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Seed germination responses to low-level narrow-band light spectra for 14 ornamental plant genotypes

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Abstract

To investigate whether low-level lighting is necessary and which narrow-band light spectra are effective in seeds germination, seeds of 14 genotypes from begonia, echinacea, gerbera, petunia, and vinca were germinated under ultraviolet-B (UVB), blue (B), green (G), red (R), far-red (FR) light, or darkness. Light-emitting diode (LED) fixtures provided all spectrum treatments except for UVB, which was provided by a narrow-band fluorescent light. The photon flux density at seed level was $\approx 18 \mu\text{mol m}^{-2} \text{s}^{-1}$ for B, G, R, and FR, and $0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ for UVB. Based on daily germination observations, final germination percentage, germination onset time, germination time spread, and germination speed were compared among different spectrum treatments for each of the plant genotypes. There were no promotive effects on final germination percentage, germination onset time, and germination time spread under the narrow-band lights compared to darkness. For all plant genotypes, B had a similar effect as darkness on seed germination. FR inhibited seed germination relative to darkness by reducing final germination percentage by 31–88%, delaying germination onset time by 30–40%, and decreasing germination speed by 11–48% in some genotypes. Under R, G, and UVB compared to darkness, germination speed was promoted for begonia ‘Apricot Shade’, a light-requiring genotype, and inhibited for vinca ‘Burgundy’, a light-inhibited genotype. Therefore, lighting at low levels used in our study is unnecessary for seed germination of the tested species except light-requiring genotypes, where R, G, and UVB are the most promotive among the tested narrow-band lights.

Key words: controlled environment production, germination parameters, light-emitting diode, light spectra

Introduction

Controlled environments with electric light as the sole light source have been increasingly used for commercial seedling transplant production of ornamental plants (Lopez et al. 2017; Gómez et al. 2019). Consistent and uniform seed germination is the first step for successful production of seed-propagated ornamental plants (Hartmann et al. 2002a). Seeds of most ornamental plant species can germinate in darkness, but for others it is unclear whether electric light can promote germination, and if so, which spectra are the most effective.

Different spectra can affect seed germination through their respective photoreceptors such as phytochromes, cryptochromes, phototropins, and unknown green light receptors (Casal et al. 1998; Goggin and Steadman 2012; Demotes-Mainard et al. 2016). Red light (R; 600–700 nm) can activate phytochromes, but far-red light (FR; 700–800 nm) can deactivate phytochromes (Demotes-Mainard et al. 2016). Blue (B; 400–500 nm) and ultraviolet-A (UVA; 320–400 nm) light have common photoreceptors such as cryptochromes and phototropins, which can be activated by either B or UVA (Huché-Thélier et al. 2016). Green light (G; 500–600 nm) can reduce cryptochromes activity and may also activate unknown green light photoreceptors (Golovatskaya and Karnachuk 2015). Although it is unknown whether UVR8, the photoreceptor for ultraviolet-B light (UVB; 280–320 nm) (Neugart and Schreiner 2018), is involved in seed germination, UVB can promote germination by causing the seed coating to break down (Kovács and Keresztes 2002; Noble 2002). Based on the potential photoreceptors involved in seed germination, at least R, B, FR, G, and UVB should be considered for studying seed germination responses to narrow-band light.

Seed germination responses to some different narrow-band light spectra have been investigated in some previous studies (Sharma and Sen 1975; Lindig-Cisneros and Zedler 2001; Basto and Ramírez 2015). However, the traditional light sources used (e.g., fluorescent lights or

coloured filters) provided broad-band light spectra, which may contain low levels of other spectra (Bergstrand et al. 2014). Light-emitting diodes (LEDs) can emit narrow-band lights with different spectra and provide the opportunity to re-evaluate the effects of narrow-band light on seed germination (Smith et al. 2017; Gómez et al. 2019).

