Environmental and Experimental Botany 193 (2022) 104700

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Interaction of gravitropism and phototropism in roots of *Brassica oleracea*

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ARTICLE INFO

Keywords:
Blue light
Gravitropism
Hypergravity
Light quality
Phototropism
Random positioning machine
Red light
Root tropisms
Simulated microgravity

ABSTRACT

Gravitropism and phototropism play a primary role in orienting root growth. Tropistic responses of roots mediated by gravity and light have been extensively investigated, and a complex mutual interaction occurs between these two tropisms. To date, most studies have been conducted in 1 *g*, microgravity, or simulated microgravity, whereas no studies investigated root phototropism in hypergravity. Therefore, we studied the effects of several gravity treatments with those of different light wavelengths on root growth orientation. Here, we report growth and curvature of *Brassica oleracea* roots under different *g* levels, from simulated microgravity up to 20 *g*, and unilateral illumination with different spectral treatments provided by light emitting diodes. Microgravity was simulated with a random positioning machine whereas hypergravity conditions were obtained using the Large Diameter Centrifuge at the laboratories of the European Space Agency in the Netherlands. Four light treatments (white light, blue light, red light, and dark) were used in this study. Overall, roots of seedlings grown in the dark were longer than those developed under unilateral light treatments, regardless of the gravity level. Unilateral blue light or white light stimulated a negative phototropism of roots under all *g* levels, and root curvature was not affected by either hypergravity or simulated microgravity compared to 1 *g*. Results also confirmed previous findings on the effect of light intensity on root curvature and highlighted the relevance of blue-light photon flux density in root phototropism. Roots illuminated with red light showed a weak curvature in simulated microgravity but not in hypergravity. Moreover, root curvature under red light was similar to dark-grown roots in all *g* levels, suggesting a possible involvement of surface-dependent phenomena in root skewing under either red light or dark conditions. Further studies can confirm phototropic responses of *B. oleracea* in the weightless environment of orbiting spacecraft. Nevertheless, according to our findings, directional lighting represents an effective stimulus to guide root growth in a wide range of gravity conditions.

1. Introduction

Gravitropism and phototropism are directional growth responses of plant organs to gravity and light, respectively. Numerous studies have focused on plant tropisms since those of Darwin who pioneered modern research on gravitropism and phototropism (Darwin and Darwin, 1880). During the water-to-land transition, seed plants evolved rapid gravitropic responses of roots which facilitated the adaptation to the terrestrial environment (Zhang et al., 2019). Generally, to harvest light while anchoring to the substrate, plants orient shoots toward the light (positive phototropism) and away from the gravity vector (negative gravitropism) and, conversely, orient roots into the soil, away from light (negative phototropism) and toward the direction of gravity (positive gravitropism) (Gilroy, 2008). Plant roots have also evolved several other tropisms (e.g., hydrotropism, chemotropism, thigmotropism, magnetotropism, electrotropism, and phonotropism) to orient their growth

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https://doi.org/10.1016/j.envexpbot.2021.104700
Received 29 July 2021; Received in revised form 19 October 2021; Accepted 20 October 2021
Available online 6 November 2021
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according to a wide range of environmental stimuli, and phototropism is among the most studied together with gravitropism (Muthert et al., 2020).

As already reported in the late 1800s by Darwin and Darwin (1880), the root tip acts as a "brain" in governing organ orientation by actively perceiving and responding to tropistic stimuli. To date, four different zones with distinct cell populations in the root tip of *Arabidopsis thaliana* have been reported (Verbelen et al., 2006; Baluška et al., 2010), as well as the location of all known sensor and action regions involved in root tropisms (Muthert et al., 2020). Specifically, sensors for root gravitropism and phototropism are located in the root cap and in the elongation zone, whereas action regions for root gravitropism and phototropism are the transition zone and the elongation zone, respectively (Blancaflor et al., 1998; Briggs and Christie, 2002; Mullen et al., 2002; Sakamoto and Briggs, 2002; Wolverton et al., 2002; Kiss et al., 2003). Although gravitropism and phototropism have completely different sensors for tropistic stimuli (i.e., statoliths for gravity, phototropins and phytochromes for light), their transduction pathways exhibit a complex interaction in the control of organ orientation (Correll and Kiss, 2002). According to the Cholodny-Went theory, auxin accumulation in the root tip inhibits cell elongation at the lower side of the root, causing the root to bend in the direction of gravity (Geisler et al., 2014). Similarly, root phototropism acts through differential auxin distribution mediated by asymmetrical distribution of PIN FORMED 2 (PIN2) proteins upon phototropin 1 (phot1) activation (Pedmale et al., 2010; Zhang et al., 2014). In this model, NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3) influences PIN2 distribution and is a point of interaction for gravitropic and phototropic signaling (Wan et al., 2012). Nevertheless, a study on *A. thaliana* by Kimura et al. (2018) suggests that the asymmetrical increase in auxin on the illuminated side of the root is a gravitropic reaction following the initial phototropic bending. It is also noteworthy that light can operate not only as a tropistic signal in plants, but possibly also as a tonic (turgor) signal affecting graviresponse as has been documented in fungi (Galland, 1998; Grolig et al., 2000).

Interaction of root gravitropism and phototropism also involves the phytochrome-dependent regulation of PHYTOCHROME KINASE SUBSTRATE 1 (PKS1) which negatively regulates gravitropism and contributes to phototropin-mediated phototropism (Boccalandro et al., 2008). Phytochromes are also directly involved in the regulation of root phototropism and the different responses of phytochrome A and phytochrome B allow the integration of multiple environmental stimuli including gravity (Kiss et al., 2003). Phytochromes are involved in several responses of plants and their photosensory activity relies on a reversible switching between inactive and active form mediated by red and far-red light (Quail, 2002). However, phytochromes are photoreceptors that also absorb blue light, which can influence the phytochrome photoequilibrium with implications in plant photomorphogenesis (Smith, 2000; Meng and Runkle, 2017; Kong et al., 2018; Kong et al., 2019), possibly affecting also phototropic responses.

