Research Article

Plant perception of light: the role of indoleamines in *Scutellaria* species

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ABSTRACT

Light mediates plant growth through diverse mechanisms and signaling networks including plant growth regulators (PGRs). We hypothesized that a novel class of PGRs, the indoleamines, are plant signaling molecules that perceive changes in light composition and initiate a cascade of metabolic responses. We used three *Scutellaria* model species (skullcap): *S. lateriflora*, *S. galericulata* and *S. racemosa* that produce high levels of melatonin and serotonin to investigate this hypothesis. Axenic *Scutellaria* cultures were exposed to red, blue, green or full spectrum white light spectra provided by light emitting diode (LED) lighting systems, or daylight fluorescent bulbs. Melatonin (MEL), serotonin (5HT), abscisic acid (ABA), auxin (IAA), and jasmonic acid (JA), were quantified by liquid chromatography with tandem mass spectrometry. Melatonin was detected consistently in plants grown under blue light in all species of *Scutellaria*. In *S. galericulata*, significant quantities of ABA were detected in plants grown under white light but not detected in plants grown under other light spectra. In timeline studies of *S. racemosa* plants exposed to limited red or blue light spectra had significantly reduced levels of tryptamine (TRM), 5HT and MEL in the shoots initially but melatonin was detected after 12 hours and quantifiable amounts of 5HT were detected after 7 days. Supplementation of the culture medium with MEL or 5HT did not change the pattern of MEL in blue light grown cultures but did change patterns of 5HT accumulation. 5HT was highest in plants grown under red light immediately after culture and decreased over 7 days. These data indicate that the relative amounts of MEL and 5HT are responsive to light spectra and redirect metabolic resources to enable plant adaptations to changing environments.

Keywords: melatonin, serotonin, *Scutellaria racemosa,* light signal transduction.

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1. INTRODUCTION

The genus *Scutellaria* in the Lamiaceae family includes about 360 species that are widely distributed across temperate, subtropical, and tropical regions of Europe, North America and Eastern Asia (1). *Scutellaria* species have been used in traditional medicine to treat diverse ailments, including cancers, hepatitis, cirrhosis, jaundice, anxiety, and nervous disorders (2). *S. lateriflora* L. is native to North America and has been used traditionally to stimulate blood flow,

reduce nervous tension and to treat mental or neurological illnesses, such as epilepsy, neurological damages from bacterial infections, insomnia, and anxiety (2). *S. racemosa* Pers. is a tropical species in the genus, native to Central and South America (3). Traditionally, *S. racemosa* has been used for its neuroprotective activity (4). *S. galericulata* L. is native to China and is an important part of traditional medicines for treatment of edemas (5, 6).

Previous research has identified indoleamine neurotransmitters in *Scutellaria* species (3, 7). Melatonin (MEL; *N*-acetyl-5-methoxy-tryptamine) and the related metabolite serotonin (5HT; 5-hydroxytryptamine) are human indoleamine neurotransmitters first described in *S. baicalensis* in 1997 (7). They have since been reported in *S. lateriflora, S. racemosa* (3), and more than 300 other plant species (8–10). MEL and 5HT are tryptophan (TRP) derived molecules whose biosynthesis in plants was first described by Murch et al. (2000) in *Hypericum perforatum* L. (St. John's wort) (11). Their roles in mediating plant growth were subsequently described in the same species with MEL generally promoting root induction while 5HT promoted shoot induction (12). Since then, MEL and 5HT have been found to mediate plant growth and development at every stage in the plant growth life cycle from promotion of germination, to mediation of morphogenesis, to protection and development of embryos (9). It was first proposed that their effects on plant growth were due to their structural similarity to the phytohormone auxin. Recent work, however, has refuted this hypothesis (13) and has instead shown that the indoleamines are a distinct class of plant growth regulator (PGR) working in a careful balance with each other to fine tune other plant signaling networks, including traditional classes of PGRs such as auxins and cytokinins (9, 14, 15). This classification has been supported by the recent discovery of a phytomelatonin receptor PMTR1(16) as well as Hyp-1 a potential mediator of MEL action and MEL/cytokinin cross-talk (17, 18).

It has been hypothesized that 5HT and MEL are plant signaling molecules that perceive and initiate responses to environmental cues such as insect feeding (19, 20) and light intensity and wavelength (10, 21). In general MEL levels have been reported to be highest in dark or low light conditions in St. John's wort (etiolated hypocotyls) (11) and *Arabidopsis* (22) ; however, other light environments have also been found to influence MEL levels. In licorice, MEL levels were highest after 3 months of growth under a red spectrum when compared to blue and white spectra after 3 months of growth (23). MEL has also been effective in stimulating growth of dark treated tissues, for example, stimulating growth and expansion of etiolated lupin hypocotyls (24).