To date, limited information has been available for ornamental plants on seed germination responses to narrow-band lights. For stevia (*Stevia rebaudiana*) under continuous 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ LED lighting, B resulted in 8–15% and 15–30% higher final germination percentage than R and darkness, respectively (Simlat et al. 2016). In contrast, continuous 57.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ B LED lighting inhibited germination of barley (*Hordeum vulgare*) compared to darkness (Hoang et al. 2014). Another study on barley indicated that germination was approximately 25% higher under FR or darkness compared to R, B, and G from 16-h LED lighting at 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Hawley 2013). Continuous G LED lighting at 57.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ promoted germination of barley compared to B, despite a similar effect as darkness (Hoang et al. 2014). In *Bletilla ochracea*, continuous G LED lighting at 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ led to approximately 13% higher germination percentage compared to darkness, but B and R showed no difference from G and darkness (Godo et al. 2011). Continuous UVB lighting promoted seed germination in kale (*Brassica napus*), cabbage (*Brassica oleracea*), radish (*Raphanus sativus*), and agave (*Agave americana*) (Noble 2002). However, there was no study in the literature comparing the effects of R, B, FR, G, UVB, and darkness on seed germination of ornamental plants.

Begonia (*Begonia* $\times$ *tuberosa*), echinacea (*Echinacea* spp.), gerbera (*Gerbera jamesonii*), petunia (*Petunia* $\times$ *hybrida*), and vinca (*Catharanthus roseus*) are commercially important seed-propagated ornamental species. However, some genotypes from these species have low seed germination in darkness, based on communication with commercial seed suppliers and

propagators. Using low-intensity, continuous (i.e., 24-h) narrow-band lighting (e.g., LED) is a common way of producing plants in a controlled environment (Sysoeva et al. 2010). Therefore, it is necessary to know whether low-intensity, continuous narrow-band light with specific wavebands can promote seed germination. High light intensity (e.g., $\geq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) has been shown to inhibit seed germination due to photoinhibition in many species, including light-requiring species (Pons 2014; Carta et al. 2017). Under continuous lighting, the inhibitory intensity can be even lower than $50 \mu\text{mol m}^{-2} \text{s}^{-1}$, but the inhibitory effect was reduced with intensities decreasing within the range of $\approx 35\text{--}55 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Hoang et al. 2014). Possibly, continuous lighting at a low intensity between 0 and $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ (e.g., $20 \mu\text{mol m}^{-2} \text{s}^{-1}$) can be used for testing seed germination under narrow-band light compared to darkness. For UVB, even intensities lower than $1 \mu\text{mol m}^{-2} \text{s}^{-1}$ are enough to trigger normal biological responses in plants (Dotto and Casati 2017). The objective of this study was to investigate whether narrow-band B, G, R, or FR at approximately $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ or UVB at $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ can promote seed germination relative to darkness for different genotypes from the above five ornamental plant species.

Materials and Methods

Plant materials and germination conditions

The study was conducted in a 29 m^2 walk-in growth chamber at the University of Guelph, Guelph, ON, Canada in 2019. In total, 14 genotypes (2–4 genotypes from each of the five plant species) were selected for the germination experiment (Table 1).

A simple germination system was developed for this study to compare seed germination of the 14 genotypes under the different treatments. Rockwool sheets (1" A-OK; Grodan,

Kingsville, ON, Canada) were cut into 8 cm × 9 cm pieces and soaked in tap water for at least 24-h before seeding. Filter paper discs (P5; Fisher Scientific, Ottawa, ON, Canada) were cut into 6.4 cm × 6.4 cm squares and placed on top of the fully soaked rockwool pieces (one filter paper per rockwool piece) within a plastic tray (27 cm × 56 cm × 5 cm). When the filter paper pieces were fully wet, seeds were manually sown on the surface of the filter paper. Once the seeding of a genotype was completed, the sown seeds were placed under the different spectrum treatments (Supplementary Fig. S1). For each of the three replicates, in each treatment compartment there were two plastic trays, and each tray contained 14 pieces of rockwool with filter paper (two pieces for each genotype and 24 seeds for each piece; total of 48 seeds per genotype).

Prior to seeding the seeds were stored in a dark room at 4°C. When seeding, each plastic tray was filled with ≈1500 mL of tap water to ensure the filter papers on the top of the rockwool pieces were kept moist. After commencing spectrum treatments, more water was added to the trays as needed to maintain the moisture level of the filter papers. During the spectrum treatment period, the environmental conditions in the growth chamber were controlled by an Argus control system (Argus Controls Systems Ltd., Surrey, BC, Canada). Across all three replications the air temperature and relative humidity were 23.9 ± 0.1 °C and 69.4 ± 5.4 % (mean ± standard deviation (SD)), respectively.