Although positive gravitropism of roots is ubiquitous in higher plants (e.g., Ge and Chen, 2016), root phototropic responses exhibit considerable variability. Systematic studies on a total of about 300 species reported that about a half did not react to unilateral white light, whereas the other half showed a negative phototropism of roots, and a few species displayed a positive response (Schaefer, 1911; Hubert and Funke, 1937). More recently, tropism research has focused on disentangling the molecular pathways in *A. thaliana* (Muthert et al., 2020), whose roots show negative phototropic responses to white and blue light like most species of the Brassicaceae family tested by Schaefer (1911) and Hubert and Funke (1937). To date, it is known that *A. thaliana* roots can exhibit negative and positive phototropism to blue and red light respectively (Kiss et al., 2012), but also that phototropic responses may not be predictable in altered gravity as in the case of the blue-light positive phototropism discovered in microgravity (Vandenbrink et al., 2016). Specifically, the blue-light positive phototropism of roots was only detectable at gravity levels below 0.3 g and pre-treatment with 1 h of red light enhanced the response (Vandenbrink et al., 2016). Similarly, other tropisms have been revealed in microgravity such as the chemotropism of *Daucus carota* roots toward disodium phosphate (Izzo et al., 2019), and the red-light phototropism of *A. thaliana* hypocotyls (Millar et al., 2010; Kiss et al., 2012), indicating that the relatively strong gravitropic responses typically mask other tropisms.

According to the vector hypothesis, the actual degree of root bending in *A. thaliana* depends on the phototropic response and a counteracting gravitropic response, resulting as the sum of gravity and light vectors (Okada and Shimura, 1992; Vitha et al., 2000). Phototropic responses involve deviation of the growth direction from the gravity vector and generate a gravitational stimulus that partially counteracts phototropism. Consequently, mutants with deficient gravitropic response show enhanced root phototropism (Okada and Shimura, 1992; Vitha et al., 2000). Similarly, it has been shown that attenuating the effects of gravity or using mutants that are impaired in gravisensing, the red-light positive phototropism of roots can be revealed (Ruppel et al., 2001; Kiss et al., 2003; Kiss et al., 2012). Tropism research has also shown that both positive and negative phototropic responses of *A. thaliana* roots are dependent on photon flux density at very low values and saturate at about 10 μmol m^{-2} sec^{-1}, whereas responses are constant at higher photon flux densities for both blue- and red-light phototropism (Sakai et al., 2000; Kiss et al., 2003).

Overall, these findings suggest that changes in the quality and magnitude of light and gravity stimuli can influence the interaction of gravitropism and phototropism in roots and that tropistic responses can vary strongly between species. Despite the extensive research conducted under gravity conditions ranging from microgravity to 1 *g*, few experiments investigated root tropisms in hypergravity (Muthert et al., 2020). It has been shown that an acceleration of 5 *g* is required for the restoration of root gravitropism in starchless mutants of Arabidopsis, and this response was associated with increased sedimentation of plastids (Fitzelle and Kiss, 2001). Still, to date, no studies investigated the interaction of gravitropism and phototropism of roots under hypergravity conditions.

Thus, it remains to be verified whether the same relationship between root gravitropism and phototropism holds when increasing the magnitude of gravity using different light spectra. Furthermore, it is necessary to expand the knowledge on plant tropisms to other species, particularly considering candidate crops for cultivation in space where gravity conditions can alter the interaction between the different tropisms (Izzo and Aronne, 2021). Recently, the increasing possibility of performing experiments in altered gravity, together with the development of narrow-band Light Emitting Diodes (LEDs), is paving the way toward a better understanding of gravitropism and phototropism interaction (Borst and van Loon, 2009; Gómez and Izzo, 2018).

In this study, we tested the hypothesis that changes in light quality and magnitude of gravity can influence the net effectiveness of root gravitropism and phototropism of *Brassica oleracea* seedlings. We analyzed growth and curvature of roots under different *g* levels, from simulated microgravity up to 20 *g*, in combination with different spectral treatments provided by LEDs and dark conditions.

2. Materials and methods

2.1. Experimental design and facilities

The experiment was performed using the Ground Based Facilities at the European Science and Technology Center in Noordwijk (NL) (Frett et al., 2016) in the framework of the ROOTROPS project funded by the European Space Agency. The experiment consisted of two runs, each including 6 gravity levels (1 *g*, 5 *g*, 10 *g*, 15 *g*, 20 *g*, and simulated microgravity) and 4 light treatments (white light, blue light, red light, and dark). Conditions of 1 *g* and hypergravity were obtained within gondolas of the Large Diameter Centrifuge (LDC) (van Loon et al., 2008), whereas a Random Positioning Machine (RPM) (Fokker / Dutch Space /

EADS, Leiden, the Netherlands) was used to simulate microgravity. The experiment was performed using square Petri dishes (12 × 12 × 1.5 cm) as seedling experimental container (EC) and specifically developed hardware to hold the ECs (Aronne et al., 2022). The external hardware consisted of a multi-slot box with an adjustable LED system to provide a stable housing for the ECs, gravity direction according to the seedling root/shoot axis and unilateral light treatments perpendicular to the gravity vector (Fig. 1A). Each box housed five ECs per light treatment for a total of 20 ECs which were randomly distributed within the box.

2.2. *Plant material and EC setup*

Seeds of *B. oleracea* (Bavicchi S.p.A., Italy, batch n. 181654) were surface sterilized in 3% (v/v) sodium hypochlorite/water solution for 5 min and then rinsed with sterile water. Seeds were subsequently germinated on wet filter paper (cellulose; 67 g/m^2) in a growth chamber at 26 °C under continuous fluorescent white light for 24 h. During germination, seeds were placed within Petri dishes on a 45° inclined plane to facilitate a straight downward protrusion of roots.

The ECs were lined with black paper (cellulose; 21 g/m^2) which was successively wetted with deionized water. Seedlings with a root length of approximately 10 mm were placed in the ECs using a strip of filter paper punctured in five aligned points and folded to form a pocket aimed to insert the roots and anchor the seedlings (Fig. 1B). Five seedlings of *B. oleracea* were then placed within each EC (Fig. 1C) for a total of 1200 seedlings tested during the two experiment runs (5 seedlings × 5 ECs × 4 light treatments × 6 gravity levels × 2 experiment runs). The ECs with the seedlings were then placed within the multi-slot box and kept in vertical position for 12 h under dark conditions to promote root anchoring to the black paper. Each experiment run was then performed at an ambient temperature of 26 °C for 24 h and was monitored by means of internal cameras.

2.3. *Gravity treatments*

Four different hypergravity levels were tested using the gondolas of the LDC. Overall set up of the gondolas was defined to achieve the nominal gravity values, namely 5 *g*, 10 *g*, 15 *g*, and 20 *g*, at the center of the holder boxes. The 1 *g* control treatment was set up using a static gondola placed in the same room as the centrifuge to have the same environmental conditions.

For simulated microgravity, all seedlings were located less than 10 cm from the center of rotation of the RPM to reduce residual centrifugal force due to rotation (van Loon, 2007; Hasenstein and van Loon, 2015). Running at a maximum random speed of 60°/s the residual acceleration was less than $10^{-4}g$. The system was also set to random direction and interval.