We hypothesized that MEL is a plant signaling molecule that perceives changes in the environment to initiate a cascade of metabolic responses. To investigate this hypothesis, we constructed a simple system of light emitting diodes (LEDs) that deliver targeted spectra of light. We grew 3 species of *Scutellaria*: *S. lateriflora, S. galericulata* and *S. racemosa*, in sterile axenic culture. These species were chosen to represent diverse geographical regions with different native light spectra. Our objectives were to test the effects of 1) different light spectra, and 2) exposure time to these spectra on PGR levels and 3) to examine the impact of these light environments on uptake and utilization of exogenous indoleamines.

2. MATERIALS AND METHODS

2.1. Establishment of *in vitro* cultures.

Commercially available seed was used to initiate *in vitro* stock cultures of *S. lateriflora* (Richter's Herb's Inc., Goodwood, ON, Canada) (3) and *S. galericulata* (Prairie Moon Nursery,

Winona, MN, USA). Seeds of *S. racemosa* were collected from wild populations in December 2005, from three locations in North and Central Florida as listed on herbarium vouchers from the University of South Florida's Atlas of Florida Vascular Plants database (3). Seeds were surface sterilized by dipping in 70% ethanol for 30 s, then immersed in a solution of commercial bleach (1.5% sodium hypochlorite) containing a few drops of either Tween 20 or zinc pyrithione (8 mg/L) for 18 min, followed by 3 rinses with sterile distilled water (25). Seeds were cultured in 60 x 100 mm Petri dishes containing (VWR, PA, USA) 25 mL of water-agar (0.8%) and once germinated, were transferred to a culture medium containing MS salts (Murashigue & Skoog 1962), B5 vitamins (27) and 3.0% (w/v) sucrose, hereafter referred to as MSO. To control microbial growth, 8 mg/L zinc pyrithione (Sigma, St. Louis, MO, USA) was added to the initial medium. The pH of the media was adjusted to 5.75 with 1 N sodium hydroxide (Sigma, St. Louis, USA) and 0.3% Phytagel (Sigma, St. Louis, USA) was added before autoclaving at 121 °C, 1.4 kg cm^{-2} for 20 min. The resulting seedlings were transferred to Magenta boxes containing 25 mL of MSO medium and plantlets were maintained by regular subculture of nodal segments on MSO, every three weeks. All cultures were maintained in a controlled consistent environment with a 16-h photoperiod under daylight fluorescent bulbs (Sunblaster Holdings ULC, Langley; 30–45 μmol $m^{-2}s^{-1}$) (Figure 1b) at 28 °C.

2.2. Box lighting design (BLD).

A custom designed system used standard light emitting diodes (LED; Xcellent Global Multi-color Remote 12 Inch 18 RGB LED 16 Color Underwater Submersible Aquarium LED Light with Air Pump Bubble Light Strip) installed into simple lab-designed reflective mylar-lined (Easygrow Reflective Film; Sunlight Supply, Vancouver WA) plastic bins to ensure even exposure to light (Figure 1a). Air circulation in the boxes was provided by a standard aquarium air pump (Air Tech 2KO) using the bubble system of the LED strips. Light treatments were applied to compare the standard daylight fluorescent spectrum with the LED standard settings for full white, blue, red and green spectra and light intensity and spectrum were measured at culture height inside the box (Figure 2; Sekonic Spectromaster C-700; Sekonic Corp. Japan) (Figure 1b – f).

2.3. Chamber lighting design (CLD).

Replicate controlled environment chambers (Percival AR36L3; Percival Scientific, Iowa) maintained at 28 °C with a 16-hour photoperiod were fitted with either (a) red light (CLD-Red) provided by the LEDs in the growth chamber (Figure 1g) or (b) blue light (CLD-Blue) was provided by a PowerPAR LED bulb (Hydrofarm, California) (Figure 1h).

2.4. Experimental design.

S. lateriflora, S. galericulata and *S. racemosa* plantlets in Magenta boxes were transferred to the experimental setup and exposed to selected spectra of LED lights for 67.5 ± 0.5 hours. Other than the light environment, growth conditions were kept the same as in the stock culture room. Tissues were aseptically harvested and immediately flash frozen with liquid nitrogen to halt and preserve metabolic processes and stored in a – 80 °C freezer until extraction.