Experimental design and treatment arrangement

The experiment was conducted as a randomized complete block design with one factor (spectrum treatment) and three consecutive replicates over time. The treatments were: 1) Dark (D); 2) Ultraviolet-B (UVB; 310 nm, full width at half maximum (FWHM) = 3 nm); 3) Blue (B; 449 nm, FWHM = 17 nm); 4) Green (G; 525 nm, FWHM = 35 nm); 5) Red (R; 660 nm, FWHM = 18 nm); and 6) Far-red (FR; 735 nm, FWHM = 35 nm) light. The average photon flux density

(PFD) at seed level was approximately $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ for B, G, R, and FR, and $0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ for UVB. Continuous 24-h lighting was used for all the above spectrum treatments. The UVB light was provided by a narrow-band fluorescent tube (PL-L-36W; Philips, Solarc Systems Inc., Minesing, ON, Canada) which provided UVB as the dominant waveband (50%) and small amounts of other wavebands (UVA-14%, B-19%, G-13%, R-1%, and FR-3%). LED fixtures (YNS9810-820A; Yunustech Inc., Mississauga, ON, Canada) provided the other spectrum treatments. The LED and UVB light fixtures were hung 60 cm and 76 cm above the bench, respectively. For the dark treatment, no light fixtures were used and black cloth was used to prevent light contamination from neighbouring treatments.

For each replicate, the six treatments were randomly allocated to six compartments inside the chamber, which were separated from each other by opaque white curtains to prevent neighbouring light effects. The spectrum treatments were replicated by changing their locations within the six compartments during the three replications of the experiment. To check light uniformity, light intensity and spectrum were measured at 16 spots at seed level within the treatment area (two trays; 0.3 m^2) in each compartment for each replicate using a radiometrically-calibrated spectrometer (UV/Vis Flame-S-XR; Ocean Optics, Inc., Dunedin, FL, USA) coupled to a CC3 cosine corrector with a $1.9 \text{ m} \times 400 \mu\text{m}$ optical fibre. Light spectra and intensities were also checked at the end of each replicate. The target intensity was achieved at seed level by adjusting fixture height or applying layers of neutral-density mesh for the UVB treatment, and by changing electric power input for the other spectrum treatments. For the spectrum treatments, the light spectral distributions and phytochrome photostationary state (PPS) values are presented in Supplementary Fig. S2. The PPS values for each spectrum treatment were

calculated using the protocol (Mah et al. 2019) developed by our lab according to Sager et al. (1988).

Germination measurements

After sowing, the number of germinated seeds (defined as having visible radicle protrusion) on each piece of filter paper was counted and recorded daily until all seeds had germinated or the germination percentage was stable for five consecutive days. The germination check for all treatments was assisted with a handheld magnifier under a green safe light (peak wavelength of 520 nm, $<1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$).

At the end of the experiment, the final germination percentage, germination onset time, germination time spread, and germination speed were determined. Final germination percentage (%) was calculated as number of germinated seeds out of 48 seeds sown for each genotype under each spectrum treatment for each replicate. Germination onset time (d) was calculated as the number of days from the sowing date to the first day of observed seed germination. Germination time spread was calculated as the time (d) from the first day of observed seed germination to the day germination percentage became stable (i.e., the first of the five days). Germination speed was calculated as the inverse of t_{50} , which was the time (d) required to reach median germination (around 50% of the final germination percentage).

Statistical analysis

Data were subjected to analysis of variance using SAS statistical software (University Edition; SAS Institute, Cary, NC, USA) and were presented as means $\pm$ standard error (SE). Separation of means for different spectrum treatments was performed separately for each genotype using Tukey's HSD test at the significance level of $P < 0.05$.