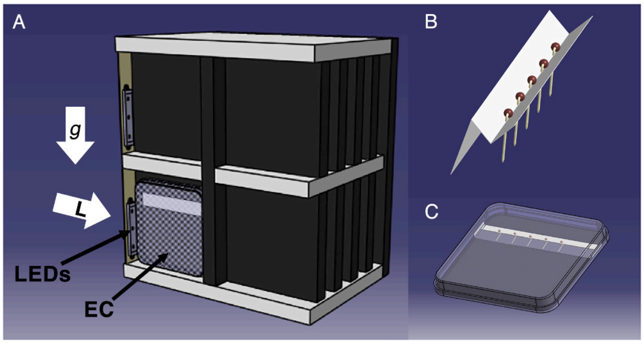

Fig. 1. Experimental device used for the ROOTROPS experiment. A) multi-slot box with light emitting diodes (LEDs) and the experimental container (EC). White arrows labeled *g* and L indicate the direction of gravity and light vectors, respectively; B) strip of white filter paper punctured and folded to hold the seedlings; C) experimental container with five seedlings (Aronne et al., 2022).

2.4. *Light treatments*

Four light treatments were tested in this study: white light, blue light, red light, and dark conditions. A spectroradiometer (SS-110, Apogee Instruments Inc.) was used to determine the emission spectrum of each LED source in the range of 340–820 nm and to generate a light-intensity map within the EC for each light treatment (Fig. 2). The blue and red LED lamps had peak wavelengths of 443 nm and 632 nm, respectively, whereas white light provided a broad spectrum consisting of 25% blue (400–500 nm), 53% green (500–600 nm), and 21% red (600–700 nm) (Fig. 2).

Light intensity was controlled using dimmers connected to the LED light sources. Light treatments using white, blue, and red LEDs had an average photon flux density (PFD) of ≈ 80, 50, 40, 35, and 30 μmol m^{-2} s^{-1} at 2, 4, 6, 8, and 10 cm distance from the light source, respectively (Fig. 3). Dark conditions were assured by wrapping ECs with aluminum foil and the light mixing between spectral treatments was avoided separating the ECs with a black foam. To test root phototropic responses, unilateral light treatments were provided by LEDs placed on the side of the ECs with light direction perpendicular to the direction of gravity

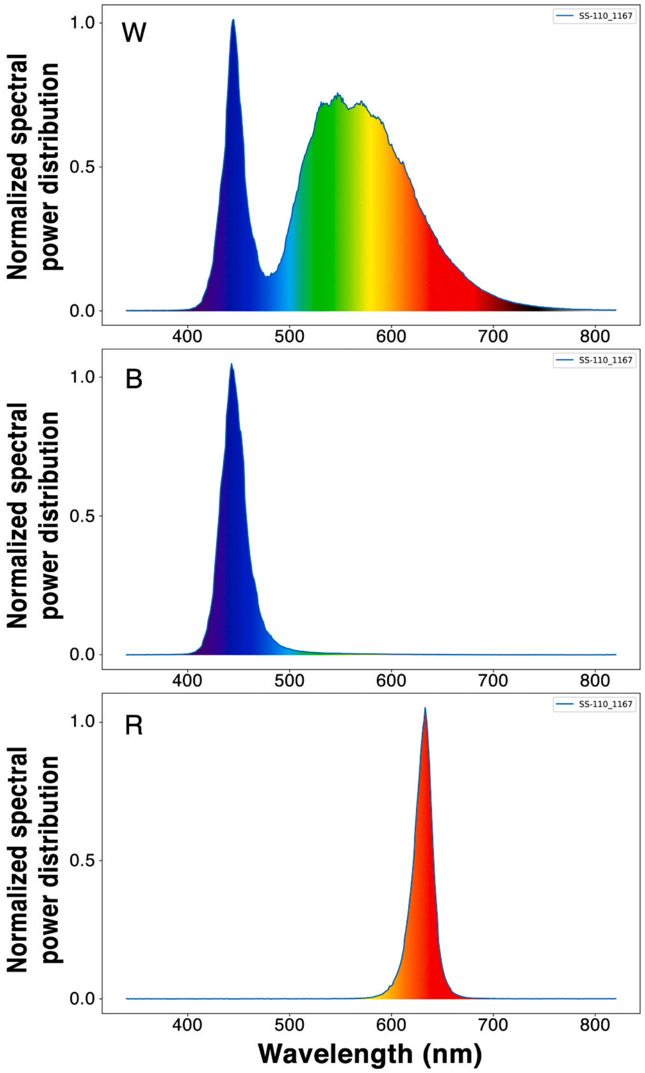

Fig. 2. Normalized spectral power distribution of the light-emitting diode lamps used in this study: (W) white light; (B) blue light; (R) red light. Spectral scans were recorded at 5 cm distance from the light sources with a spectroradiometer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

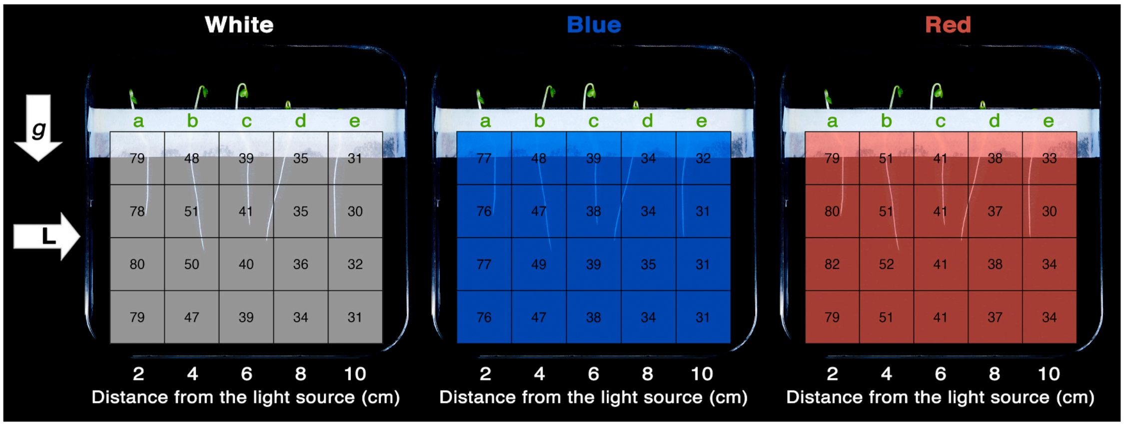

Fig. 3. Light-intensity map for white, blue, and red LEDs within square Petri dish (12 × 12 cm) used in this study. The values reported in the tables refer to the photon flux density (μmol m^{-2} s^{-1}). White arrows labeled *g* and L indicate the direction of gravity and light vectors, respectively. Green letters indicate the different positions of seedlings according to their distance from the light source (a = 2 cm; b = 4 cm; c = 6 cm; d = 8 cm; e = 10 cm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Figs. 1 and 3). The configuration of the light source and the EC determined a range of light intensity decreasing from ≈ 80 μmol m^{-2} s^{-1} (seedlings placed at 2 cm distance from the LEDs) to ≈ 30 μmol m^{-2} s^{-1} (seedlings placed at 10 cm distance from the LEDs) for all light treatments (Fig. 3).

