Growth of micropropagated *Pontederia cordata* using broadband white light with or without far-red radiation

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Abstract

***Pontederia cordata* is a herbaceous aquatic plant commonly used for wetland restoration and mitigation projects. Aiming to reduce negative ecological impacts from plant overcollection, mass production of *P. cordata* has transitioned to indoor micropropagation with sole-source lighting, which typically lacks far-red (FR) radiation. The objectives of this study were to 1) quantify growth of micropropagated *P. cordata* in response to five sole-source lighting treatments with or without FR radiation; and 2) assess treatment effects after finishing in a common greenhouse. Four treatments provided 64±2 µmol m^{-2} s^{-1} of broadband white light from cool-white fluorescent (FL) or light-emitting diode (LED) lamps with ($FL_{64}FR_{17}$ or $LED_{64}FR_{17}$, respectively) or without (FL_{64} or LED_{64}, respectively) 17±2 µmol m^{-2} s^{-1} of FR radiation. Another treatment provided 47±2 µmol m^{-2} s^{-1} from FL + 17±2 µmol m^{-2} s^{-1} of FR ($FL_{47}FR_{17}$). Our findings show that without FR radiation, explants of *P. cordata* grown under FL had larger leaves than those grown under LEDs, but produced a similar number of shoots and roots, and had the same shoot FM and DM. In addition, regardless of intensity, 17 µmol m^{-2} s^{-1} of FR supplemented to FL increased leaf length and root formation compared to FL_{64}, but the highest number of shoots per explant were produced under LED_{64}. In general, $FL_{47}FR_{17}$ produced a higher shoot biomass than all other treatments, demonstrating the potential benefit of adding FR to broadband white light during micropropagation, particularly under low daily light integrals. Furthermore, our results show that FR radiation applied in vitro may benefit growth and morphology during subsequent transplant production of *P. cordata*.**

Keywords: LEDs, light-emitting diodes, micropropagation, native plants, red to far-red ratio, young plants

INTRODUCTION

Pontederia cordata is a herbaceous aquatic plant that can serve several functions in natural ecosystems and is therefore, popular for habitat restoration or mitigation projects that typically require tens of thousands of plants (Burlakova et al., 2009; McConnell et al., 1990). Increased demand for *P. cordata* led to over-collection and subsequent environmental damage to donor wetland sites. Kane and Philman (1997) first proposed the use of in vitro culture (micropropagation) with sole-source lighting as an ecologically sound method to mass produce wetland plants, and several studies since have used micropropagation to rapidly screen, select, and store germplasm of *P. cordata*.

Fluorescent lamps used to be the light source of choice for micropropagating plants (Chen, 2005). However, light-emitting diodes (LEDs) are continuously being used to replace fluorescent lamps for sole-source lighting, partly due to their high efficacy and large spectral availability (Mitchell and Stutte, 2015; Gómez and Izzo, 2018). Although early LED systems for plant production were equipped with red and blue LEDs alone, research has shown that plants can use most of the light within the photosynthetic active radiation spectrum (400-700

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nm) for photosynthesis and growth (Ouzounis et al., 2015). Therefore, commercial fixtures with broadband white LEDs are increasingly being used for sole-source lighting (Kozai, 2016). Nonetheless, far-red radiation (700-800 nm; FR) is often disregarded as a useful waveband for photosynthesis and growth despite findings that show positive direct and indirect effects when used in combination with other wavebands (Christiaens et al., 2016; Park and Runkle, 2017; Zhen and van Iersel, 2017). Therefore, the objectives of this study were to 1) quantify growth of micropropagated *P. cordata* in response to five sole-source lighting treatments with or without FR radiation; and 2) assess treatment effects after finishing transplants in a greenhouse. We hypothesized that FR radiation could increase growth and quality of *P. cordata* and that positive results would carry-over to greenhouse-grown transplants.

MATERIALS AND METHODS

The experiment was conducted during the in vitro multiplication stage (stage II) of *P. cordata*. Shoot explants from previously established stock cultures were multiplied prior to starting the experiment and only newly established plant material was used for treatment comparisons. Shoot explants were transferred to 350 mL (77×77×97 mm) Magenta GA-7 polycarbonate vessels (Magenta Corp., Chicago, IL, USA), which contained 40 mL of shoot multiplication medium consisting of full-strength Murashige and Skoog basal salts (Murashige and Skoog, 1962), 3% sucrose, 0.4 mg L^{-1} thiamine HCL, 100 mg L^{-1} myo-inositol, 50 mg L^{-1} citric acid, 50 mg L^{-1} ascorbic acid, 2 mg L^{-1} BA, 1 mg L^{-1} IAA, and solidified with 0.8% TC agar (PhytoTechnology Laboratories, Shawnee Mission, KS, USA). The medium was adjusted to pH 5.7 with 0.1 N KOH prior to autoclaving for 20 min at 118 kPa and 121°C. Cultures were established for three weeks in a growth room set at a constant 25°C; cool-white fluorescent lamps provided 40 µmol m^{-2} s^{-1} for 16 h d^{-1} (from 06:00-22:00 $_{HR}$). One day before starting the experiment, four uniform shoot explants were transferred into single vessels containing 40 mL of fresh medium.

