Fisher Scientific) equipped with a CarboPac PA1 column (250 × 2 mm) (Thermo Fisher Scientific) eluted with 100 mM NaOH at a flow rate of 0.25 mL min^{-1} at 25 °C. Chromeleon 7.2 (Thermo Fisher Scientific) was used for data analysis. Total soluble sugar was calculated as the sum of glucose, fructose, and sucrose.

The stored pellet was used for starch analysis. After washing three times with 80 % ethanol, the pellet was dried in a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific, Waltham, USA) at 55 °C and 5.1 mbar for 25 min, then resuspended in 2 mL of 1 g L^{-1} thermostable alpha-amylase (SERVA Electrophoresis GmbH) in Milli-Q water and incubated for 30 min at 90 °C in a shaking water bath. Then 1 mL of 0.5 g L^{-1} amyloglucosidase (Sigma 10,115) in 50 mM citrate buffer (pH 4.6) was added and incubated at 60 °C for 10 min in a shaking water bath. After centrifugation at 21,100 rcf for 10 min and 20–50 times of dilution with Milli-Q water, glucose was quantified using HPEAC-PAD as described above.

2.4. Statistical design and analysis

Experiment 1 consisted of 5 replicate trials that were executed consecutively, each time with new randomization, representing 5 blocks. Per replicate 15–25 cuttings were used per treatment with split-

split-plot design; the main factor being light, the subfactor was with/without IBA and the sub-subfactor was cultivar. Experiment 2 consisted of 2 replicate trials, one was conducted in a commercial facility while the other was conducted at Wageningen University and Research. In trial 1 and 2, 40 and 70 cuttings were used per treatment with a split-plot design; the main factor was light and the subfactor was cultivar. The exact numbers of cuttings in each trial are shown in Table S2. IAA and carbohydrate contents were determined in trial 2 of Experiment 2, where 6 pooled samples per treatment were analysed and considered as replicates. Analysis of variance (ANOVA) was conducted followed by mean separation by Fisher's protected LSD test at $P = 0.05$ in Genstat (v.19; VSN International, London, UK). The normality and homogeneity of the residuals were examined with Shapiro-Wilk and Bartlett's tests, and in all cases both assumptions were met, except for the residuals of rooting parameters in Experiment 2, where homogeneity was assumed as it could not be tested because of the small number of replicates ($n = 2$).

3. Results

3.1. Rooting

Adding far-red to a background of red:blue (R88B12) did not significantly affect the rooting of cannabis stem cuttings in any of the two cultivars in Experiment 1 where cuttings were placed in sand substrate (Fig. 1A-F). Changing the fraction of blue light from 12 % (R88B12+FR50) to 0 % (R100+FR50) did not affect the rooting. The application of IBA enhanced the percentage of rooting and root dry weight which did not interact with light nor cultivar (Table S3). Plant height tended to increase with far-red intensity, although this was not statistically significant (Fig. 1C,F). The cultivar 'WR' rooted better than 'CCBD'; on average, the percentage of rooted cuttings was about 12 % higher in 'WR' than in 'CCBD'.

Adding far-red to either a background of red:blue (R45B45) or a sole red of 45 μmol $m^{-2}s^{-1}$ (R45) significantly increased the percentage of rooted cuttings in both cultivars, in Experiment 2 where cuttings were placed in stonewool substrate (Fig. 2A). Blue light did not affect the rooting. Light treatments did not affect the rooting scores of rooted cuttings, which on average had few roots that appeared outside the plug (Fig. 2B). Plant height was strongly increased by adding far-red to either red:blue (R45B45) or sole red light (R45) (Fig. 2C). For the dynamic treatment, cuttings were exposed to far-red added to sole red light for the first 7 days, followed by equal red:blue without far-red. This dynamic treatment significantly increased the percentage of rooted cuttings, while the plant height was not statistically significantly different from the cuttings not receiving far-red. No significant difference between cultivars was shown, and no interaction between light spectrum and cultivar was found (Table S4).