2.5. *Image analysis and measurement of root curvature*

At the end of each experiment run, the samples were photographed using a camera (α7 II, SONY) mounted on a photographic workstation keeping track of ECs orientation with respect to the direction of gravity and light. Images were analyzed using the package NeuronJ within the software ImageJ v1.53e (Schneider et al., 2012).

The image analysis traced a line for each root developed within the ECs providing the root length and the spatial coordinates (x, y) of the points forming the line (Fig. 4). The starting point of each root was set as origin (x = 0; y = 0) considering the Y axis parallel to the gravity vector and perpendicular to the direction of light. For each root tracing, the subsequent points were detected at about 1 mm distance from each other. We then calculated the angles between the Y axis and each vector between two consecutive points of the root tracing starting from the root tip (Fig. 4). The plus (+) or minus (-) sign preceding the degree of curvature indicates whether the phototropic response was positive or negative, respectively.

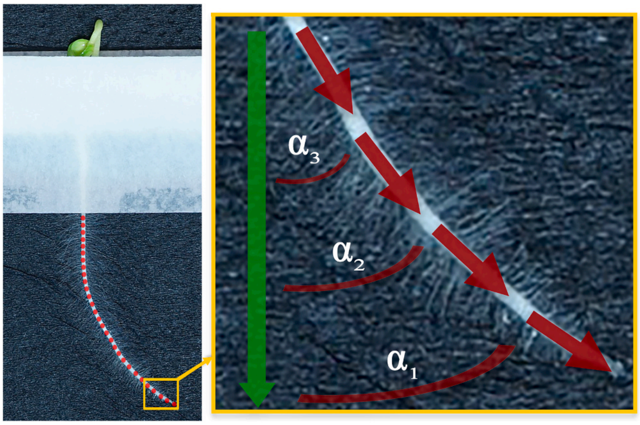

Fig. 4. Image analysis of root growth and curvature. Red dots represent the points of the root tracing. Green arrow indicates the direction of gravity vector (Y-axis). Red arrows represent the vectors between two consecutive points of the root tracing. Angles (α_i) were calculated between Y-axis and each vector. Illumination is from the left side of the figure. The plus (+) or minus (-) sign preceding the degree of curvature (α) indicates whether the phototropic response was positive or negative, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6. *Data analyses*

The influence of gravity and light, and their interaction on growth and curvature of *B. oleracea* roots was analyzed by means of generalized linear mixed model. We set the experimental replications and their interaction with treatments as random effects for the model. Pairwise comparisons were performed using Tukey’s post-hoc test ($P < 0.05$) to identify differences among individual treatments.

For each light treatment, a regression analysis was then used to evaluate the quantitative response of root curvature to light intensity (PFD) or gravity (*g*), setting the treatment of simulated microgravity as 0 g. All data were processed and analyzed using Excel ver. 16 (Microsoft Corp.) and SPSS Statistics ver. 21 (IBM Corp.).

3. Results

Light treatments significantly affected the degree of curvature of *B. oleracea* roots grown under all gravity conditions tested in this study (Fig. 5). Overall, results showed that root bending reached a photo-gravitropic equilibrium prior to the end of the 24 h treatments and the degree of curvature was steady in the last portion of the root starting from the tip (alpha 1) (Fig. 5). Unilateral blue or white light stimulated a negative phototropic response of roots. The effect after 24 h treatment was similar between blue and white light with a mean response ranging from − 40° to − 41° among the six gravity treatments tested in this study. Differently, at the end of the experiment, roots grown under unilateral red light showed an average degree of curvature approximately equal to 0° under hypergravity conditions (5–20 *g*), an angle of 13° under simulated microgravity and 5° at 1 *g*. Similar to roots under red light, dark-grown roots showed a straight growth under hypergravity, and an average degree of curvature of 16° and 5° under simulated microgravity and 1 *g*, respectively.

Root length was not affected by gravity level ($P = 0.83$) but resulted significantly reduced under white, blue, and red light if compared to dark conditions (Fig. 6). Overall, dark-grown roots were 18% longer compared to roots developed under light.

We analyzed data to highlight possible effects of increasing light intensity of white, blue, and red LEDs on root curvature. For each light treatment, the regression analysis showed no significant effect of the