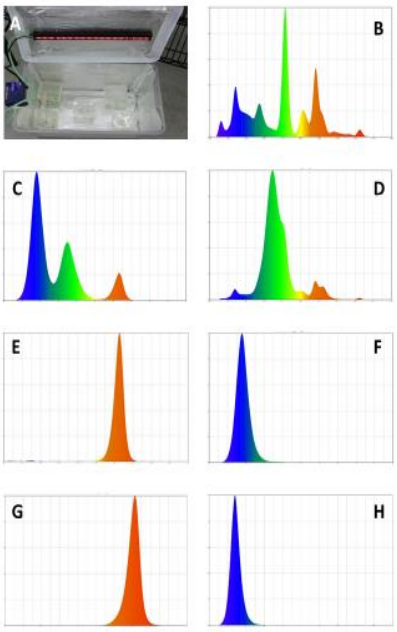

Fig. 1. Lighting box and lighting designs.

(*a) Box light design (BLD) setup; (b) emission spectra of daylight fluorescent Sunblaster™ bulbs; (c) emission spectra of LED white light BLD; (d) emission spectra of LED green light BLD; (e) emission spectra of LED red light BLD; (f) emission spectra of LED blue light BLD; (g) emission spectra of LED red light CLD; (h) emission spectra of LED blue light CLD.*

2.5. Effects of time.

S. racemosa plantlets were subcultured into 25 mm glass culture tubes (Phytotech Labs, Kansas) containing 10 mL of MSO media and grown for 21 days in the same controlled environment stock culture room as described above until transfer. Established plantlets were incubated in the CLD for 21 days with interval harvesting with the same growth conditions were kept the same as in the stock culture room – other than the light environment. Initial harvesting (t = 0) took place at 21:00 and time interval harvesting was kept consistent with t = 0. Tissues were harvested by immediate immersion in liquid nitrogen. Shoots and roots were excised prior to storage at – 80 °C.

2.6. Effects of exogenous indoleamines.

S. racemosa plantlets were subcultured into 25 mm glass culture tubes (Phytotech Labs, Kansas) containing 10 mL of solid MSO media and grown for 21 days in the same controlled environment stock culture room as described above until transfer. Day 0 control plants were harvested, and the remaining plants were dosed by adding 100 mM stock solutions of MEL or 5HT to the top of the solid media creating either a 100 µM or 300 µM concentration of MEL or 5HT – these plants were then transferred to CLD-Red/Blue or back to the control white light. The control plants for each treatment were grown for 7 days on MSO media in the initial growth environment or specified light environment provided by Setup version 2 in the Percival controlled environment chambers.

2.7. Quantification of indoleamines and stress metabolites.

TRP, MEL, 5HT, tryptamine (TRM), indole-3-acetic acid (IAA), abscisic acid (ABA), and jasmonic acid (JA) were extracted, detected, and quantified as previously described (28–30). In brief, samples were prepared in a dark room under a single red light to avoid light degradation of the indoleamines during extraction. Eppendorf tubes containing samples were accurately weighed and sample weight determined by difference and homogenized in a solution of 80% methanol (Optima Grade, Fisher Scientific, Mississauga, ON) and 20% 0.5 N tricholoracetic acid (TCA; Sigma, Mississauga, ON) in 18 mΩ E-Pure water™ (Millipore) with a disposable tissue grinder (Kontes Pellet Pestle; Fisher). Samples were centrifuged (13,000 x g) for 3 minutes, the supernatants were decanted and filtered (0.2 mm, Ultrafree-MC filtered centrifuge tubes; Millipore) prior to chromatography. Analytes were separated on a reverse phase column (30 x 3 mm, 2.6 µm C18 100 Å, Phenomenex, Torrance, CA) using an Acquity I-Class BSM UPLC (Waters, Mississauga, ON) over a gradient of 0.1% formic acid (Eluent A) and acetonitrile (Eluent B) [(A%:B%)]: 0.0–0.5 min, 90:10; 0.5–3.5 min, 40:60; 3.5–4.2 min, 5:95; 4.2–6.5min, 5:95; 6.5–7.0 min, 90:10] with a flow rate of 0.3 mL/min. Analytes were identified and quantified with a tandem mass spectrometer (Xevo TQ-S triple quadrupole Mass Spectrometer, Waters) using optimized settings as follows: capillary voltage at 3500; desolvation gas rate at 800 L/hr; cone gas at 150 L/hr; desolvation temperature at 550 °C; source temperature at 150 °C. Parent and daughter ions were detected using the appropriate optimized Multiple Reaction Monitoring (MRM) transitions (Table 1). Quantification was done by comparison to a dilution series of authentic standards (Sigma) for each compound (0, 0.001, 0.025, 0.5, 10, 200, 400, 4000 ng/mL in the homogenizing solution) (30). Since the dynamic range of the data spread over 4 orders of magnitude, data for the external standard curve underwent a LOG transformation for linearity. The Limit of Detection (LOD) was determined at ≈ 10 pg on column by the lowest concentration with an observed signal (> 3 S/N) for all compounds. Positive signals in extracts were quantified within the linear range of 40 – 2000 pg on column.