3.2. IAA content

To investigate whether the effects of far-red on rooting are related to endogenous auxin, IAA content was measured in the young leaf of cuttings at 5, 10 and 21 days after start of treatments. In the early phase (5 days after start of treatments), the IAA content in the leaf of cuttings grown under far-red was lower than for the control (no far-red), though this effect was only statistically significant in cultivar 'CCBD' (Fig. 3A, D). Also at 10 days after start of treatments, far-red decreased leaf IAA content; however, this time the reduction was not statistically significant in 'CCBD'. The lowest leaf IAA content was found when far-red was added to sole red in the 'WR' cuttings (Fig. 3B,E). Leaf IAA content at the end of the experiment (21 days after start of treatments) was not significantly affected by light treatments (Fig. 3C,F).

3.3. Carbohydrate content

The soluble sugar and starch contents in the young leaf and stem of cuttings at 10 and 21 days after start of treatments were measured to determine whether far-red improved rooting by changes in carbohydrates accumulations. Total soluble sugar content in the young leaf was not influenced by light treatments (Fig. 4A,B,E), except for cultivar 'WR' at 21 days after start of treatments, where sugar was decreased by exposure to additional far-red (Fig. 4F). Total soluble sugar in the stem of cultivar 'CCBD' was significantly increased by additional far-red at 10

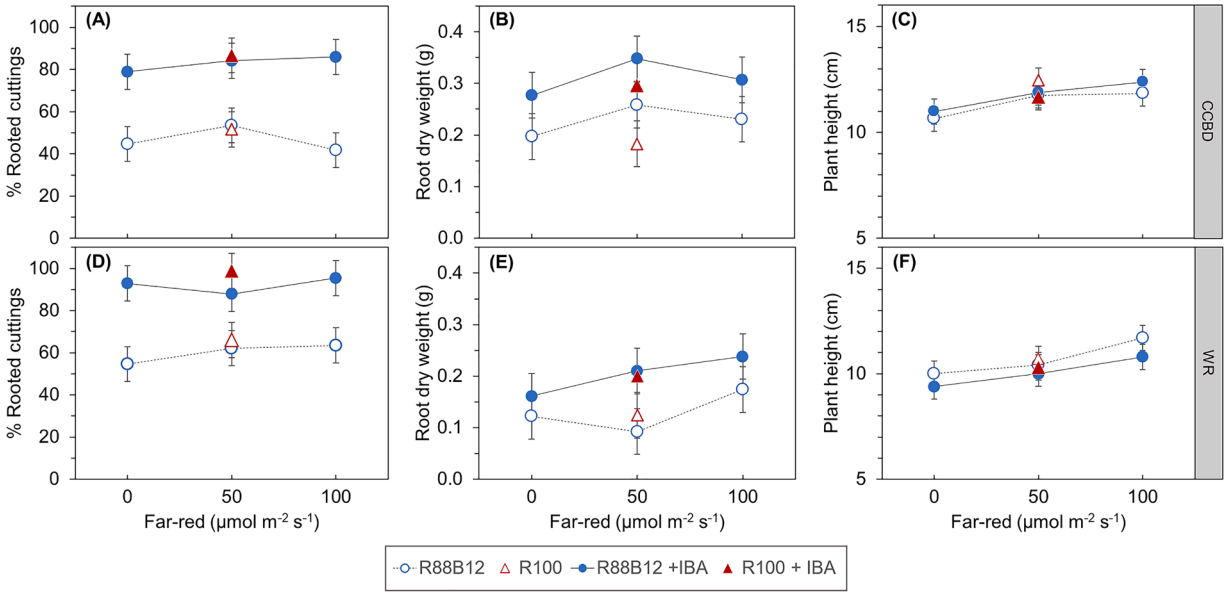

Fig. 1. Effect of far-red on percentage of rooted cuttings (A,D), root dry weight (B,E) and rooted plant height (C,F) of cultivars 'CCBD' (A-C) and 'WR' (D-F) with (closed symbol) or without IBA application (open symbol) in Experiment 1. Different intensities of far-red light were added to a background of red:blue light (R88B12, 88 μmol $m^{-2}s^{-1}$ red + 12 μmol $m^{-2}s^{-1}$ blue) (blue circles) and at the intermediate far-red level the red:blue was compared with sole red background light (R100, 100 μmol $m^{-2}s^{-1}$ red) (red triangle). Data were obtained 21 days after start of treatments. All data are the means of 5 trials. The error bars indicate ± standard error of means. There is no significant effect of light on all parameters for each cultivar and each IBA application separately ($n = 5$, $P = 0.05$). F-Probability values are shown in Table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