Results and Discussion

The spectrum treatments did not promote seed germination compared with darkness for the tested species based on final germination percentage, germination onset time, and germination time spread (Fig. 1–3). Particularly, B light had a similar effect as darkness on all the germination parameters and all the tested species (Fig. 1–4). Similar results were observed in soybean (*Glycine max*) (Hawley 2013), chickpea (*Cicer arietinum*) (Vasilean et al. 2018), and 18 vegetable genotypes (Kong and Zheng 2018) where light treatments did not promote seed germination compared to darkness. From a commercial standpoint, the knowledge that lighting is unnecessary during the seed germination period (i.e., until germination onset time) is important for minimizing energy input costs. The lack of germination response to spectrum treatments relative to darkness may be due to a cold storage (around 4°C) of the seeds before seeding, which is commonly used for maintaining seed viability. Low temperatures can reduce the light requirement for seed germination by breaking seed dormancy (Hartmann et al. 2002b; Pons 2014), which consequently increased seed germination in darkness after a low temperature treatment in many plant species (Pons 2014). However, it is worthwhile to note that darkness can also promote plant elongation and may compromise seedling quality. The negative effect of darkness on seedling quality would depend on the uniformity of seed germination and the duration of the dark treatment. In other words, if the seeds germinate uniformly, then light would not be needed until germination onset time has elapsed.

In many cases, FR negatively affected seed germination compared to darkness. This was demonstrated by 31–88% lower final germination percentage (Fig. 1B–C, E), 30–40% longer germination onset time (Fig. 2C), and 11–48% slower germination speed (Fig. 4A–C) in some

plant genotypes from begonia, echinacea, gerbera, or vinca in response to FR. FR light has also been reported to inhibit seed germination of tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), pepper (*Capsicum annuum*), and kale (*Brassica oleracea*) compared to darkness (Hawley 2013). A FR-rich environment is indicative of a shade signal, and the inhibitory seed germination observed in the present study could be considered a shade-avoidance response in plants, since the environmental conditions during seed germination are what the subsequent seedling experiences after germination is complete (Goggin and Steadman 2012). Another explanation is that the light response of seed germination in the present study can be considered a long duration exposure response, since the light exposure lasted for several days (Pons 2014). For this light response type, FR light has been shown to be the most effective for inhibiting germination (Pons 2014; Demotes-Mainard et al. 2016).

Germination speed was 11%, 14%, and 11% higher under R, G, and UVB, respectively, compared to darkness for begonia ‘Apricot Shade’ (Fig. 4A). However, germination speed was 51%, 48%, and 46% lower under R, G, and UVB, respectively, compared to darkness for vinca ‘Burgundy’ (Fig. 4E). The light response in germination speed of the above two genotypes support begonia and vinca belonging to light-requiring and light-inhibited species, respectively (Ball 1998; Carta et al. 2017). In addition to germination speed, the other three parameters describe the germination response to spectrum treatments from different perspectives. Final germination percentage indicates the germination capacity; onset time reflects whether germination starts early or late; time spread, indirectly via germination speed, indicates the germination synchrony (i.e., uniformity) (Hartmann et al. 2002b; Ranal and De Santana 2006). For commercial plant production, a large amount of uniformly germinated seedlings in a short time is ideal. The contrasting germination responses between begonia ‘Apricot Shade’ and vinca

‘Burgundy’ under R, G, or UVB relative to darkness were not observed in the other three parameters, but only in germination speed. Based on the contrasting response between a light-requiring (begonia) and light-inhibited (vinca) species, germination speed could be the most useful parameter for growers and R, G, and UVB are optimal spectra to evaluate the species’ sensitivity in germination responses to light spectra.

In summary, for the tested species, spectrum treatments did not promote seed germination compared to darkness in terms of the four germination parameters, except germination speed. In many cases, FR negatively affected seed germination, which was demonstrated by lower final germination percentage, delayed germination onset time, and decreased germination speed in some plant genotypes. Germination speed was promoted for begonia ‘Apricot Shade’, a light-requiring genotype, and inhibited for vinca ‘Burgundy’, a light-inhibited genotype, under R, G, and UVB compared to darkness. Therefore, lighting at low levels used in our study is unnecessary during the germination period until the onset time has elapsed for the tested ornamental genotypes, except for begonia ‘Apricot Shade’. For this light-requiring genotype, R, G, and UVB are the most promotive on seed germination speed among the narrow-band lights.

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Table 1. Ornamental plant genotypes used in seed germination experiment.

