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Spaninks, K.; Lamers, G.E.M.; Lieshout, J. van; Offringa, R.

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Light quality regulates apical and primary radial growth of *Arabidopsis thaliana* and *Solanum lycopersicum*

Kiki Spaninks [a], Gerda Lamers [b], Jelmer van Lieshout [a], Remko Offringa [a,*]

[a] Plant Developmental Genetics, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE Leiden, Netherlands
[b] Core Facility Microscopy, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE Leiden, Netherlands

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ABSTRACT

For a horticultural crop such as tomato (*Solanum lycopersicum*), the initial growth phase of young plants can take place in multi-layer systems to reduce space. Here LEDs form the ideal lighting system, as they decouple light intensity from heating and can thus be placed in close proximity to the plants. Moreover, the spectral quality control of LEDs may be utilized to steer the plants towards a desired compact and sturdy phenotype. To achieve this, we must understand how light quality affects plant elongation and stem thickness during early plant development. Therefore, we assessed apical and radial growth of tomato and *Arabidopsis thaliana* (Arabidopsis) plants grown in white, red, or blue LED conditions. Our analysis revealed that in both species the red LED condition increased cell elongation in hypocotyls and stems, whereas the blue LED condition decreased cell elongation, compared to the white light condition. In seedlings, hypocotyls were thinner in the red LED condition, and thicker in the blue LED condition, compared to white light. However, in flowering plants, Arabidopsis showed sensitivity of primary radial growth to light quality, while tomato plants appeared indifferent. Finally, analysis of Arabidopsis photoreceptor mutants suggested that cryptochromes and type II phytochromes are the main regulators of light-mediated apical and primary radial growth. To summarize, LEDs can be used to regulate both apical and primary radial plant growth, but the resulting phenotypes may be plant age- or species-specific.

1. Introduction

Before the production cycle of a horticultural crop, such as tomato or cucumber, in greenhouses is started, young plants can be grown in growth chambers (nurseries) until the appearance of the first truss. During this initial phase, multi-layer systems can be applied to reduce space. Light-emitting diode (LED) lights decouple light intensity from heating and can therefore be applied in these systems without the risk of overheating the plants, and additionally help to reduce the energy input for cooling systems. Moreover, spectral quality control of LEDs may be used to steer plant development into desired phenotypes. For tomato nurseries, uniform young plants that flower early and remain sturdy and compact until transport are the desired end product. To achieve this, both apical and radial stem growth should be tightly controlled. To avoid misinterpretation, we distinguish between "primary radial growth", indicating an increase in stem thickness caused by cell growth in primary stem structures, and "secondary growth" where an increase in stem thickness results from cell divisions in the vascular or cork cambium.

The stem is initiated in the rib zone (RZ) of the shoot apical meristem (SAM), where the central region gives rise to the pith, the boundary region to the vasculature, and the peripheral regions produce epidermal and cortex cells (Sachs, 1965). Apical stem growth depends on cell division as well as on cell elongation. Cell division in the RZ is mainly promoted by signaling pathways that respond to the phytohormones gibberellic acid (GA) and brassinosteroids (BR) (Davière et al., 2014; Gallego-Bartolomé et al., 2012), while cell elongation in stem tissues depends mainly on the interplay between auxin, abscisic acid and GA (Ross et al., 2003; Seo et al., 2006; Oh et al., 2007). Modulation of apical stem growth by light quality and intensity has been linked to these

Abbreviations: BR, brassinosteroids; Col-0, Arabidopsis ecotype Columbia; CRY, cryptochrome; DAG, days after germination; DAS, days after sowing; FO, tomato (hybrid) cultivar Foundation; GA, gibberellic acid; LED, light-emitting diode; Ler, Arabidopsis ecotype Landsberg erecta; LMP, low melting point; MM, tomato cultivar Moneymaker; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PHY, phytochrome; PHOT, phototropin; R:FR, red/far red; RZ, rib zone; SAM, shoot apical meristem; SEM, scanning electron microscope.

* Corresponding author.
E-mail address: r.offringa@biology.leidenuniv.nl (R. Offringa).

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phytohormones, and has already been reported in several species (Zheng et al., 2019; Gawronska et al., 1995; Hisamatsu et al., 2005; Paradisoand Proietti, 2021), with the most well-known example being the shade avoidance syndrome where apical stem growth is increased under low light intensity or low red/far-red (R:FR) ratios. Plants detect changes in light quality through specialized chromoproteins called photoreceptors, of which each class is activated by a specific wavelength range. Phytochromes (PHYs) respond to (far-)red light, whereas cryptochromes (CRYs), phototropins (PHOTs), and Zeitlupes respond to blue light (Galvão and Fankhauser, 2015). While PHYB and CRY1 photoreceptors have been reported to inhibit apical growth of hypocotyls (Neff and Chory, 1998), it remains unclear if this is driven by cell division or elongation, and whether or not the response is similar in the adult stem. Moreover, changes in light quality modulate the activity of multiple photoreceptors simultaneously, thus adding another layer of complexity.