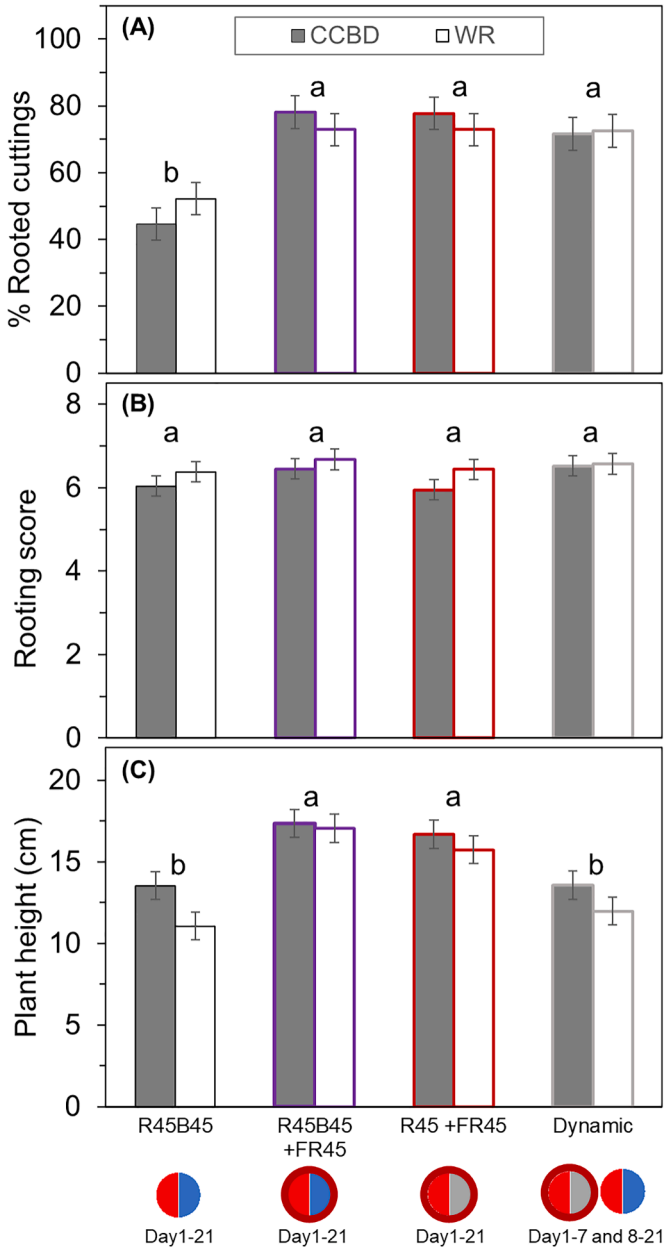

Fig. 2. Effect of light spectrum on percentage of rooted cuttings (A), rooting score of rooted cuttings (higher score means more roots) (B) and height (C) of 'CCBD' and 'WR' cuttings in Experiment 2. Far-red (FR45) was added to red:blue (R45B45) and sole red (R45). The dynamic treatment means the cuttings were exposed by R45FR45 until day 7, followed by R45B45 on day 8–21. External auxin was not applied in any of the treatments. Data were obtained 21 days after start of treatments. All data are the means of 2 independent trials. The error bars indicate ± standard error of means. Since there was no cultivar effect, and no interaction between light spectrum and cultivar, the different letters indicate the significant differences of the means of both cultivars according to Fisher's protected LSD test ($n = 2$, $P = 0.05$). F-Probability values are shown in Table S4.

days (Fig. 4C), but later (21 days after start of treatments) there was no effect of light (Fig. 4D). In contrast, the stem of 'WR' cuttings under additional far-red had higher sugar content at 21 days after start of treatments (Fig. 4H). Among all four light treatments, cuttings placed under red:blue with additional far-red showed the highest starch content in both leaf and stem at 10 days after start of treatments (Fig. 5A,E,C,G). At 21 days after start of treatments, additional far-red tended to reduce starch in stem but not in dynamic treatment (Fig. 5D,H).