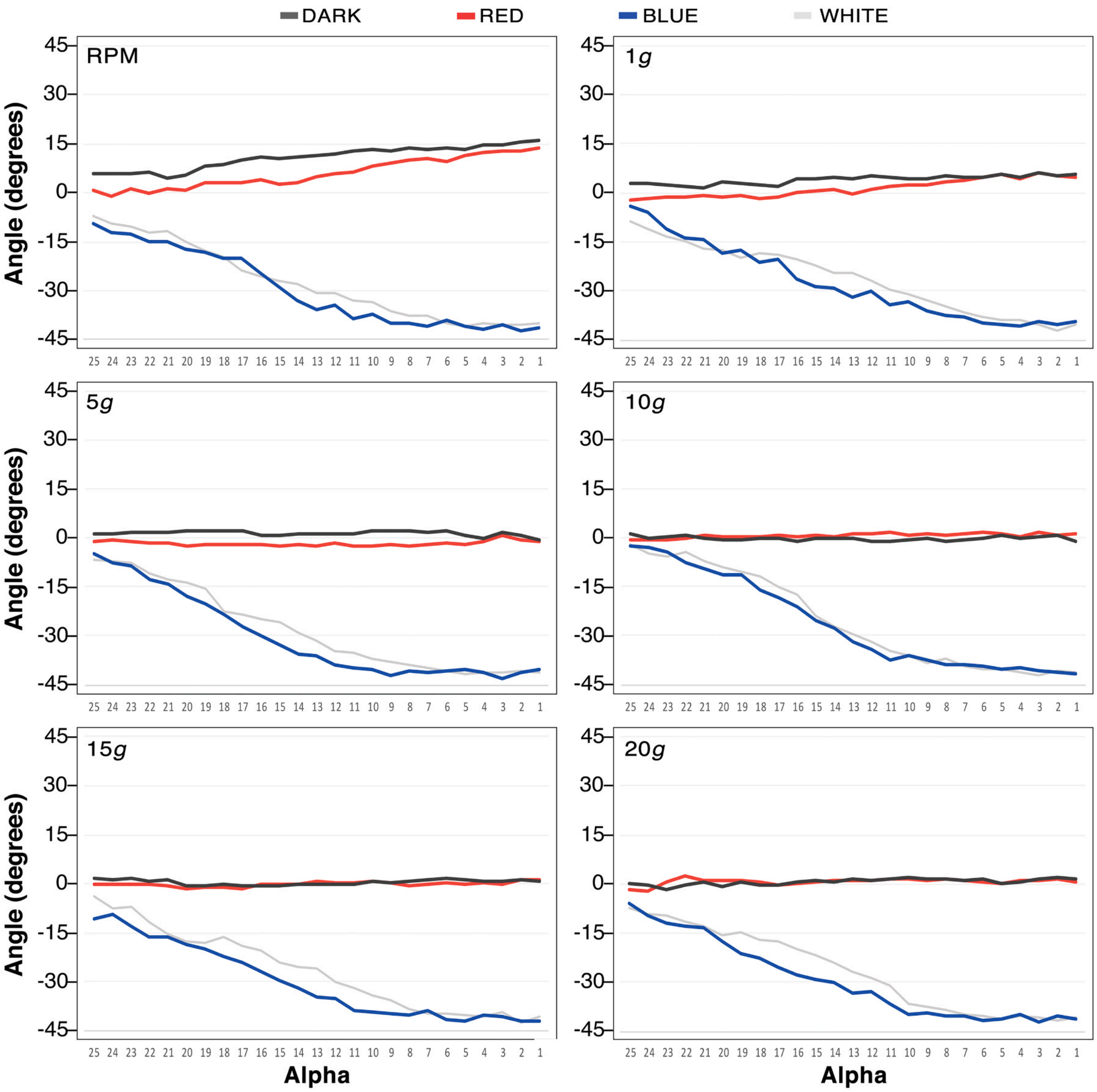

Fig. 5. Root curvature of *Brassica oleracea* seedlings grown at different gravity levels (RPM, 1 *g*, 5 *g*, 10 *g*, 15 *g*, 20 *g*) under dark conditions (dark-grey line), unilateral red light (red line), blue light (blue line), or white light (light-grey line) for 24 h. Angles were measured between the gravity vector and each vector of the root tracing starting from the root tip (alpha 1–25). Each data represents the mean of two replications with 25 plants. Illumination is from the top side of the figure. Photon flux density of blue, red, and white light was ≈ 30–80 μmol m^{-2} s^{-1}. The plus (+) or minus (-) sign preceding the degree of curvature indicates whether the response was towards or away from the light source, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

applied PFD on the degrees of curvature of *B. oleracea* roots and this occurred under all gravity conditions tested in this study (Fig. 7).

The quantitative response of root curvature to the magnitude of gravity was tested in the range of simulated microgravity to 20 *g*. The regression analysis showed no significant effect of gravity on root phototropic responses stimulated by white light and blue light (Fig. 8) and root curvature was not affected even at 20 *g* (Fig. 9). Overall, the degree of curvature of *B. oleracea* roots was − 41° for white- and blue-light negative phototropism, regardless of gravity. Conversely, root curvature decreased with increasing gravity under red light with an angle ranging from 13° to 0°. A similar result was also found for dark-grown roots (Fig. 8).

4. Discussion

It is well known that a complex mutual interaction between gravitropism and phototropism determines the form and orientation of plant roots under gravity conditions ranging from microgravity to 1 *g* (Kiss et al., 2003; Kiss et al., 2012; Vandenbrink et al., 2016). To further study this interaction, we evaluated growth and curvature of *B. oleracea* roots to unilateral light treatments under different *g* levels, from simulated microgravity up to 20 *g*, expanding for the first time phototropism research to hypergravity conditions.

Previous studies showed that *A. thaliana* roots exhibit negative phototropism in response to unilateral blue or white light, whereas red light can induce a positive phototropism when attenuating the effects of gravity or using mutants that are impaired in gravisensing (Okada and Shimura, 1994; Sakai et al., 2000; Ruppel et al., 2001; Kiss et al., 2012). In our study, roots of *B. oleracea* grown with unilateral white or blue light showed a negative phototropic response regardless of gravity conditions. Remarkably, the negative phototropism of roots was comparable between simulated microgravity, 1 *g*, and hypergravity conditions, with an average degree of curvature of − 41° measured at photo-gravitropic equilibrium. Although root curvature was similar to

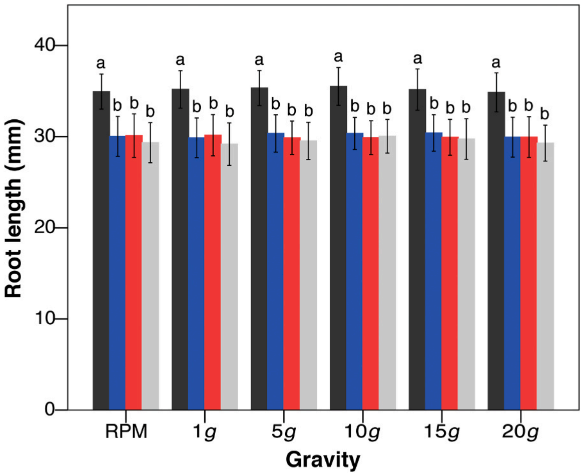

Fig. 6. Effect of light treatments (dark conditions = dark-grey bars; red light = red bars; blue light = blue bars; white light = light-grey bars) on root length of *Brassica oleracea* seedlings grown at different gravity levels (RPM, 1 *g*, 5 *g*, 10 *g*, 15 *g*, 20 *g*). Each data represents the mean and the standard deviation of two replications with 25 plants. Different letters indicate significant differences between treatments according to Tukey's multiple comparison test ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

what was found in previous studies under 1 *g* conditions, our findings do not support the vector hypothesis reported by Okada and Shimura (1994). Indeed, our data showed that hypergravity did not affect root phototropism stimulated by blue or white light, which resulted effective in orienting roots even at 20 *g* (Fig. 7). More specifically, Okada and Shimura (1994) reported a degree of curvature of – 44° to unilateral illumination in vertically grown *A. thaliana* roots under 1 *g* conditions and using a PFD of 50 μmol m^{-2} s^{-}. Indeed, in their study, the light and gravity vectors were at right angles and the mean root angle was intermediate (−44°), suggesting the strength of both tropisms being equal.