Different from apical growth, radial growth is initiated in lateral meristems that consist of a xylem- and phloem-producing vascular cambium, and a cork- and phelloderm-producing cork cambium (Barra-Jiménez and Ragni, 2017). Stem thickness is determined by cell size, stem cell proliferation, and their differentiation into secondary tissues. Vascular stem cell proliferation and differentiation rely on the phytohormones auxin, cytokinin, BR, and their downstream signaling components (Suer et al., 2011; Etchells et al., 2013, 2016). Further towards the outside of the stem, phloem formation is promoted, whereas xylem is produced towards the inside of the stem (Kubo et al., 2005; Guo et al., 2008; Yordanov et al., 2010). Similar to apical stem growth, radial growth has been shown to respond to alterations in light quality, intensity, and day length. For example, low light intensity, short days, and low R:FR ratios all result in thicker stems in Arabidopsis and potato plants, respectively (; Botterweg-Paredes et al., 2020; Li-Li et al., 2020). While the red light responsive PHYB photoreceptor has been reported to promote radial stem growth in maize (Wies et al., 2019), the underlying molecular and cellular mechanisms remain unclear. In addition, radial growth studies that include other photoreceptors, or are conducted in other species are limited.

In order to steer plant growth towards the desired sturdy and compact phenotypes, a better understanding of light-regulated stem growth and development is required. In a previous study, we observed that hypocotyl and stem elongation can be modulated by red or blue light in both *Arabidopsis thaliana* (Arabidopsis) and *Solanum lycopersicum* (tomato) (Spaninks et al., 2020). To further investigate these observations, we performed histological and microscopic analyses on the hypocotyls and stems of Arabidopsis and tomato plants grown in white, red, or blue LED conditions. Here we show that apical growth of both hypocotyls and stems in response to light quality mostly relies on cell elongation. Furthermore, we confirm that primary radial growth of hypocotyls and stems can be controlled by light quality in Arabidopsis, and that the stem phenotype is affected both by the formation of vascular bundles and by primary xylem production. However, while tomato hypocotyls responded to light quality in a similar way, the primary radial growth in the stems of 30-day-old tomato plants was indifferent to light quality, once again demonstrating that phenotypes from a genetic model organism may not always be translated to horticultural crops.

2. Materials and methods

2.1. Growth conditions and led treatments

In all experiments, plants were grown at a 16 h photoperiod, under white (#9290 008 43,003), deep red (#9290 006 32,003), or blue (#9290 006 32,203) Philips Greenpower LED research modules (Signify B.V., Eindhoven, Netherlands) with a measured photon flux density of 120±10 μmol m^{-2} s^{-1} at the top of the canopy, a temperature of 21 °C, and 70% relative humidity. The percentages of blue, green, red, and far-red wavelengths for the different LED modules are listed in Table S1. Experiments with the different LED treatments were performed simultaneously in the same growth chamber in separate compartments enclosed by white plastic screens with a proximal distance of 50 cm to the plants.

2.2. Plant lines and seed germination

Arabidopsis thaliana (Arabidopsis) ecotypes Columbia (Col-0) and Landsberg *erecta* (*Ler*), and *Solanum lycopersicum* (tomato) cultivars Moneymaker (MM) and Foundation (FO) were used as wild-type accessions. All wild-type and mutant lines that were used have been described before and are listed in Table S2. All Arabidopsis mutants (T-DNA insertion lines in Col-0 background) were genotyped with the primers listed in Table S3. Arabidopsis seeds were sown on the soil surface and stratified for 5 days at 4 °C in darkness before they were placed in white light to allow simultaneous germination. After one day in white light, the seeds were moved to the LED conditions. Tomato seeds were planted approximately 2 cm under the soil surface and placed directly in the LED conditions. The age of tomato plants was expressed in days after sowing (DAS), whereas the age of Arabidopsis plants was expressed in days after germination (DAG).

2.3. Imaging of hypocotyl epidermis

Hypocotyls of 7-day-old Arabidopsis seedlings were cut directly below the transition zone and fixed in 2% paraformaldehyde (PFA) and 1% glutaraldehyde in 1x phosphate-buffered saline (PBS) solution for 2 h at 21 °C, and overnight at 4 °C. Fixed hypocotyls were washed twice with 1x PBS and dehydrated in a graded acetone series (70, 80, 90, 96 and 100%) under vacuum. Acetone suspensions were transferred to a Bal-Tec CDP030 critical point dryer where acetone was replaced by liquid carbon dioxide. Samples were fixed to stubs and sputter coated with gold using the SEM Coating unit 5100 (Polaron Equipment Ltd.). Gold was discharged by admitting pressurized argon in a low vacuum environment, and coated samples were kept under dry vacuum conditions. Samples were imaged with a scanning electron microscope (JSM-6400, JEOL, Tokyo, Japan) at magnifications of 25x, 80x, and 250x. Hypocotyls of 5-day-old tomato seedlings were cut directly below the transition zone and mounted onto a glass slide using 1% low melting point (LMP) agarose (#16,520,050, Thermo Scientific™) and imaged with a stereomicroscope (MZ16FA, Leica, Heerbrugg, Switzerland) equipped with a Leica DFC420C camera.

2.4. Fixation and epon embedding of hypocotyls and stems

Hypocotyls of 7-day-old Arabidopsis seedlings and 5-day-old tomato seedlings were cut directly below the transition zone using a razorblade (Wilkinson Sword) and fixed overnight in a 4% PFA in 1x PBS solution. Stems of Arabidopsis plants at 1 and 4 weeks after bolting were cut at the base of the main inflorescence using a razorblade and kept on ice. Stem segments of at least 1 cm in size were fixed in 4% PFA in 1x PBS for 2 h at 21 °C, and overnight at 4 °C. Fixed hypocotyls and stems were washed twice with 1x PBS and dehydrated in a graded ethanol series (70, 80, 90, 96 and 100%) under vacuum. After dehydration, the tissues were placed in propylene oxide and subsequently in a 1:1 propylene oxide: Epon mixture overnight. The Epon-drenched tissues were embedded in epoxy resin molds that dried overnight at 60 °C, and were stored at 21 °C. At 30 DAS, tomato stems were cut directly below the third leaf using a razor blade. The stems were kept on ice until fixation in 4% PFA in 1x PBS for 2 h at 21 °C, and overnight at 4 °C. The fixed stems were washed twice with 1x PBS and stored in 70% ethanol at 4 °C.