Five treatments were evaluated in the experiment. Four treatments provided a 3.7 mol m^{-2} d^{-1} daily light integral (DLI) achieved by using a photon flux density of 64±2 µmol m^{-2} s^{-1} of broadband white light from cool-white fluorescent (FL) (Model 64243; GE Lighting, Cleveland, OH, USA) or LED (Model 40803; Green Creative, San Bruno, CA, USA) lamps with ($FL_{64}FR_{17}$ or $LED_{64}FR_{17}$, respectively) or without (FL_{64} or LED_{64}, respectively) 17±2 µmol m^{-2} s^{-1} of FR radiation (RAY66 lamps; Fluence Bioengineering, Austin, TX, USA), and a constant 16 h d^{-1} photoperiod (06:00-22:00 HR) (Figure 1). Another treatment consisted of a 2.7 mol m^{-2} d^{-1} DLI from 47±2 µmol m^{-2} s^{-1} of FL + 17±2 µmol m^{-2} s^{-1} of FR ($FL_{47}FR_{17}$) using the same photoperiod. Photon flux density was adjusted based on the average of nine measurements recorded at mid-canopy height within each experimental area using a spectroradiometer (SS-100; Apogee Instruments, Inc., Logan, UT, USA). Lamps hung from different compartments (183-cm long × 41-cm wide × 61-cm tall) within multilayer shelves placed inside a growth room set at a constant temperature of 21°C, ambient CO_2 levels, and 50-70% relative humidity. Ten randomly selected vessels were placed within each treatment compartment. Light pollution (≤5 µmol m^{-2} s^{-1}) within treatments was minimized by covering the sides and back of the shelves with a double layer of 0.3-mm white polyethylene film. Vessels were randomly rotated daily to minimize location effects within each treatment compartment. A shielded temperature and RH sensor (RC-4HA/C; Elitech, Milpitas, CA, USA) was placed at the center of each compartment to provide real-time data monitoring and ensure that ambient temperature differences among treatments were ≤1°C.

The experiment was replicated three times; all treatments were re-randomized within the growth room before the start of each experimental replication. Leaf length, leaf area, number of shoots and roots per explant, and shoot fresh (FM) and dry mass (DM) were destructively measured from two microcuttings per vessel 21 d after treatment initiation. In addition, for each treatment, two microcuttings per vessel were rinsed and transplanted into individual cells within 48-cell plug trays (100-mL individual cell volume) filled with horticultural grade substrate composed (v/v) of 60% peat and 40% perlite (Sunshine Mix #4, Sun Gro Horticulture, Agawam, MA, USA). Transplants were placed in a greenhouse in Gainesville, FL (lat. 29°N, long. 86°W; US Department of Agriculture hardiness zone 9a) and

grown for 21 d under 5-10 mol m^{-2} d^{-1}, 20-27°C, ambient CO_2, and 95-70% relative humidity provided by overhead intermittent mist. For each transplant, shoot and root number, length of the longest root, and total leaf length were measured destructively at harvest.