Table 1. Fragmentation patterns and optimized voltages.

Compound	Transitions (m/z)	Cone Voltage (V)	Collision Voltage (V)	Retention Time (min)
Serotonin (5HT)	177>160 177>115	45	10 27	0.77
Melatonin (MEL)	233>159 233>174	30	23 15	2.49
Indole-3-Acetic Acid (IAA)	176>103 176>130.1	30	25 13	2.60
Jasmonic Acid (JA)	211>133 211>151	22	14 10	3.16
Abscisic Acid (ABA)	265>135 265>147	20	22 6	2.77
Tryptophan (TRP)	205.1>188.1 205.1>146	18	1 18	1.13
Tryptamine (TRM)	161>117 161>161	15	15 1	1.45

2.8. Statistical design and analyses.

Experiments characterizing the *Scutellaria* model systems, 9 biological replicates of each of the *Scutellaria* species were grown and 3 were chosen randomly and analyzed. For detailed studies of *S. racemosa* responses, 3 individual plantlets were analyzed per treatment per time point. Normality of data was confirmed prior to analysis by a one-way analysis of variance (ANOVA) with a post-hoc Tukey multiple means comparison using JASP 0.8.5.1 (Eric-Jan Wagenmakers, Amsterdam). Significance was determined at 95 % confidence ($\alpha = 0.05$). Statistical analyses for CLD experiments were performed using Prism 8.0.2 (GraphPad Prism LLC). Data were found to be normally distributed as were compared using a two-way analysis of variance (ANOVA) with a post-hoc Dunnett's multiple comparisons test roots with a = 0.05.

3. RESULTS

3.1. Characterization of the *Scutellaria* model system.

All cultures were grown on media containing adequate sugar, vitamins and minerals to support growth of the plantlets. The plantlets appeared vigorous and were not visibly damaged by 3 days exposure to restricted light spectra.

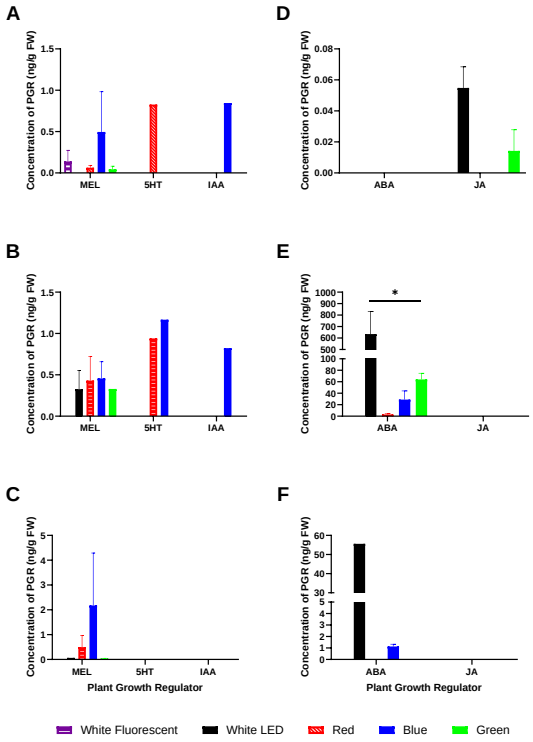

Fig. 2. Characterization of the responses of three *Scutellaria* species to varying light conditions.

*Top row: Scutellaria racemosa; Middle row: Scutellaria galericulata; Bottom row: Scutellaria lateriflora. (A, B, C) melatonin, serotonin and auxin. (D, E, F) abscisic acid and jasmonic acid. Error bars represent standard error for n=3. Bars without standard error bars were detected in < 3 samples. Significance is shown with stars comparing between the light treatments. * $p \leq 0.05$.*

3.2. Growth under white LED (BLD) and daylight fluorescence light.

MEL was detected in plantlets of all three species grown under white or daylight spectra (Figure 2a, b, c). *S. racemosa* plantlets produced detectable levels of MEL under daylight fluorescent bulbs but not when LED lights were used (Figure 2a). *S. galericulata* and *S. lateriflora* plantlets had detectable MEL when grown under white LED spectra (Figure 2b, c). IAA and 5HT were not detected in plantlets grown under white or green light for any of the 3 species (Figure 2a, b, c). ABA was found in *S. galericulata* and *S. lateriflora* plantlets grown under BLD-White light (Figure 2e, f). JA was detected in *S. racemosa* plantlets grown under BLD-white light (Figure 2d).