2.5. Sectioning and imaging of hypocotyls and stems

Epon-embedded hypocotyls and stems were trimmed and 3–4 μm

sections were cut with a Leica RM2265 rotary microtome equipped with a glass knife. The sections were mounted on a glass slide and stained with filtered 0.01% aqueous toluidine blue. A droplet of Epon was placed on the stained sections and covered with a glass coverslip. For tomato stems, sectioning was performed using a razor blade (very thin free-hand sectioning). The sections were stained with 6% aqueous toluidine blue, washed with MilliQ water, and mounted on a glass slide without cover slip. All stem sections were imaged with a stereomicroscope (MZ16FA, Leica, Heerbrugg, Switzerland) equipped with a Leica DFC420C camera, and all hypocotyl sections were imaged with a light microscope (Axioscope A1, Zeiss, Oberkochen, Germany) equipped with a Zeiss AxioCam MRc5 camera.

2.6. Plant phenotyping

To measure hypocotyl length, Arabidopsis seedlings were grown in the LED conditions and photographed at 7 DAG. Plant height was measured with a tape-measure in Arabidopsis plants after termination of the primary inflorescence meristem. For both species, microscopic images of longitudinal sections were used to measure pith cell length, whereas cross section images were used to measure the stem area and xylem width, and to count the number of vascular bundles. All image measurements were performed using image-processing software ImageJ (Fiji, Wisconsin, United States of America) (Schindelin et al., 2012).

2.7. Statistical analysis and figures

All experiments were performed with two technical replicates of 15 or 20 biologically independent plants for tomato and Arabidopsis, respectively. Measurements under different LED conditions, or comparing different plant lines, were statistically analyzed using a one-way ANOVA followed by a Tukey's honestly significant different (HSD) post hoc test and plotted into graphs using 2D scientific graphing and statistics software GraphPad Prism 5 (Dotmatix, San Diego, Unites States of America) software. In the graphs, the colors of the dots and lines indicate white, red, and blue LED conditions. Microscopic images were edited in ImageJ software (Fiji, Wisconsin, United States of America). Schematic models were generated with illustrator software (BioRender, Toronto, Canada). Final figures were assembled using Microsoft PowerPoint.

3. Results

3.1. Apical growth of Arabidopsis and tomato hypocotyls is regulated by light quality

To assess the effect of light quality on hypocotyl elongation in Arabidopsis, seedlings of ecotype Columbia (Col-0) were grown for seven days in white, red, or blue LED conditions. As described previously, hypocotyls were longer in red light, and shorter in blue light, when compared to white light conditions (Spaninks et al., 2020; Fig. 1A, B). Scanning electron microscopy analysis revealed that the hypocotyl epidermis cells were greatly affected by light quality (Fig. 1C). Epidermal cells of seedlings grown in monochromatic red light were extremely elongated and appeared to be flaccid due to a possible loss of turgor, or rapid elongation. In contrast, seedlings grown in monochromatic blue light showed very small and turgid epidermal cells (Fig. 1C). Next, we used stereomicroscopy to visualize the hypocotyls of 5-day-old wild-type tomato seedlings of both Moneymaker (MM) and the commercial hybrid Foundation (FO) grown in the different LED conditions. Hypocotyls of seedlings grown in monochromatic red light appeared to be greener, while hypocotyls of blue light-grown seedlings appeared slightly purple, compared to white light (Fig. 1D). At a higher magnification, we observed that hypocotyl epidermis cells of red light-grown seedlings were elongated, whereas epidermis cells of seedlings grown in monochromatic blue light were shorter, compared to white light (Fig. 1E). Together, these results show that hypocotyl elongation of both Arabidopsis and tomato seedlings can be modulated by light quality.