4. Discussion

4.1. Adding far-red improved rooting in some conditions

In our study on cannabis stem cuttings, a positive effect of far-red on adventitious rooting was found in one of two experiments (Experiment 2). Adding far-red to either a red:blue (R45B45) or a sole red (R45) background promoted rooting. The presence of far-red during only the first 7 days had a similar positive effect on rooting as providing far-red for 21 days. A positive effect of far-red on rooting has also been reported for other species such as *Rhododendron*, Chinese *Thuja, Leucothoe* (Park et al., 2022), and *Chrysanthemum* in *vivo* (Christiaens et al., 2019) and in *vitro* (Kurilčik et al., 2008). It has been claimed that far-red upregulates the biosynthesis of auxin (Tao et al., 2008) which plays a crucial role in root formation (Muday and DeLong, 2001; Vanneste and Friml, 2009); therefore, far-red may lead to rooting enhancement (Christiaens et al., 2016).

In commercial cannabis propagation, synthetic auxins such as indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are used to enhance the success rate of rooting (Blythe et al., 2007; Caplan et al., 2018). This was confirmed by our results, where quick-dipping the cuttings in 0.25 % IBA increased rooting by 20 %−40 % under all light treatments. Moreover, genotypes of cannabis may differ in rooting ability (Campbell et al., 2021), which was also shown in Experiment 1 (Fig. 1, where 'WR' rooted better than 'CCBD' with about 12 % higher rooted cuttings on average). However, in Experiment 2, there was no difference in rooting between cultivars; the positive effect of far-red on rooting was similarly found in both cultivars. The presence of blue (12 % or 50 %, while red was 88 % or 50 % and in both cases with additional far-red) did not affect the rooting of cannabis cuttings. Similarly, Moher et al. (2023) reported that changing the fraction of blue between 15 %−75 % (with the fraction red changing concomitantly from 85 % to 25 %) did not the alter rooting of cannabis cuttings. However, in *Chrysanthemum* and rosemary cuttings, blue light increased rooting and increased the expression of IAA synthesis-related genes (Christiaens et al., 2019; Gil et al., 2021, 2020).

There were several differences between the experimental conditions of Experiment 1 and 2 that we should consider as possible reasons for a positive effect of far-red on rooting in Experiment 2 but not in Experiment 1. First of all, PFD was different. The better rooting found in Experiment 2 may have resulted from the lower PFD at 90–135 μmol $m^{-2}s^{-1}$, while the PFD in Experiment 1 was 100–200 μmol $m^{-2}s^{-1}$. Zheng et al. (2019) showed that a high light intensity (PPFD) caused a delay in the rooting of strawberry runners. Second, the ratio of red:blue background light differed between Experiment 1 (R88B12) and 2 (R45B45). However, we assume that this was not causing the different influence of far-red on rooting, since red:blue resulted in similar rooting as sole red light. The rooting substrate and temperature also differed between the two experiments. In Experiment 2, the cuttings were placed in stonewool at 28 °C, while they were placed in river sand at 25 °C in Experiment 1. As a substrate, river sand allows for the easy removal of substrate such that roots can be observed, but stonewool is more often used in commercial production. Campbell et al. (2021) found that the rooting success of cannabis cuttings was 10-fold higher in stonewool than in peat-based substrates. Lastly, the mother plants did not grow under the same light spectrum in both experiments, potentially influencing the quality of the produced cuttings. In Experiment 1, the mother plants were grown under solar light with supplemental light from HPS lamps, whereas in Experiment 2 the supplemental light was from LED lamps with a spectrum consisting of 86 % red, 6 % green and 8 % blue. Thus, we suspect that these factors might interact with far-red, causing positive effects of far-red on rooting to be observed in some conditions while not in other conditions.