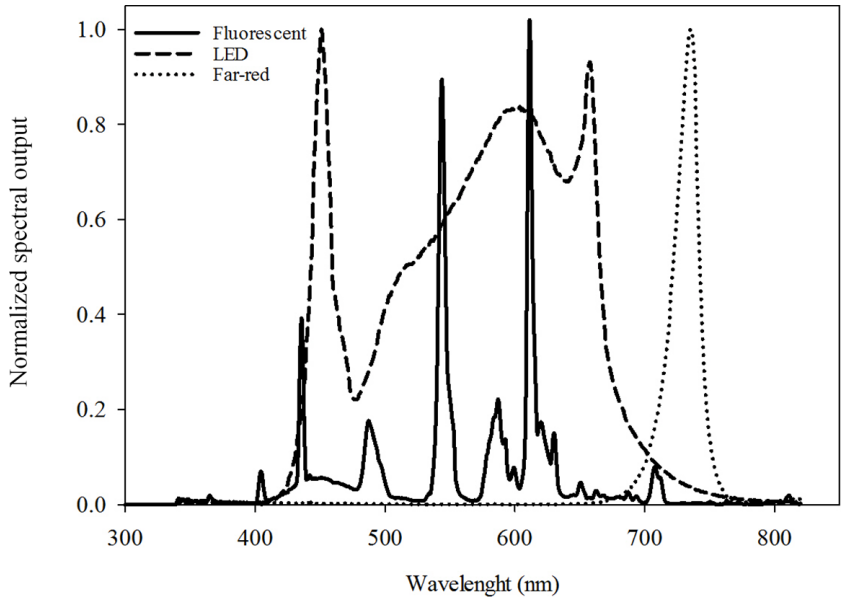

Figure 1. Normalized spectral output of the lamps used in the experiment.

The experiment used a randomized complete block design with three blocks (replication of time) and ten sub-samples per block (mean of two microcuttings per vessel). All microcuttings under each treatment compartment were regarded as the experimental unit. Analysis of variance and mean separation by Tukey's honestly significant difference test ($P \leq 0.05$) were performed for all response variables using the SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA) PROC MIXED procedure.

RESULTS AND DISCUSSION

Explants grown under FL_{64} produced slightly longer and significantly larger leaves than those grown under LED_{64}, and leaves developed under $FL_{64}FR_{17}$ were longer than those under $LED_{64}FR_{17}$ (Table 1). Regardless of PPFD, 17 µmol m^{-2} s^{-1} of FR supplemented to FL increased leaf length and root number compared to FL_{64}. This corresponds with previous findings that have reported an increase in leaf expansion and adventitious rooting with FR radiation supplemented to other light-quality treatments (Christiaens et al., 2019; Park and Runkle, 2017). Explants grown under LED_{64} were exposed to the highest R/FR ratio and the lowest percentage of blue light (Table 2; Figure 1). A high R/FR ratio can reduce stem elongation and leaf area expansion (Christiaens et al., 2019). Similarly, blue light often inhibits cell division and expansion and thus, tends to reduce leaf area (Bugbee, 2016). Therefore, the different responses in leaf length and leaf area measured between FL_{64} and LED_{64} could be attributed to the different spectral composition of each lamp and its resulting R/FR ratio. Furthermore, the larger number of roots per explant produced under $FL_{64}FR_{17}$ compared to FL_{64} or LED_{64} could be related to its lower R/FR ratio and higher yield photon flux density, which weighs photons according to photosynthetic responses. Christiaens et al. (2016) explained that adventitious rooting increases when FR is used as a supplement to other wavelengths for both in vivo and in vitro propagation of plants, possibly as a response to higher auxin biosynthesis.

Table 1. Growth responses to five sole-source lighting treatments used during the in vitro multiplication stage of *P. cordata*.

Treatment[a]	Leaf length (cm)	Leaf area (cm^2)	Shoots per explant (no.)	Shoot FM (g)	Shoot DM (g)	Roots per explant (no.)
FL_{64} (control)	2.3 ab[b]	18.7 a	8.5 ab	1.8 ab	0.12	2.8 c
LED_{64}	2.1 b	15.8 b	9.4 a	1.9 ab	0.12	2.5 c
$FL_{64}FR_{17}$	2.5 a	17.6 ab	7.1 b	1.7 b	0.12	4.5 a
$LED_{64}FR_{17}$	2.2 b	17.7 ab	6.1 c	1.8 ab	0.12	2.7 c
$FL_{47}FR_{17}$	2.5 a	17.5 ab	5.8 c	2.2 a	0.15	3.7 b

[a]FL or LED = broad spectrum cool-white fluorescent or light-emitting diode lamps; FR = far-red LEDs. Values are in µmol m^{-2} s^{-1}.

[b]Means within column with different letters are significantly different by Tukey's honestly significant difference test (P<0.05).

Table 2. Spectral characteristics of five sole-source lighting treatments used during the in vitro multiplication stage of *P. cordata.*.

Treatment[a]	Blue (400-499 nm)	Green (500-599 nm)	Red (600-699 nm)	Far-red (700-820 nm)	PPFD[b]	YPFD[c]	TPFD[d]	R/FR[e]
FL_{64}	15 (24)[f]	26 (41)	22 (35)	2	63	56	65	2.9
LED_{64}	12 (19)	27 (42)	24 (39)	1	63	55	64	13.6
$FL_{64}FR_{17}$	17 (26)	26 (40)	22 (34)	18	65	61	81	1.3
$LED_{64}FR_{17}$	14 (21)	27 (41)	24 (38)	17	65	61	82	1.6
$FL_{47}FR_{17}$	11 (26)	20 (41)	16 (33)	18	47	44	65	1.2

[a]FL or LED = broad spectrum cool-white fluorescent or light-emitting diode lamps; FR = far-red LEDs. Values are in µmol m^{-2} s^{-1}.