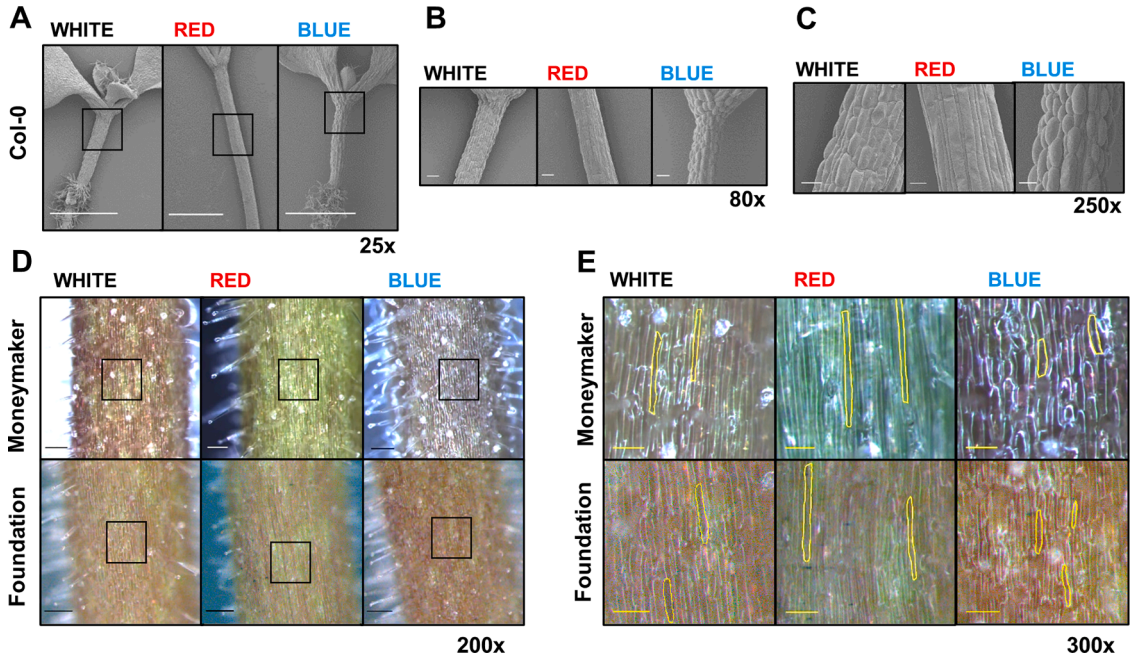

Fig. 1. Epidermal cell elongation in Arabidopsis and tomato hypocotyls is regulated by light quality.
A-C: Scanning electron microscopy images of hypocotyls from 7-day-old Arabidopsis seedlings of ecotype Columbia (Col-0) that were grown in white, red, or blue LED conditions. D-E: Stereomicroscopy images of hypocotyls of 5-day-old tomato seedlings of cultivars Moneymaker and Foundation that were grown in the different LED conditions. Hypocotyls were imaged at 25x (A), 80x (B), 250x (C), 200x (D), and 300x (E) magnification. Black boxes in A and D indicate the area that was further magnified in B, C and E, respectively. Single epidermal cells were highlighted in yellow in E. Scale bars indicate 1 mm (A), 100 μm (B), 50 μm (C), 200 μm (D) and 150 μm (E). Images shown are of representative seedlings (n=15), and similar results were obtained from two independent experiments.

3.2. Primary radial growth of Arabidopsis and tomato hypocotyls is regulated by light quality

Next, we investigated radial growth in hypocotyls of 7-day-old Arabidopsis seedlings of ecotypes Col-0 and Landsberg *erecta* (L*er*) that were grown in the different LED conditions. Toluidine blue stained cross sections showed that, both for Arabidopsis Col-0 and L*er* ecotypes, in monochromatic red light, hypocotyls were thinner, whereas in blue light hypocotyls were thicker than in white light (Fig. 2A, B). This difference in hypocotyl thickness appears to rely on cell size rather than cell number (Fig. 2A). Analysis of the hypocotyls of 5-day-old tomato seedlings of MM and FO cultivars grown in white, red, or blue light showed that, similar to Arabidopsis, treatment with monochromatic red light resulted in thinner hypocotyls, and treatment with monochromatic blue light in thicker hypocotyls (Fig. 2C, D). Furthermore, like in Arabidopsis, primary radial growth of tomato hypocotyls appeared to rely mostly on cell size (Fig. 2C). Secondary growth was not observed in any of the Arabidopsis or tomato hypocotyls. To summarize, these results show that radial hypocotyl growth is affected by light quality in both Arabidopsis and tomato seedlings.

3.3. Apical stem growth of reproductive Arabidopsis and tomato plants is regulated by light quality

Next, we investigated the effect of light quality on apical stem growth in Arabidopsis and tomato plants. At 4 weeks after bolting, longitudinal sections of the basal internode of the main inflorescence of Arabidopsis plants of ecotypes Col-0 and L*er* suggested that the size of cortex, vascular, and pith cells were affected by the LED conditions (Fig. 3A). Measurements of the pith cells confirmed that the red LED condition increased pith cell length, whereas the blue LED condition decreased pith cell length, compared to white light. This difference was observed in both ecotypes, although for blue light it was less pronounced in L*er* stems (Fig. 3B). Longitudinal sections of the basal part (just above the epicotyl) of stems of 30-day-old tomato plants of MM and FO cultivars showed that treatment with monochromatic red or blue light affects the pith cells (Fig. 3C). Quantification of pith cell length showed that, compared to white light, the red LED condition significantly increased pith cell length in the stems of both MM and FO, while the blue LED condition significantly increased pith cell length only in MM (Fig. 3D). The longitudinal sections revealed that light quality can be used to modulate apical stem growth in both Arabidopsis and tomato, and that, similar to apical hypocotyl growth, these phenotypes rely (at least in part) on cell elongation.

3.4. Primary radial growth of Arabidopsis, but not of tomato stems, is regulated by red and blue light

For analysis of radial stem growth in Arabidopsis, we made cross-sections from the most basal internode of the primary inflorescence of Arabidopsis plants at 4 weeks after bolting. Stems of both Col-0 and L*er* were thicker when grown in monochromatic red light, and thinner in monochromatic blue light, when compared to white light (Fig. 4A). Quantification of the surface area of each cross-sectioned stem confirmed a statistically significant increase in red light, and decrease in blue light, when compared to white light (Fig. 4B). At higher magnification, we observed structural differences in the different stem tissues. For example, the cortex and pith of stems from plants that were grown in blue light consisted of less layers than those from plants grown in white or red light (Fig. 4C). But also the primary xylem width, and the number of vascular bundles were enhanced in red light and reduced in blue light compared to white light (Fig. 4D, E). Interestingly, most of these differences in primary radial growth of Arabidopsis stems could already be observed at 1 week after bolting (Figure S1). Although no secondary xylem or phloem was observed in any of the samples, stems of Arabidopsis plants grown in red light contained interfascicular cambium, whereas no early signs of secondary growth were observed in plants grown in the white and blue LED condition. Next, we analyzed cross