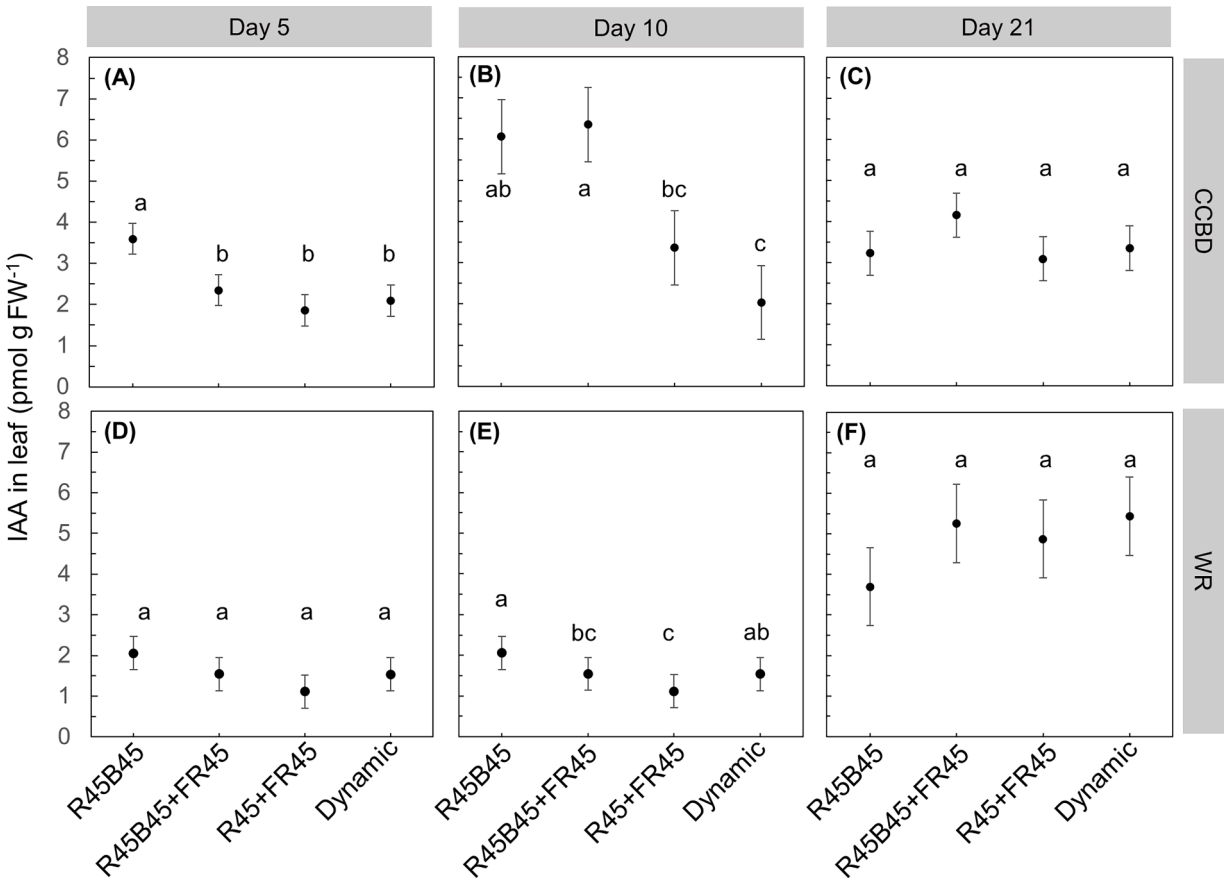

Fig. 3. Effect of light spectrum on IAA content in young leaf of 'CCBD' (A-C) and 'WR' (D-F) cuttings after start of treatments for 5, 10 and 21 days in Experiment 2. Far-red (FR45) was added to red:blue (R45B45) and sole red (R45). The dynamic treatment means the cuttings were exposed to R45FR45 until day 7, followed by R45B45 on day 8–21. In none of the treatments external auxin was applied. All data are the means of 6 pooled samples per treatment from the same trial. The error bars indicate ±standard error of means. The different letters indicate the significant differences between means for each cultivar and timepoint separately according to Fisher's protected LSD test ($n = 6$, $P = 0.05$). F-Probability values are shown in Table S5.