3.3. Effects of red light (BLD-Red).

MEL was detected in all three species when they were grown under red LED (Figure 2a, b, c). Detectable levels of 5HT were observed in one replicate of *S. racemosa* and *S. galericulata* under red LED lights (Figure 2a, b). IAA was not detected in any of the Scutellaria plantlets grown under red LED lights (Figure 2a, b, c). *S. galericulata* plantlets grown under red LED lights had detectable levels of ABA but ABA was not measured in the other species under the same conditions (Figure 2e).

3.4. Effects of blue light (BLD-Blue).

All of the *Scutellaria* species accumulated MEL when grown under blue LED lights (Figure 2a, b, c). 5HT was detected in one biological replicate of *S. galericulata* grown under blue light (Figure 2b). IAA was detected in *S. racemosa* and *S. galericulata* plantlets grown under blue light (Figure 2a, b). ABA was found in *S. galericulata* and *S. lateriflora* plantlets grown under the blue LED lights (Figure 2e, f).

3.5. Effects of green light (BLD-Green).

MEL was above detection limits in plantlets of all 3 species grown under green LED spectra (Figure 2a, b, c). 5HT and IAA were not detected in any of *Scutellaria* plants grown under green LED lighting (Figure 2a, b, c). *S. galericulata* plantlets grown under green LED lights had detectable levels of ABA (Figure 2e). *S. racemosa* plantlets grown in the green LED system had detectable concentrations of JA (Figure 2d).

3.6. Plant signaling responses over time in S. racemose.

Following the preliminary characterization experiments, *S. racemosa* was selected as the best model for determining how plants responded to selected red and blue light spectra and a time course study was conducted to determine how the plantlets adapted to the restricted light conditions. The flow of metabolites through the pathway from tryptophan and auxin to 5HT and MEL was characterized (Figure 3). None of the metabolites were detected in the tissues at the initiation of the experiment when explants were cut, and nodal cuttings were subcultured onto basal media (Figure 3). The patterns of accumulation of indoleamine metabolites varied in response to the red and blue light spectra (Figure 3).

3.7. Effects of red light (CLD-Red).

TRP accumulated in *S. racemosa* plants grown under red LED spectra from 0.5 days to 5 days of culture (Figure 3a). Between 5 – 21 days of culture under the same conditions, the metabolic pool of TRP plateaued with a decline in total concentration as the plantlets grew (Figure 3a). Concentrations of IAA were below the limits of detection in the *S. racemosa* shoot cultures grown under red LED spectra, with the exception of a single sample collected after 2 days of culture (Figure 3b). TRM concentrations gradually increased throughout the culture period (Figure 3c). A single plantlet sample was found to have a sporadic spike in TRM after 48 hours of culture under red LEDs (Figure 3c). 5HT was below detectable concentrations in S. racemosa plantlets grown under red LEDs for the first 7 days of the culture period but was consistently detected during plantlet growth (Figure 3d). MEL was detected after one day of plantlet culture under red LEDs and increased with a roughly linear trend throughout the culture period (Figure 3e).