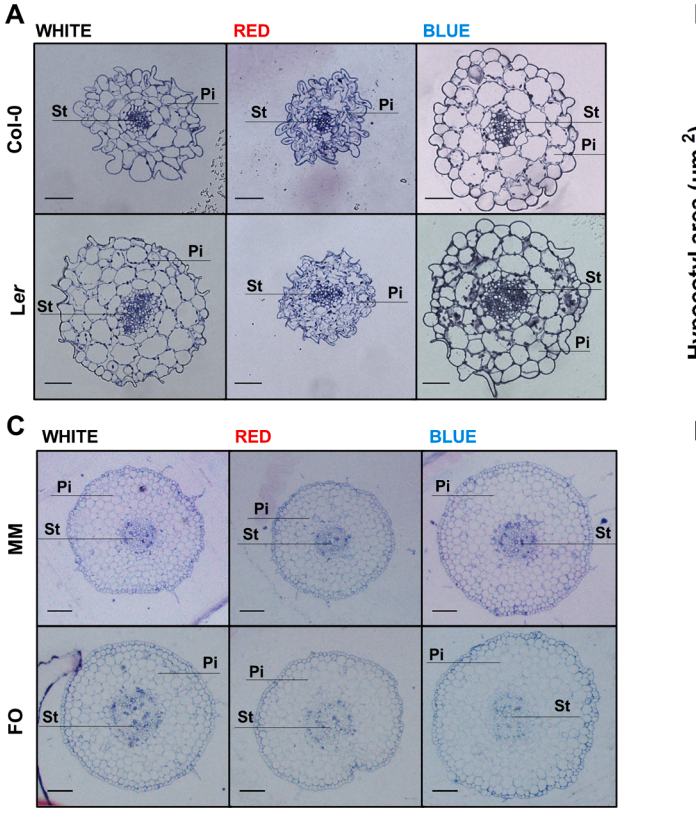

Fig. 2. Primary radial growth of Arabidopsis and tomato hypocotyls is regulated by light quality.
A. Light microscopy images of toluidine blue stained cross sections from hypocotyls of 7-day-old Arabidopsis seedlings of ecotypes Columbia (Col-0) and Landsberg *erecta* (L*er*) grown in white, red, or blue LED conditions. B. Dot plot presenting the surface area of Col-0 and L*er* hypocotyl cross sections in square micrometres (μm^2). C. Light microscopy images of toluidine blue stained cross sections from the hypocotyl of 5-day-old tomato seedlings of cultivars Moneymaker (MM) and Foundation (FO) grown in white, red, or blue LED conditions. D. Dot plot presenting the surface area of MM and FO hypocotyl cross sections in square millimetres (mm^2). Scale bars indicate 10 µm in A and 150 µm in C. Pi=pith and St=stele in A and C. LED conditions and ecotypes were compared using a one-way ANOVA followed by a Tukey's test (letters a, b, c, and d indicate statistically significant differences, $p<0.05$) in B and D. Error bars represent standard error from mean (n = 10) in B and D. Similar results were obtained from two independent experiments.