Similarly, in our case, *B. oleracea* roots showed a degree of curvature of – 41° stimulated by blue or white light under 1 g conditions. However, changes in the magnitude of gravity did not affect root curvature, suggesting that the gravitropic responses saturate at 1 *g* or even at a lower level, and hypergravity does not reduce the effectiveness of phototropism in orienting *B. oleracea* roots. Furthermore, our results under simulated microgravity differ from previous studies on *A. thaliana* reporting either an enhancement of blue-light negative phototropism in microgravity with a significant attenuation at 0.3 *g* (Kiss et al., 2012) or that a blue-light positive phototropism of roots can be revealed in microgravity (Vandenbrink et al., 2016). Nevertheless, it must be considered that the mentioned studies were performed in the near-weightless environment of the International Space Station (ISS), a condition much different from that achievable on Earth using RPM where plants constantly change their orientation with respect to the gravity vector (Kiss et al., 2019). In this regard, further studies are needed to assess phototropic responses of *B. oleracea* in real microgravity to shed light on possible differences at species level.

The phototropic response stimulated by blue or white light was not affected by light intensity in the range of 30–80 μmol m^{-2} s^{-1} PFD. Accordingly, previous studies using *A. thaliana* found that the phototropic response increases with increasing PFD up to about 10 μmol m^{-2} s^{-1} and has a maximal curvature in the range of 10–100 μmol m^{-2} s^{-1} (Sakai et al., 2000; Kiss et al., 2003). Similar results have also been found in *Zea mays* by Mullen et al. (2002) who reported that blue-light phototropic response saturate at 10 μmol m^{-2} s^{-1}. In our study, white light and blue light showed a similar effect and this can be explained considering that white LEDs provided 25% blue light which is the main driver for negative phototropism of roots. Specifically, blue-light photon flux densities of white LEDs were ≈ 8 μmol m^{-2} s^{-1} and ≈ 20 μmol m^{-2} s^{-1} at the maximum and minimum distance from the light source respectively. It is also known that other wavelengths such as green, which represented more then 50% of the emission spectrum of the white LEDs, do not affect root phototropism in a significant way (McCoshum and Kiss, 2011).

Therefore, we hypothesize that in the case of white LEDs, the root curvature of *B. oleracea* was determined by blue light component and that other wavelengths had negligible effect. Interestingly, although peak wavelength of blue (443 nm) was similar for blue and white LEDs, the intensity of blue-light photon flux was lower in white compared to blue treatment. This suggests that root curvature was not affected even at lower PFD (≈8 μmol m^{-2} s^{-1}). In agreement with previous studies on *A. thaliana* and *Z. mays* (Sakai et al., 2000; Mullen et al., 2002; Kiss et al., 2003), our findings suggest that the response of root curvature to light intensity might be a conserved trait among numerous plant species. Nevertheless, further studies using lower PFD in the range of 0–10 μmol m^{-2} s^{-1} must determine the light-intensity threshold for root phototropism of *B. oleracea* and assess whether this response is affected by altered gravity.

As discussed above, either blue or white light stimulated a negative phototropic response of roots which was comparable among gravity treatments and light intensities. Conversely, roots under red light showed a weak curvature in simulated microgravity that was abolished when increasing the gravity level. It is known that red light can trigger a weak positive phototropism in *A. thaliana* roots which is detectable in microgravity and fractional gravity, or using mutants impaired in gravisensing (Ruppel et al., 2001; Kiss et al., 2012). However, considering that in our study a weak curvature was observed also in dark-grown roots under simulated microgravity conditions, an alternate hypothesis is that some of the root curvature might be attributed to a surface-dependent phenomenon referred as root skewing. This phenomenon involves gravity and touch stimuli resulting in a slanted angle of roots when they are growing along a nearly-vertical surface (Oliva and Dunand, 2007; Roux, 2012).

Moreover, root skewing can show significant differences in curvature and direction due to helical circumnutation of roots that can be clockwise or counterclockwise when referring to the direction of root growth (Oliva and Dunand, 2007). Similar to our study, Millar et al. (2011) reported a skew to the right of dark-grown roots of *A. thaliana* (ecotype Landsberg) during a spaceflight experiment, and this growth response was largely masked by the 1 *g* conditions on Earth. Furthermore, the process called automorphogenesis has been described in seedlings germinated and grown under microgravity conditions. It consists of spontaneous curvatures of newly sprouted roots followed by straight root elongations in random directions (Hoson and Soga, 2003; Driss-Ecole et al., 2008). The molecular mechanism of automorphogenesis is still incompletely understood, as well as the relationships between automorphogenesis and skewing.

Overall, *B. oleracea* seedlings exhibited robust development under all gravity conditions with some differences in terms of growth due to the light treatments. It is known that seedlings are extremely sensitive to light quality and have evolved specific photomorphogenic responses to blue and red light which can influence both root and shoot development (Izzo et al., 2020; Izzo et al., 2021). Still, to date, very few studies investigated root growth response to direct illumination with spectral treatments, whereas the effect of light signals perceived by aboveground organs on root growth is more characterized (Gundel et al., 2014; Klem et al., 2019). In our study, roots were directly illuminated with white, blue, or red light from LEDs placed on one side of the EC and no significant difference was found among spectral treatments. However, dark-grown roots were longer than those grown under light, regardless of gravity conditions.