[b]PPFD: photosynthetic photon flux density (photon flux integral between 400 and 700 nm, in µmol m^{-2} s^{-1}).

[c]YPFD: yield photon flux density, which is the product of radiation intensity and relative quantum efficiency (in µmol m^{-2} s^{-1}) based on McCree (1972) and published by Sager et al. (1988).

[d]TPF: total photon flux density (photon flux integral between 400 and 820 nm, in µmol m^{-2} s^{-1}).

[e]R/FR: ratio of photon flux integral of red light (from 600 to 700 nm) and FR (from 700 to 820 nm) radiation.

[f]Numbers in parenthesis represent the percentage of each waveband from total PPFD.

The highest number of shoots per explant were produced under LED_{64} (Table 1), which corresponds with the findings of others who have reported higher axillary bud outgrowth under a high R/FR ratio (Christiaens et al., 2019). Explants under $FL_{47}FR_{17}$ produced fewer shoots, but their overall shoot FM was highest compared to those under other treatments. FR radiation has been shown to increase biomass production partly due to an increase in radiation capture (Park and Runkle, 2017). However, our findings showed no significant treatment effect for shoot DM even though explants grown under $FL_{47}FR_{17}$ produced 25% more shoot DM than those of any other treatment. Moreover, supplementing FR radiation to FL stimulated root formation. This growth trends could be related to the developmental stage of the explants used in our study. Compared with mature plants, micropropagated explants are not able to make efficient use of high light intensities (Read and Fellman, 1984). It is likely that at this young developmental stage, explants are more sensitive to light quality and its effects on plant morphology, possibly benefitting from the addition of FR radiation to low PPFDs (as the ones used in $FL_{47}FR_{17}$), which are also more efficient at driving photosynthesis compared to high PPFDs.

Although there is evidence that light-quality responses can carry-over during production (Johkan et al., 2010), to our knowledge, no studies have investigated the subsequent effect of FR radiation used during micropropagation on transplant growth. We found that FR radiation applied during the in vitro multiplication of *P. cordata* positively affects transplant growth in the greenhouse (Table 3). In particular, FR radiation increased the number of leaves and roots produced per shoot, as well as the leaf length of *P. cordata*

transplants. In addition, although $FL_{47}FR_{17}$ provided the lowest DLI during the micropropagation stage, transplant growth was generally higher than that of FL_{64} and LED_{64}, which could be related to the higher shoot FM and DM produced by $FL_{47}FR_{17}$ during the micropropagation stage.

Table 3. Growth responses of *P. cordata* transplants micropropagated under five sole-source lighting treatments and finished in a greenhouse for 21 d.

Treatment	Leaves per shoot (no.)	Leaf length (cm)	Roots per shoot (no.)	Longest root length (cm)
F_{64} (control)	2.3 b[a]	7.7 b	5.0 ab	6.4 a
LED_{64}	2.3 b	7.3 b	4.7 b	3.8 b
$F_{64}FR_{17}$	3.1 a	12.4 a	6.1 a	7.8 a
$LED_{64}FR_{17}$	3.3 a	12.1 a	6.6 a	7.6 a
$F_{47}FR_{17}$	2.7 ab	11.0 ab	6.3 a	6.8 a

[a]FL or LED = broad spectrum cool-white fluorescent or light-emitting diode lamps; FR = far-red LEDs. Values are in µmol m^{-2} s^{-1}.

[b]Means within column with different letters are significantly different by Tukey's honestly significant difference test (P<0.05).

CONCLUSIONS

Our findings show that without FR radiation, shoot explants of *P. cordata* grown under broadband FL had larger leaves than those grown under LEDs, but produced a similar number of shoots and roots, and had the same shoot FM and DM (Table 1). In addition, regardless of PPFD, 17 µmol m^{-2} s^{-1} of FR supplemented to FL increased leaf length and root number compared to FL_{64}. The highest number of shoots per explant were produced under LED_{64}. However, $FL_{47}FR_{17}$ tended to produce more shoot biomass than all other treatments, demonstrating the potential benefit of adding FR radiation to broadband white light during micropropagation processes, particularly under low PPFDs. Furthermore, adding FR during the in vitro multiplication stage of *P. cordata* resulted in positive effects after finishing transplants in a greenhouse. Findings from this experiment demonstrate growth benefits of FR radiation when used in combination with other photosynthetic wavebands. Our results could be advantageous when designing light strategies to sustainably mass produce native plants such as *P. cordata*, which are in high demand for restoration or mitigation projects.

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