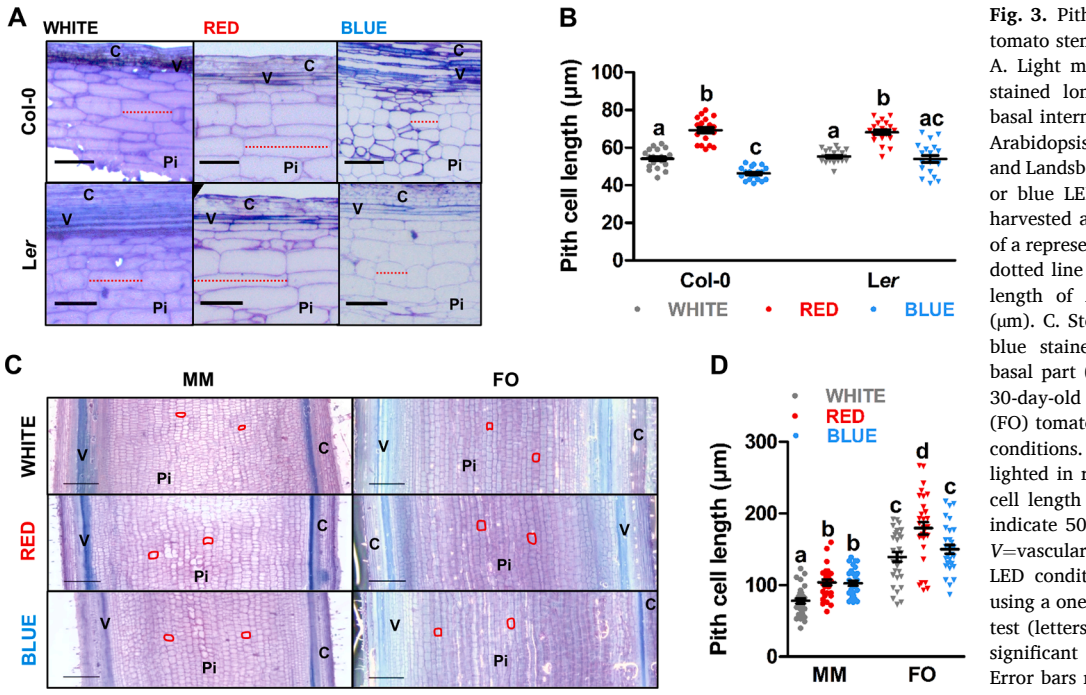

Fig. 3. Pith cell elongation in Arabidopsis and tomato stems is regulated by light quality.
A. Light microscopy images of toluidine blue stained longitudinal sections from the most basal internode of the primary inflorescence of Arabidopsis plants (ecotypes Columbia (Col-0) and Landsberg *erecta* (L*er*)) grown in white, red, or blue LED conditions. Stem segments were harvested at 4 weeks after bolting. The length of a representative pith cell is indicated by a red dotted line B. Dot plot presenting the pith cell length of Arabidopsis stems in micrometres (µm). C. Stereomicroscopy images of toluidine blue stained longitudinal sections from the basal part (above the epicotyl) of the stem of 30-day-old Moneymaker (MM) and Foundation (FO) tomato plants grown in the different LED conditions. Representative pith cells are highlighted in red. D. Dot plot presenting the pith cell length of tomato stems in µm. Scale bars indicate 50 µm in A and 1 mm in C. Pi=pith, *V*=vascular tissue and C=cortex in A and C. LED conditions and ecotypes were compared using a one-way ANOVA followed by a Tukey's test (letters a, b, c, and d indicate statistically significant differences, $p<0.05$) in B and D. Error bars represent standard error from mean (n=30) in B and D. Similar results were obtained from two independent experiments.

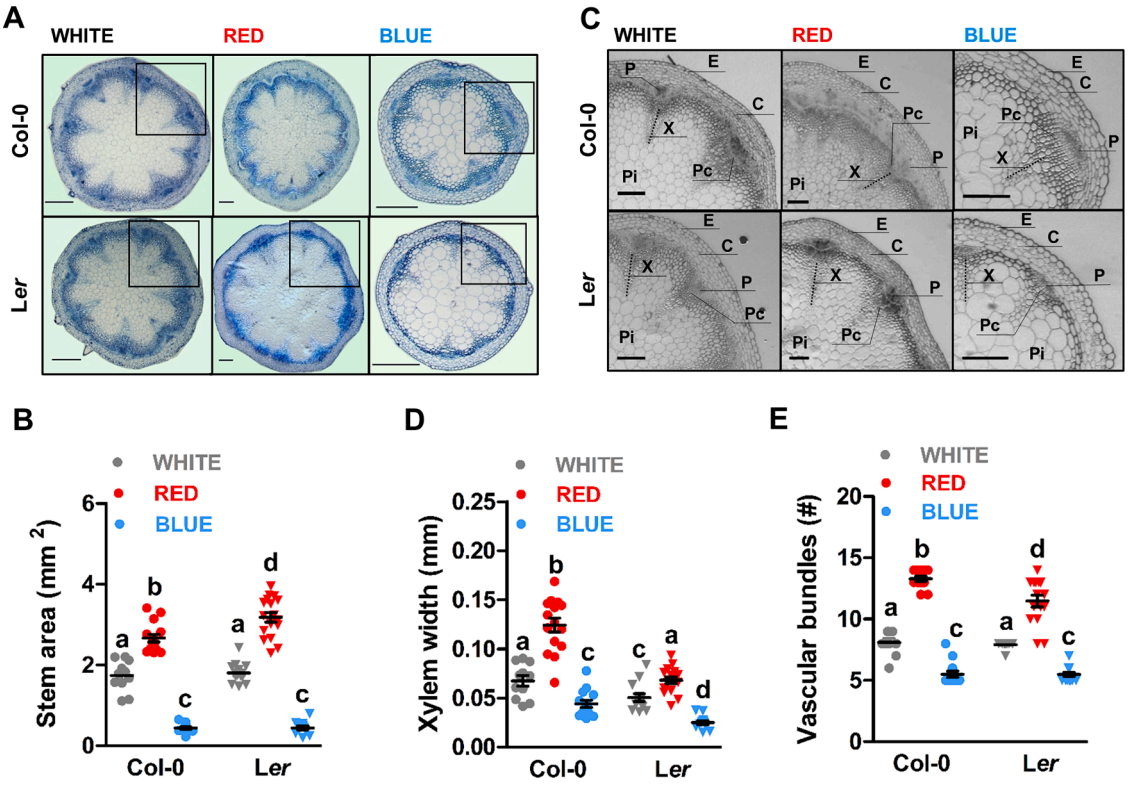

Fig. 4. Primary radial growth of Arabidopsis stems is regulated by light quality.
A. Stereomicroscopy images of toluidine blue stained cross sections from the most basal internode of the primary inflorescence of Arabidopsis plants (ecotypes Columbia (Col-0) and Landsberg *erecta* (L*er*)) grown in white, red, or blue LED conditions. Stem segments were harvested at 4 weeks after bolting. B. Dot plot presenting the surface area of Col-0 and L*er* stem cross sections in square millimetres (mm^2). C. 2x digital magnification of the boxed areas in A to show the vascular bundles in more detail (*E*=epidermis, C=cortex, *P*=phloem, Pc=procambium, *X*=xylem, and Pi=pith). D. The width (in mm) of the primary xylem tissue in Col-0 and L*er* stems. Each dot represents the average of three measurements within one stem. E. The number of vascular bundles in Col-0 and L*er* stems. Scale bars indicate 200 µm in A and 100 µm in C. LED conditions and ecotypes were compared using a one-way ANOVA followed by a Tukey's test (letters a, b, c, and d indicate statistically significant differences, $p<0.05$) in B, D and E. Error bars represent standard error from mean (n = 15) in B, D and E. Similar results were obtained from two independent experiments.

sections of the stems of 30-day-old MM and FO tomato plants. Interestingly, the tomato stems showed a similar stem surface area in all three LED conditions (Fig. 5A, B). In addition, we did not observe any differences in stem tissues (Fig. 5C), xylem width or vascular bundle number (Fig. 5D, E). These results indicate that primary radial stem growth of Arabidopsis plants can be steered by red or blue light, while this appears to be impossible in young tomato plants. In addition, the effect of red and blue light on Arabidopsis primary radial growth are opposite in seedlings and reproductive plants.