It has been shown that direct illumination of roots can shorten root length, also altering plant response to hormones or abiotic stress (Silva-Navas et al., 2015). Flavonoids, particularly quercetin, are

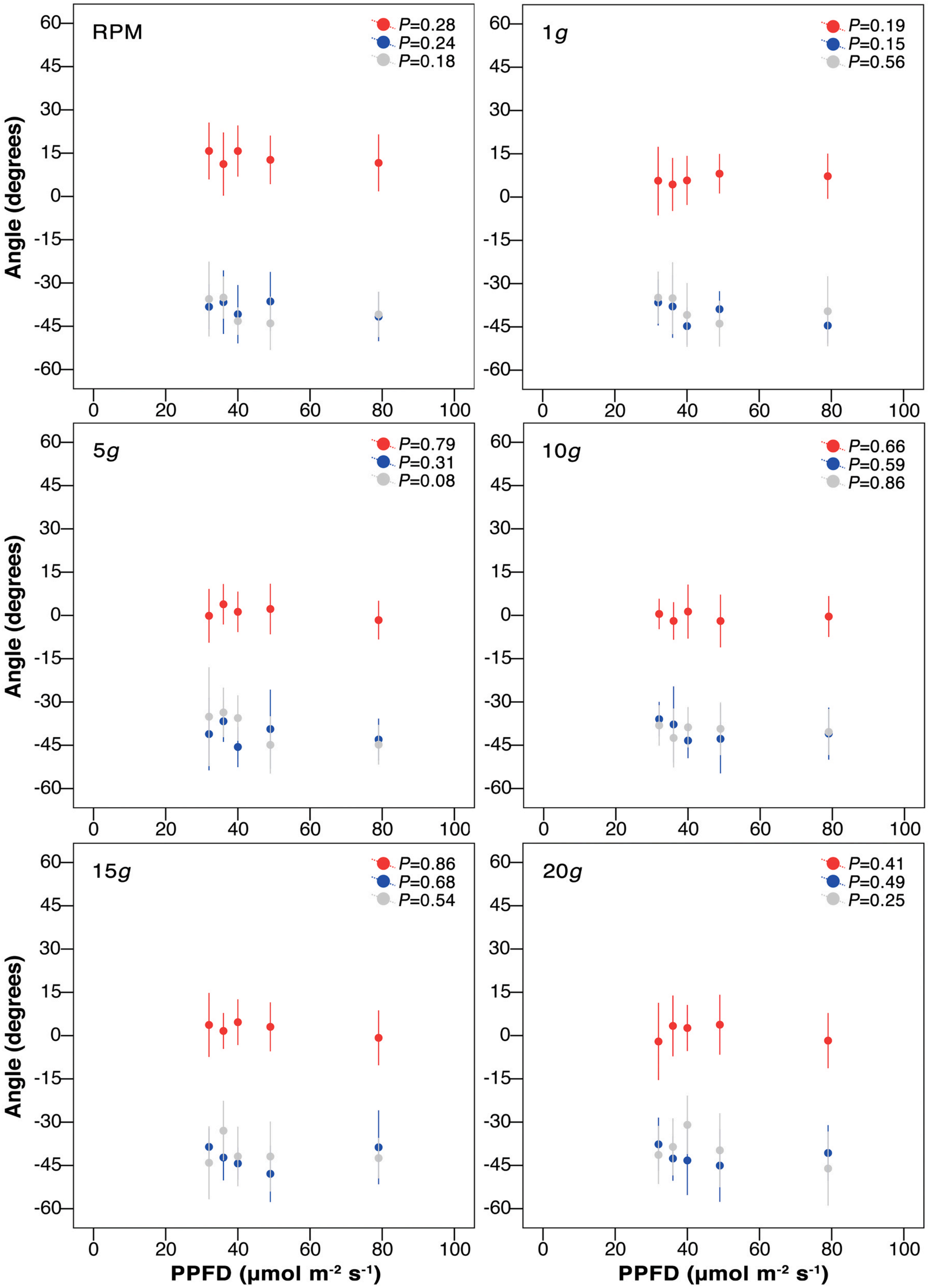

Fig. 7. Effect of photon flux density (PFD) of blue light (blue symbols), red light (red symbols), and white light (grey symbols) on root phototropic response of *Brassica oleracea* seedlings grown at different gravity levels (RPM, 1 *g*, 5 *g*, 10 *g*, 15 *g*, 20 *g*). The degree of curvature was averaged based on measurements on the last five vectors starting from the root tip (alpha 1–5). The plus (+) or minus (-) sign preceding the degree of curvature (α) indicates whether the phototropic response was positive or negative, respectively. Each data point shows the mean and the standard deviation of two replications with 25 plants. The *P*-values reported refer to the linear regression analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

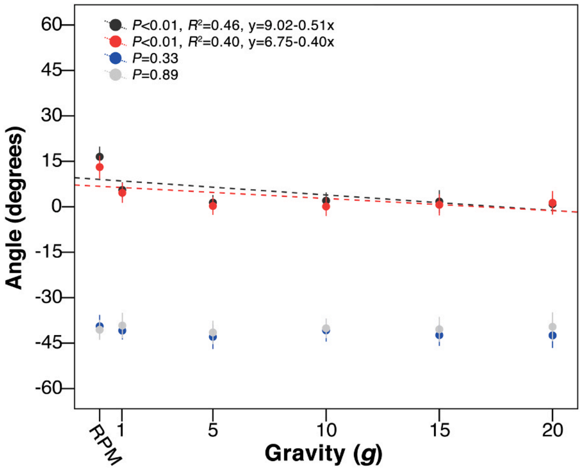

Fig. 8. Effect of gravity levels on root phototropic response of *Brassica oleracea* seedlings grown under different light treatments (dark conditions = dark-grey symbols; red light = red symbols; blue light = blue symbols; white light = light-grey symbols). Treatment using random positioning machine (RPM) was set as 0 *g*. The degree of curvature was averaged based on measurements on the last five vectors starting from the root tip (alpha 1–5). The plus (+) or minus (-) sign preceding the degree of curvature (α) indicates whether the phototropic response was positive or negative, respectively. Each data point shows the mean and the standard deviation of two replications with 25 plants. The *P*-values reported refer to the linear regression analysis. Dotted line represents significant linear regression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

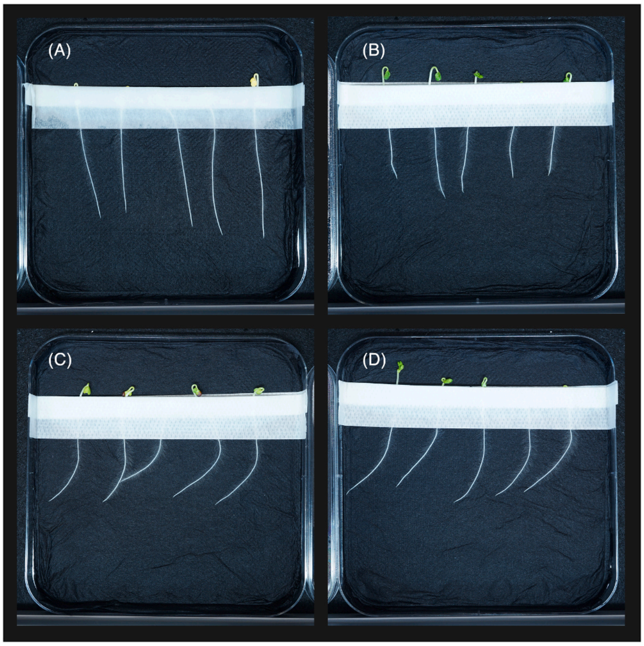

Fig. 9. Seedlings of *Brassica oleracea* grown at 20 *g* under: (A) dark conditions; (B) red light; (C) blue light; and (D) white light. Illumination is from the right side of the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