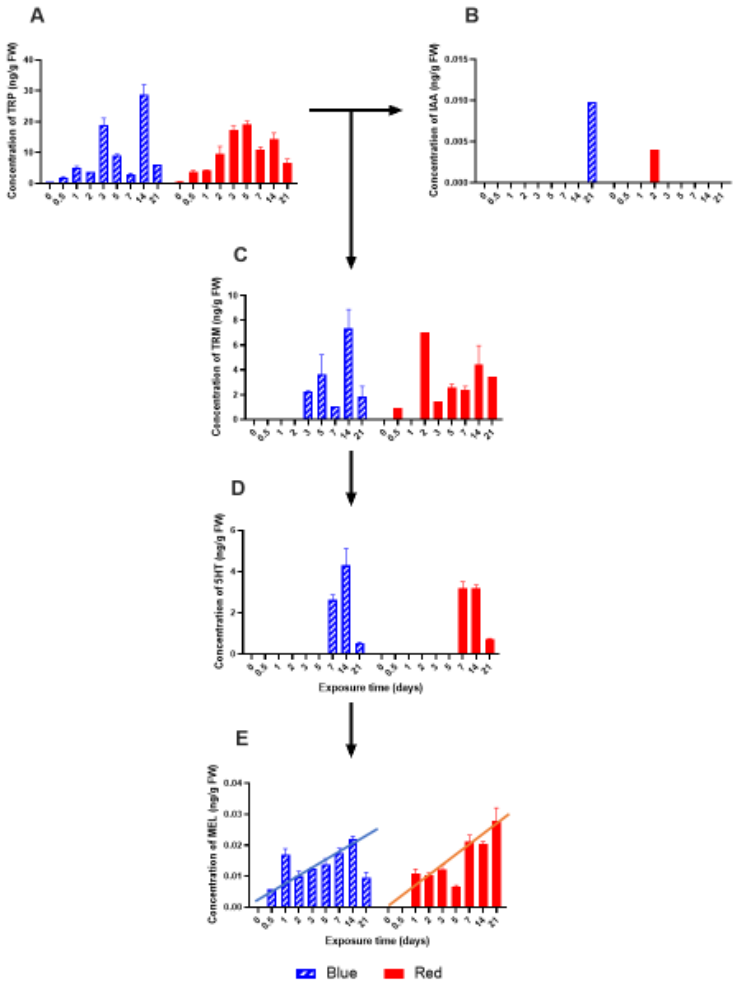

Fig. 3. Effect of red and blue light on indole pathway metabolites in *Scutellaria racemosa* shoot cultures.

Error bars represent standard error for n=3. Bars without standard error bars were detected in < 3 samples. No statistically significant relationships were detected between the different light treatments.

3.8. Effects of blue light (CLD-Blue).

Concentrations of TRP fluctuated throughout the culture period in *S. racemosa* plantlets grown under blue LED spectra with a maximum amount after about 2 weeks of culture when the plantlets were expressing the most vigorous growth (Figure 3a). Similar to the shoot cultures grown under red lights, the S. racemosa cultures grown under blue lights were not found to have significant concentrations of IAA with the exception of a single sample harvested after 21 days (Figure 3b). TRM was detected in *S. racemosa* plantlets after 5 days of culture under blue LED spectra and fluctuated throughout the 21-day incubation (Figure 3c). 5HT was detected in S. racemosa plantlets after 7 days of growth under blue LED spectra (Figure 3d). MEL concentrations in *S. racemosa* shoot explants incubated under blue LED lights were below detection limits and increased in a linear fashion with the growth of the plantlets (Figure 3e).

3.9. Effects endogenous application of indoleamines.

In follow-up experiments, to understand the role of 5HT and MEL in the growth and development of *S. racemosa* plantlets under restricted red and blue light conditions, the culture media was supplemented with exogenous 5HT and MEL. Plantlets grown under full spectrum daylight fluorescence bulbs (white) did not initially have detectable amounts of 5HT or MEL (Figure 4). Low concentrations of MEL were detected in the control plantlets after 7 days of culture on basal media (Figure 4b, d). Supplementation of the culture media with 100 µM or 300 µM 5HT resulted in significant amounts of 5HT in the plantlets grown under daylight fluorescent lights for 1 and 7 days indicating that 5HT was taken up from the media by the plantlets (Figure 4a). The plantlets incubated on 5HT enriched media and grown under white lights also produced detectable levels of MEL after 1 and 7 days (Figure 4b). Supplementation of the culture medium with 100 µM or 300 µM MEL resulted in detectable levels of MEL in plantlets after 1 and 7 days of incubation under full spectrum fluorescent bulbs (Figure 4d). MEL conversion to 5HT was not observed in any of the plantlets grown under white lights (Figure 4c).

3.10. Effects of red light (CLD-Red).

5HT was below detection limits in plantlets grown on the control MSO media and incubated under red LED spectra for 1 or 7 days (Figure 4a). When 5HT was supplemented into the media at 100 µM and 300 µM, 5HT accumulated in the plant tissues after one day and was metabolized, conjugated or eliminated by 7 days of culture under red LED spectra (Figure 4a). Supplementation of the media with 5HT also resulted in significant accumulations of MEL in cultures grown under red LEDs for 1 or 7 days (Figure 4b). Low concentrations of MEL were detected in plantlets grown on basal MSO media under red LED lights for 1 and 7 days (Figure 4d). When MEL was supplemented into the culture media and the plantlets were grown under red LEDs, the amounts of detected MEL in plantlets grown under red LEDs significantly increased (Figure 4d). Supplementation of the culture media with MEL and incubation under red LEDs resulted in an initial spike of 5HT after 1 day of culture but the increased levels were not sustained through the culture period (Figure 4c).

3.11. Effects of blue light (CLD-Blue).

5HT was not detected in plants grown on basal MSO media incubated under 1 day of blue light (Figure 4a, b). Supplementation of the culture medium with 100 µM or 300 µM 5HT resulted in significant accumulations of 5HT in plantlets grown under blue light indicating uptake from the media (Figure 4a). The level of 5HT in the plantlets was consistent after 7 days of culture under blue LED spectra indicating that the 5HT was not metabolized (Figure 4a). Supplementation of the culture media with 5HT and incubation under blue LEDs also led to the accumulation of low levels of MEL in *S. racemosa* plantlets (Figure 4b). Supplementation of the culture media with MEL and incubation under blue LED lights resulted in accumulation of MEL in the plantlets (Figure 4d) but none of the MEL was converted to 5HT (Figure 4c).