3.5. *Light-regulated apical and primary radial growth of Arabidopsis plants relies mostly on cryptochromes and type II phytochromes*

To investigate the role of photoreceptors in apical and primary radial growth, single mutants of the phytochrome (PHY), cryptochrome (CRY), or phototropin (PHOT) type of photoreceptors were grown in the different LED conditions. Like wild type, all mutants developed a longer hypocotyl in red light, and a shorter hypocotyl in white or blue light (Fig. 6A). *cry1* seedlings had a longer hypocotyl than wild-type seedlings grown in white and monochromatic blue light, but not in monochromatic red light. The hypocotyl length of *phyB* seedlings was increased in all three LED conditions. In contrast, *phyD* and *phot1* seedlings had a slightly shorter hypocotyl in respectively white and monochromatic red light, compared to wild-type seedlings. All other photoreceptor mutants showed a hypocotyl phenotype similar to that of wild type in the different LED conditions (Fig. 6A). The final plant height of all mutants, like wild type, was increased in red light and decreased in blue light. *cry2* plants showed an increase in plant height, compared to wild-type plants grown in white and monochromatic blue light. *phyB* and *phyC* plants were taller than wild-type plants grown in white and monochromatic red light. In contrast, *phyD* plants were shorter than wild-type plants grown in all three LED conditions. (Fig. 6B). The stem thickness of most mutants, like wild type, was increased in red light and decreased in blue light. Only *cry1* and *cry2* plants had a thinner stem compared to wild-type plants in all three LED conditions, while *phyB* stems were only thinner in white and monochromatic red light. (Fig. 6C). Altogether this data suggests that apical and primary radial growth of Arabidopsis is regulated by light quality, mostly via CRYs and type II PHYs (PHYB to PHYE) (Fig. 6D). However, the positive effects of CRYs on primary radial growth are also observed in the red LED condition, suggesting putative interactions with PHYs, or light-independent functions of CRYs in this process.

4. Discussion

If we wish to use LEDs to steer plants towards a short and compact architecture that is desired during the initial growth phase, we must understand how light quality regulates plant growth. Previously, we showed that treatment with monochromatic red light increased hypocotyl length and plant height in Arabidopsis and tomato, while the effect of monochromatic blue light was opposite (Spaninks et al., 2020). Here we performed histological and microscopic analyses to further investigate apical and radial growth of stems and hypocotyls under different LED conditions.

4.1. *Regulation of apical growth by light quality*

Scanning electron microscopy revealed that the hypocotyl epidermal cells of Arabidopsis seedlings grown in monochromatic red light are extremely elongated (3–4-fold compared to white light) and appear