4.2. Far- red stimulated stem elongation

The addition of far-red light tended to increase plant height, which is in line with the general shade avoidance response (Demotes-Mainard et al., 2016). Likewise, stem elongation by far-red occurred in our cannabis experiments. Far-red modulates stem elongation by inactivating phytochrome, which leads to auxin biosynthesis in the top of the plant and transport downward through the stem (Keuskamp et al., 2010), causing stem elongation. The stem elongation caused by additional far-red occurred regardless of the presence of blue (Fig. 2C, Fig. S1). The application of far-red to improve the rooting of cannabis cuttings, might not be adopted by growers if it would lead to too much stem elongation. Interestingly, applying far-red only during the first 7 days improved rooting without the stimulation of excessive stem elongation.

4.3. Far-red lowered endogenous IAA content in young leaf

Our study demonstrates that exogenous auxin application of IBA was sufficient to the improve rooting of cannabis cuttings (Fig. 1), confirming previous reports (Campbell et al., 2021; Caplan et al., 2018). It is well established that far-red stimulates auxin biosynthesis in the young leaves of *Arabidopsis* (Ljung et al., 2001; Tao et al., 2008). This suggests that far-red may induce auxin biosynthesis in cannabis young leaves, which could result in the improved rooting of the cuttings as well. Indeed, in one of the two experiments (Experiment 2), we found that additional far-red light promoted rooting. We also observed a positive effect of far-red light on stem elongation, an effect often linked to auxin signaling (Gommers et al., 2018). However, in our experiment, far-red did not increase the endogenous IAA content in young leaves. On the contrary, exposure to far-red even decreased the level of IAA in these young leaves at 5 and 10 days after start of treatments. It is possible that, in contrast to what we found in leaves, the concentration of auxin in the lower part of stem, where root initiation takes place, would be affected by exposure to far-red light. For example, far-red may induce basipetal transport of IAA auxin away from its biosynthesis location in *Arabidopsis* leaves (Küpers et al., 2023). Such downward transport in cannabis could potentially drain the auxin from the young leaves and thus explain the lower IAA content measured in the leaves while still inducing rooting at the base of stem. However, the IAA content in the lower part of stem was not measured in our study. Therefore, the selected leaf samples for these measurements might not have been the most optimal to see the effect of far-red on IAA. On the other hand, several studies found IAA in plant tissues to be rapidly changed (2 to 5 h) after far-red exposure (Ahkami et al., 2013; Druege et al., 2016; Küpers et al., 2023; Tao et al., 2008). It could be that rapid changes in IAA levels might lead to a variation that is difficult to analyze when IAA is measured at a few selected moments. Alternatively, heightened responsiveness to a stable level of auxin in the stem where elongation or rooting occurs could lead to increased auxin signaling without changing its absolute concentration.

4.4. Light spectrum did not influence carbohydrate status

Carbohydrates contribute to rooting as it is a substrate for root formation and supply energy (Corrêa et al., 2005). For example, in eucalyptus, high carbohydrate accumulation in the stem base of cuttings positively affected adventitious rooting (da Costa et al., 2013; Ruedell