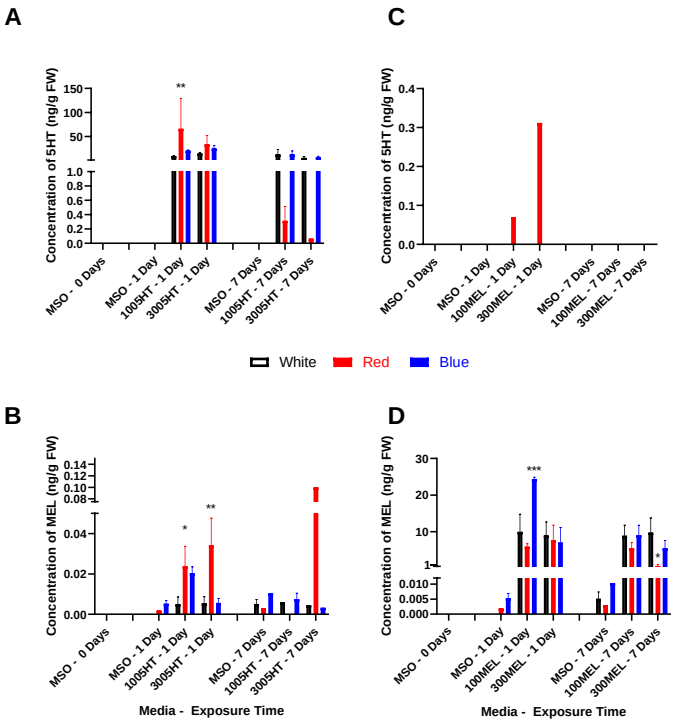

Fig. 4: **Effect of supplementation of the culture media with 5HT or MEL on indoleamine metabolism during red and blue light incubations.**

*(A) Effects of 100 or 300 µM 5HT on 5HT concentrations; (B) Effects of 100 or 300 µM 5HT on MEL concentrations; (C) Effects of 100 or 300 µM MEL on 5HT concentrations; (D) Effects of 100 or 300 µM MEL on MEL concentrations. Error bars represent standard error for n=3. Bars without standard error bars were detected in < 3 samples. Significance is shown with stars comparing between the light treatments. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.*

4. DISCUSSION

Light has traditionally been considered in plants primarily in the context of its role as the energy source of photosynthesis, however, with the discovery of the importance of the red/far red balance in the control of phytochrome and auxin signaling networks there was also an interest in the role light may play not just as an energy source, but also as a plant signal. With the advent of LED

lighting technology, there is a greater capacity using tunable lighting systems to manipulate plant growth. Red and blue light are well characterized "useful" wavelengths of light with specific photoreceptors but interactions with indoleamine plant growth regulators 5HT and MEL has not previously been described. MEL and 5HT are plant growth regulators with their diverse roles in mediating diverse aspects of plant growth and metabolism (9). Modulation of the relative amounts of 5HT and MEL enables fine control of plant processes (12, 15, 24, 31). We have previously proposed that MEL functions as a transient signal to allow plants to interpret and respond to their environment (30). MEL has been suggested to potentially function via traditional light signaling networks such as the COP9 (constitutive photomorphogenesis 9) signalsome, where a series of tryptophan residues (the indoleamine precursor) serves as the chromophore (32). The current study was designed to understand the interactions between light spectra, 5HT and MEL metabolism. MEL and 5HT responded differently to the light environments.

The process of explanting and subculturing shoots imposes a substantial physiological stress on plant cells and accumulation of reactive oxygen species (ROS) (33). In our study endogenous MEL levels recovered from explanting stress faster in plantlets cultured under blue lights. Explanting stress has also been shown to serve as an inductive signal in plants, triggering downstream signaling cascades and eventually leading to morphogenesis (33). MEL is a well-established and potent antioxidant, and previous studies have hypothesized MEL is an important mechanism to reduce metabolic stress during regeneration (34). MEL has also been found to play important roles in mediating diverse other abiotic stresses including temperature, heavy metal and salinity stress (21). In other systems, blue light has also been found to increase antioxidant potential and anthocyanin content through multiple mechanisms (35–39) and a relationship between MEL and increased anthocyanin content has been proposed (40) as well as an interaction between MEL and blue light leading to increase flavonoid content (41). These results suggest that MEL may be an early inductive signal, enhanced through blue light exposure. These are the first data that indicate regulation of MEL stress response through blue light photoreceptors.