preferentially synthesized in response to light stress and can inhibit the transport of auxin at cellular and tissue level (Brunetti et al., 2018). Indeed, the different root length between light- and dark-grown roots could be due to light-induced accumulation of flavonoids which are auxin-transport inhibitors that ultimately affect root development (Buer and Muday, 2004; Silva-Navas et al., 2016). Conversely, it has been shown that cotyledon-derived photosynthate can promote root elongation in illuminated seedlings of Arabidopsis, indicating that root development can be modulated by light through inter-organ signalling between shoots and roots (Kircher and Schopfer, 2012). However, in our experiments, the seedlings grew to an early stage of photomorphogenesis with the apical hook and cotyledons still closed which suggests that they were not fully competent to photosynthesis. Hence, it is possible that cotyledon-derived photosynthates were not significant to promote root elongation in our study, also considering the low PFD available for photosynthesis coming from the side of the EC. On the other hand, prolonged exposure to light may have inhibited root growth of illuminated compared to dark-grown roots as reported in other studies (e.g., Reboulet et al., 2010; Hopkins and Kiss, 2012; Silva-Navas et al., 2015).

It is also important to note that flavonoids have been proposed as an alternative signal gradient to auxin in mediating the differential growth response between the illuminated and non-illuminated side of the root (Silva-Navas et al., 2016). In addition, Kimura et al. (2018) concluded that the phototropic response in Arabidopsis roots does not require asymmetric auxin distribution. Indeed, it was shown that the phototropic bending preceded the establishment of asymmetric auxin distribution, then the curvature of the root acted as a stimulus for auxin accumulation in a gravitropism-dependent manner. Similarly, Shkolnik et al. (2016) reported that auxin redistribution is not required for root hydrotropism. Taken together, these findings indicate that the Cholodny-Went theory is not applicable to all root tropisms and other unknown mechanisms should be investigated. In this regard, research in altered gravity provide opportunities to better explore the mechanisms underlying plant tropisms.

We also know that cell proliferation is affected by both microgravity and hypergravity conditions which could affect root growth and elongation (Matía et al., 2010; Manzano et al., 2012). However, illumination, either in the form of red-light photoactivation in spaceflight experiments (Valbuena et al., 2018; Villacampa et al., 2021), or the incorporation of a photoperiod regime to seedlings grown in simulated microgravity (Manzano et al., 2021), was found to attenuate or suppress the effects caused by gravitational stress at the cellular level in the root meristem. Nevertheless, no studies investigated the effect of light quality on cell proliferation under hypergravity conditions and further research is needed to deepen this subject.

B. oleracea has been used as model species for tropism research since the studies on plant movements by Charles Darwin in the late 1800′s. Later studies in the early 1900′s reported a strong negative phototropism of roots stimulated by white light in most species of Brassicaceae family, including *B. oleracea* (Schaefer, 1911; Hubert and Funke, 1937). More recently, tropism research has focused on a better understanding of the molecular pathways in model species such as *A. thaliana,* fostered by the vast database of genetic information and the availability of numerous mutants. Nevertheless, *B. oleracea* belongs to the family Brassicaceae as *A. thaliana* and can represent an ideal alternative organism due to its larger size which facilitate the sampling of target tissue (Esmon et al., 2006). Moreover, there is a substantial homology between nuclear genomes of *B. oleracea* and *A. thaliana,* and both species exhibit time-dependent and saturable phototropic and gravitropic responses (Tatematsu et al., 2004).

In addition, *B. oleracea* is also a candidate crop for the production of microgreens as a component of life support systems in space because of its high content of phytonutrients and minerals to be integrated into the astronaut diet (Kyriacou et al., 2017). However, to date, no studies investigated growth and tropistic response of this species in microgravity, and our findings need to be verified in a true weightless environment such as found in orbiting spacecraft. As already discussed above, either blue or white light stimulated a negative phototropism in *B. oleracea* roots which was strong under all gravity conditions. From an applied science perspective (i.e., using plants as part of bioregenerative life support), the phototropic responses of *B. oleracea* can be exploited to guide root growth in a wide range of gravity conditions such as those of extraterrestrial environments.

5. Conclusions

Our findings provide a characterization of root gravitropism and phototropism interaction evaluating responses to different light spectra and *g* levels. We also extend phototropism research for the first time to hypergravity conditions. The use of *B. oleracea* partly confirmed results obtained in other species highlighting that some tropistic responses may be species-specific. The levels of blue or white light we applied in this study stimulated negative phototropic responses that were not affected by either simulated microgravity or hypergravity, whereas roots illuminated with red light showed a slight curvature in 1 *g* and simulated microgravity but not hypergravity. Significantly, dark-grown roots showed similar responses to red light, indicating that phototropism was not involved in root curvature under red light in a significant manner and that other phenomena (including the surface-dependent touch stimuli) need to be considered. In the light of colonizing extraterrestrial environments, a thorough knowledge of photomorphogenic and phototropic responses of candidate crops such as *B. oleracea* is indispensable for plant cultivation in altered gravity. In this framework, light can represent an effective stimulus to guide plant growth in space, and this approach can be useful in future regenerative life support systems with plants.

CRediT authorship contribution statement

LGI, LER, GA: Conceptualization. **LGI, LER, LM, MI, AM, JvL, GA**: Investigation. JvL, GA: Resources. **LGI, LER, FC**: Data curation. **LGI, FC**: Formal analysis. **LGI**: Writing – original draft. LGI, **LER, LM, MI, FC, AM, MC, RH, FJM, JZK, JvL, GA**: Writing – review & editing. **FJM, JZK, JvL, GA**: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors acknowledge Andrea Buonanno’s contribution to create all parts of the ROOTROPS device in CATIA V5. We would also like to thank Mr. Alan Dowson from ESA-ESTEC TEC- MMG LIS Lab for his support in preparation and during this study. This work was supported by the ESA-HRE CORA contract #4000127705/19/NL/PG/pt. Financial support was also provided by ESA-TEC contract #4000116223/16/NL/KML/hh to Jack J.W.A. van Loon, by Spanish Agencia Estatal de Investigación (Grant RTI2018-099309-B-I00, co-funded by EU- ERDF) to F. Javier Medina, and by NASA (Grant 80NSSC17K0546) to John Z. Kiss.

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