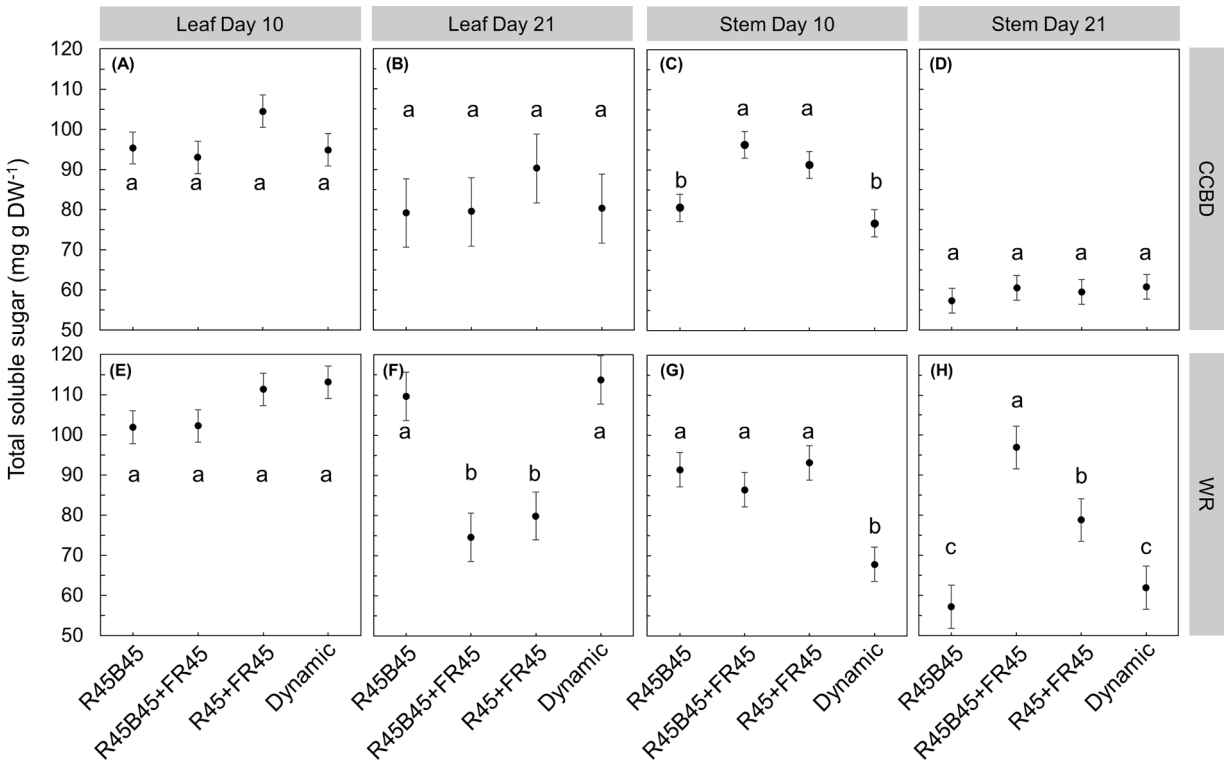

Fig. 4. Effect of light spectrum on total soluble sugar content in young leaf and stem of 'CCBD' (A-D) and 'WR' (E-H) cuttings after start of treatments for 10 and 21 days in Experiment 2. Far-red (FR45) was added to red:blue (R45B45) and sole red (R45). The dynamic treatment means the cuttings were exposed to R45FR45 on day 1–7, followed by R45B45 on day 8–21. External auxins were not applied in any of the treatments. All data are the means of 6 pooled samples per treatment from the same trial. The error bars indicate ±standard error of means. The different letters indicate the significant differences between means for each cultivar, tissue and timepoint according to Fisher's protected LSD test ($n = 6$, $P = 0.05$). F-Probability values are shown in Table S5.