MEL is best known for its function as the chemical expression of darkness in humans and other mammals as it controls the circadian clock, light-dark responses and seasonality. Biosynthesis of MEL is suppressed under light and this inhibition is relieved as light levels decrease, with blue light being up to three times more potent than higher wavelengths of light (42). Initial investigations into the function of MEL in plants therefore focused on illuminating a role for MEL in plant circadian processes, either through light mediated effects on MEL (23, 43), or daily variations in MEL levels (29, 44) . A recent development in understanding of the mechanisms of MEL actions in plants is their interactions with plant NADPH Oxidases (NOX) which play important roles in rapid ROS-mediated signaling in plant perception of insect feeding, mechanical wounding, salinity and temperature stresses (45, 46) and been found to act downstream of the MEL receptor PMTR1 (16). In the mouse system MEL was found to mimic blue light signaling at the mouse skin plasma membrane-associated ECTO-NOX protein, leading the authors to suggest that this may play a role in entrainment of the biological clock (47). Though no link between light perception and MEL mediated by NOX has been identified in plants, evidence in the mouse system suggests possible mediation of plant circadian rhythms via a NOX-MEL-ROS signaling cascade which may be downstream of blue light receptors, or may act through a novel receptor.

The second significant finding of this work is the unusual pattern of 5HT levels in plantlets exposed to red light and exogenous 5HT. 5HT is known to be a mediator of phytochrome action with exposure to 5HT being capable of mimicking red light exposure via modulation of calcium signaling, nitrate reductase and phosphatidyl inositol turnover (48–51). It was therefore expected

that while 5HT supplementation would enhance the responses to red light. The obvious route of metabolism would be conversion of 5HT to MEL, however, while there are measurable levels of MEL at 300 µM 5HT treatment, only low levels of MEL were detected in the 100 µM 5HT treatment suggesting that the process is more complex. Neff (2012) found that activation of phytochromes in the presence of red light led to decreased levels of ABA in *Arabidopsis* (52). The mechanism may be related to the activation of the CYP707A1/2 gene which is activated via red light that encodes for ABA catabolism – a gene that has also been found to be regulated by MEL levels (53).

The degradation pathways for indoleamines in plants are only beginning to be understood. Catabolism of MEL has been hypothesized to occur primarily via cyclization and hydroxylation (45). It is possible that 5HT may be converted to MEL and then rapidly degraded explaining the lack of an increase in the MEL pool observed here. Alternately it is possible that 5HT undergoes rapid degradation via a MEL-independent pathway. Another possible explanation for the decreased 5HT content at 7 days may be due accelerated conjugation or metabolism of 5HT under red light conditions as a regulatory mechanism. Several phenolic conjugates of 5HT are known to exist as cell wall and wounding responsive components, and include coumaroyl- feruloyl-, sinapoyl- and cinnamoyl-5HT (53, 54). It is also possible, however, that further previously undescribed 5HT conjugates may exist in a manner analogous to other PGRs. For example, many PGRs undergo conjugation to amino acids which either leads to their deactivation as is the case for auxin or which represents a more bioactive form as is the case for JA-Ile (55, 56). It is possible that 5HT conjugation may perform a similar function to that observed for IAA. Auxin has been shown to conjugate to sugars, peptides and proteins, with only a small fraction of auxin present in free form in plant cells. This allows plants to transport the potent signal without undesired side effects and also creates a sequestered but readily available supply should the need arise (55). It is also possible that the decline in 5HT under red light is part of a plant growth regulator cross-talk cascade.

Further research is necessary to fully understand the relationship MEL and 5HT signaling mechanisms that enable plant responses to changing light spectra. From our current data, we hypothesize that MEL metabolism is a rapid response and short time exposures to blue light systems may provide cues to signaling mechanisms. Further investigation into the relationships between MEL and reactive oxygen, nitrous oxide and other signals will provide greater understandings of plant responses to environmental cues. In addition, our data point to the necessity of a comprehensive hormonomics analysis that includes MEL and 5HT conjugates to determine the full size of the metabolic pool of bioactive indoleamine plant growth regulators. Understanding the relationship between MEL, 5HT and environmental cues will provide new insight into the resilience and vulnerability of plants in changing climates.

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AUTHORSHIP

JAF contributed to the design and conducted the experiments, acquisition, analysis, and interpretation of the data, and manuscript preparation; LAEE participated in the analysis of the

data and manuscript preparation; PRS participated in conception and design of the work; SJM developed the hypothesis and oversaw the design of the study, the resources for the project and the manuscript preparation. All authors participated in the revision of the manuscript and provided approval of the final manuscript.

CONFLICT INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be made available upon request to the corresponding author.

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