Scientific name	Genotype	Seed weight (mg seed ⁻¹)	Seed source
<i>Begonia × tuberosa</i>	Begonia ‘Tuberous Illumination Apricot Shade’ (coated)	0.47	Ball
<i>Begonia × tuberosa</i>	Begonia ‘Tuberous Nonstop Red’ (coated)	0.47	Ball
<i>Echinacea × hybrida</i>	Echinacea ‘Lakota Santa Fe’	4.56	PanAm
<i>Echinacea × purpurea</i>	Echinacea ‘PowWow Wild Berry’	3.90	PanAm
<i>Gerbera jamesonii</i>	Gerbera ‘Midi Dark Purple’	4.40	Florist
<i>Gerbera jamesonii</i>	Gerbera ‘Maxi White’	4.04	Florist
<i>Gerbera jamesonii</i>	Gerbera ‘Maxi Pink’	4.34	Florist
<i>Gerbera jamesonii</i>	Gerbera ‘Majorette Red Dark Eye’	4.19	Sakata
<i>Petunia × hybrida</i>	Petunia ‘Easy Wave White’	0.19	PanAm
<i>Petunia × hybrida</i>	Petunia ‘Easy Wave Coral Reef’	0.14	PanAm
<i>Petunia × hybrida</i>	Petunia ‘Easy Wave Red Velour’	0.11	PanAm
<i>Catharanthus roseus</i>	Vinca ‘Mediterranean XP Red Dark’	2.41	PanAm
<i>Catharanthus roseus</i>	Vinca ‘Pacifica XP Magenta Halo’	1.96	PanAm
<i>Catharanthus roseus</i>	Vinca ‘Pacifica XP Burgundy’	1.83	PanAm

Note: Three samples of ~50 seeds were weighed, and the seed weight (mg seed⁻¹) was subsequently calculated for each genotype. Ball = Ball Horticulture, Chicago, IL, USA; PanAm = PanAmerican Seed, Chicago, IL, USA; Florist = Florist Holland, De Kwakel, The Netherlands; Sakata = Sakata Seed America, Inc., Morgan Hill, CA, USA.

Fig. 1. Final germination percentage (%) of seeds in 14 ornamental plants genotypes under different spectrum treatments. Data are mean $\pm$ standard error ($n = 3$). Dark = open bar, UVB = brown dotted bar, Blue = blue horizontal striped bar, Green = green checkerboard bar, Red = red diagonal striped bar, and Far-red = dark red vertical striped bar. Within the same genotype, bars bearing the same letter are not significantly different at $P < 0.05$ level according to Tukey's HSD test. For gerbera, MR = Majorette Red, MP = Maxi Pink, MW = Maxi White, and MDP = Midi Dark Purple.

Fig. 2. Germination onset time (time from the sowing date to the first day of observed seed germination) of seeds in 14 ornamental plant genotypes under different spectrum treatments. Data are mean $\pm$ standard error ($n = 3$). Dark = open bar, UVB = brown dotted bar, Blue = blue horizontal striped bar, Green = green checkerboard bar, Red = red diagonal striped bar, and Far-red = dark red vertical striped bar. Within the same genotype, bars bearing the same letter are not significantly different at $P < 0.05$ level according to Tukey's HSD test. For gerbera, MR = Majorette Red, MP = Maxi Pink, MW = Maxi White, and MDP = Midi Dark Purple.

Fig. 3. Germination time spread (time from the first day of observed seed germination to the day germination percentage became stable (i.e., the first of the five days)) of seeds in 14 ornamental plant genotypes under different spectrum treatments. Data are mean $\pm$ standard error ($n = 3$). Dark = open bar, UVB = brown dotted bar, Blue = blue horizontal striped bar, Green = green checkerboard bar, Red = red diagonal striped bar, and Far-red = dark red vertical striped bar. Within the same genotype, bars bearing the same letter are not significantly different at $P < 0.05$ level according to Tukey's HSD test. For gerbera, MR = Majorette Red, MP = Maxi Pink, MW = Maxi White, and MDP = Midi Dark Purple.

Fig. 4. Germination speed (calculated as the inverse of median seed germination; $1/t_{50}$) of seeds in 14 ornamental plant genotypes under different spectrum treatments. Data are mean $\pm$ standard error ($n = 3$). Dark = open bar, UVB = brown dotted bar, Blue = blue horizontal striped bar, Green = green checkerboard bar, Red = red diagonal striped bar, and Far-red = dark red vertical striped bar. Within the same genotype, bars bearing the same letter are not significantly different at $P < 0.05$ level according to Tukey's HSD test. For gerbera, MR = Majorette Red, MP = Maxi Pink, MW = Maxi White, and MDP = Midi Dark Purple.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.