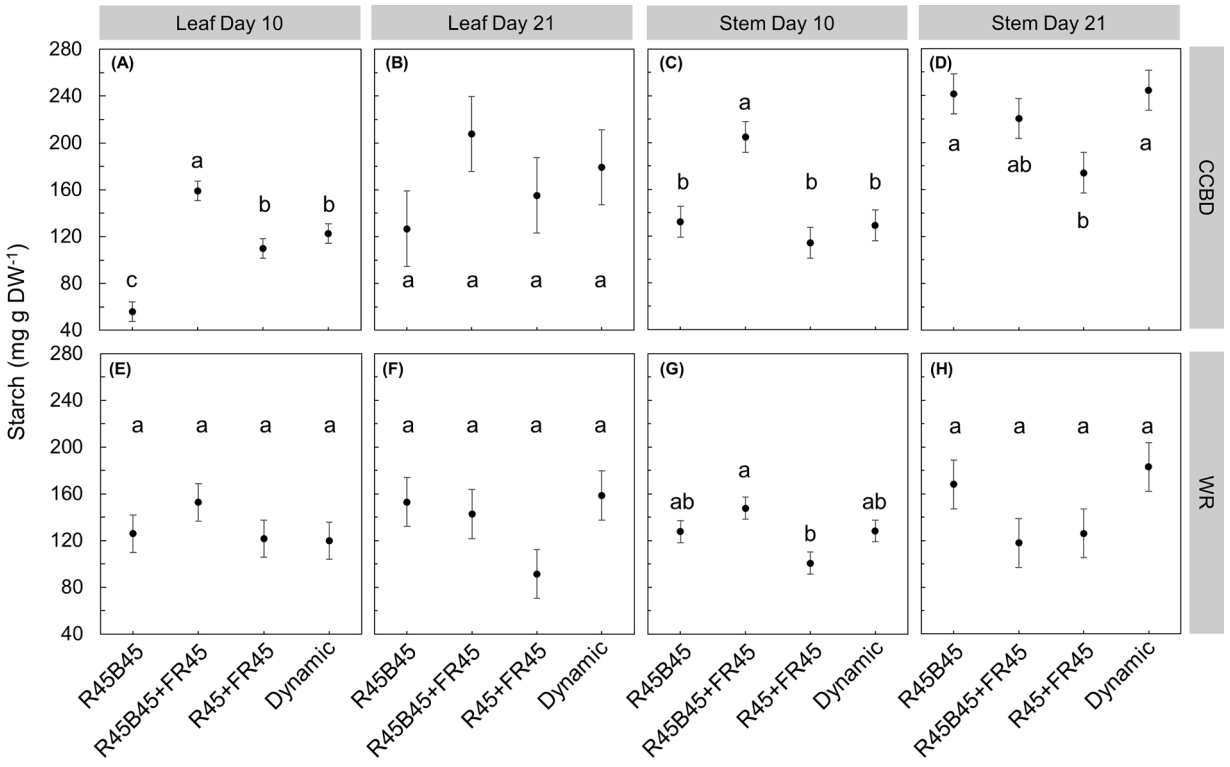

Fig. 5. Effect of light spectrum on starch content in leaf and stem of 'CCBD' (A-D) and 'WR' (E-H) cuttings after start of treatments for 10 and 21 days in Experiment 2. Far-red (FR45) was added to red:blue (R45B45) and sole red (R45). The dynamic treatment means the cuttings were exposed to R45FR45 on day 1–7, followed by R45B45 on day 8–21. All data are the means of 6 pooled samples per treatment from the same trial. The error bars indicate ±standard error of means. The different letters indicate the significant differences between means for each cultivar, tissue and timepoint according to Fisher's protected LSD test ($n = 6$, $P = 0.05$). F-Probability values are shown in Table S5.

et al., 2015, 2013). It was shown in petunia that in well-rooted shoot cuttings, the sugar level at the stem base increased continuously from 1 to 8 days after excision (Ahkami et al., 2013). The carbohydrate status of cuttings is positively influenced by leaf photosynthesis during rooting, as observed in hazelnut (Tombesi et al., 2015). Far-red benefits photosynthesis (Yang et al., 2020; Zhen and van Iersel, 2017) and thus carbohydrate accumulation. Hence, we expected that the positive effect of far-red on rooting in cannabis cuttings was partly the consequence of improved carbohydrate accumulation. Although the total soluble sugar and starch measured in cannabis cuttings did not show a substantial increase when far-red was added, their rooting was significantly improved, we cannot exclude a role for carbohydrates. The timing of the measurements may play a role, and we measured in stem and leaf, but the carbohydrates at the base of the stem are probably most relevant.

5. Conclusion

Adding far-red to either red:blue or sole red background promoted the rooting of stem cuttings, compared to applying only red:blue light in one of two experiments, which experiments differed in growth conditions such as substrate, light, and air temperature. The presence or absence of blue did not significantly affect rooting. Auxin and carbohydrate concentrations did not correlate with rooting. Adding far-red only during the initial stage of rooting was sufficient to improve rooting while it did not result in excessive stem elongation.

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CRediT authorship contribution statement

Wannida Sae-Tang: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Ep Heuvelink:** Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Wouter Kohlen:** Methodology, Investigation, Writing – review & editing. **Eleni Argyri:** Methodology, Investigation. **Céline C.S. Nicole:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Leo F.M. :** Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Leo Marcelis reports financial support was provided by Signify B.V., The Netherlands.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112614.

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