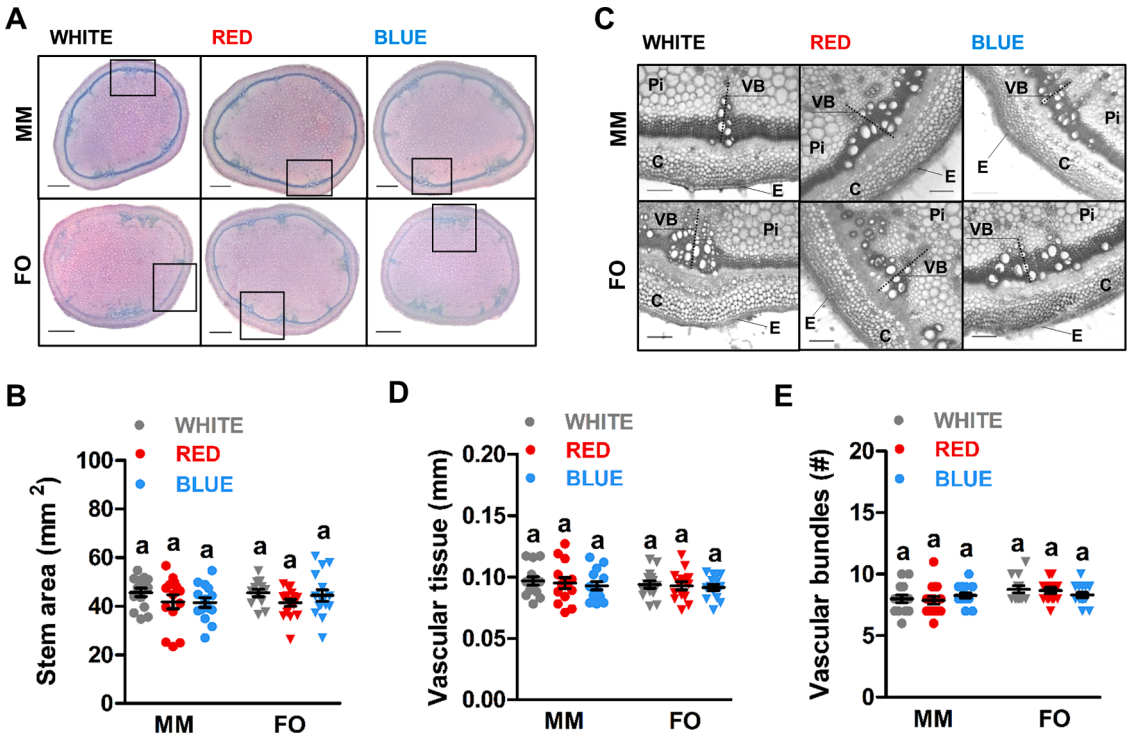

Fig. 5. Primary radial growth of young tomato stems is indifferent to light quality.
A. Stereomicroscopy images of toluidine blue stained cross sections from the basal part (above the epicotyl) of the stem of 30-day-old tomato plants (cultivars Moneymaker (MM) and Foundation (FO)) grown in white, red, or blue LED conditions. B. Dot plot presenting the surface area of MM and FO stem cross sections in square millimetres (mm^2). C. 4x digital magnification of the boxed areas in A to show the vascular bundles in more detail (*E*=epidermis, C=cortex, VB=vascular bundle, and Pi=pith). Several magnifications were reoriented for easy comparison. D. The width (in mm) of the vascular tissue in MM and FO stems. Each dot represents the average of three measurements within one stem. E. The number of vascular bundles in MM and FO stems. Scale bars indicate 2 mm in A and 500 μm in C. LED conditions and cultivars were compared using a one-way ANOVA followed by a Tukey's test (different letters indicate statistically significant differences, $p<0.05$) in B, D and E. Error bars represent standard error from mean ($n = 15$) in B, D and E. Similar results were obtained from two independent experiments.

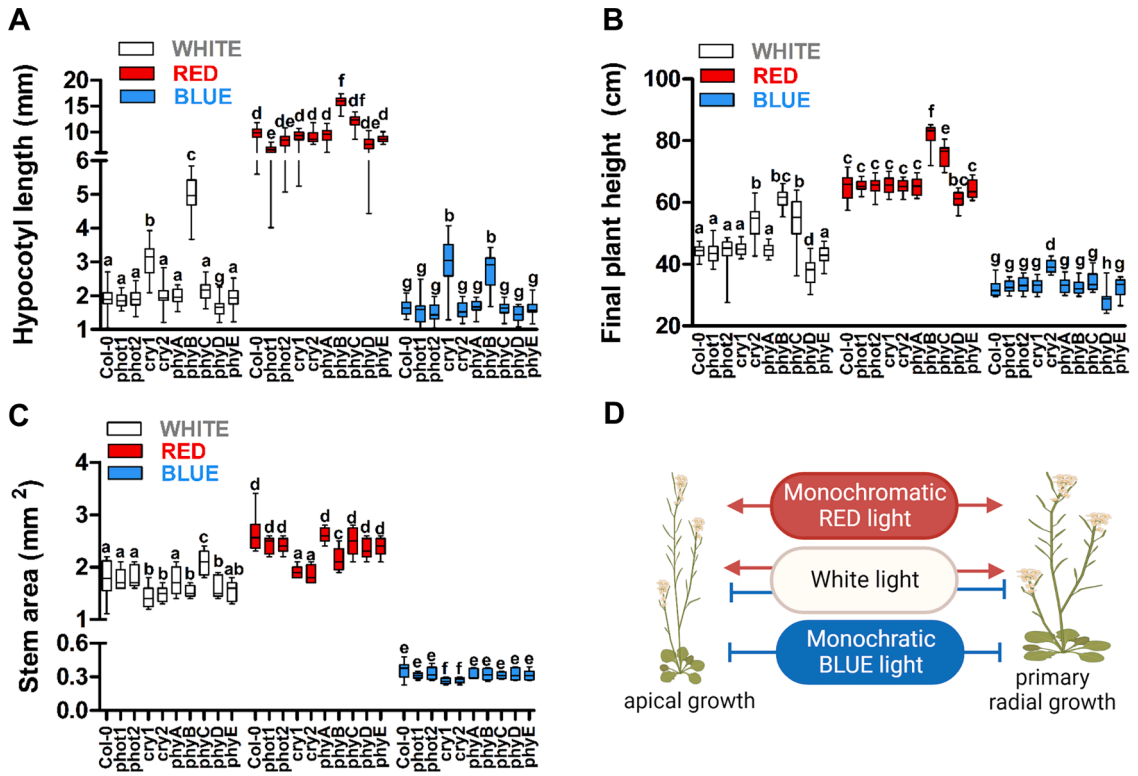

Fig. 6. Cryptochromes and Type II phytochromes regulate apical and primary radial growth in Arabidopsis.
A. Quantification of hypocotyl length of 7-day-old Arabidopsis photoreceptor mutants grown in white, red, or blue LED conditions. Single mutants of the following families were included: red / far-red light-sensing phytochromes (phys), and blue light-sensing cryptochromes (crys) and phototropins (phots). B. Plant height of Arabidopsis photoreceptor mutants grown in the different LED conditions. C. Box plot presenting the surface area of photoreceptor mutant stems in square millimetres (mm^2). D. Simplified model of apical and primary radial growth of *Arabidopsis thaliana* in the different LED conditions. Monochromatic red light results in increased plant height and primary radial growth. In monochromatic blue light plant height is decreased (most likely resulting from CRY-mediated inhibition) and primary radial growth as well. In white light, the effects of red and blue light are balanced, resulting in an intermediate phenotype. To further confirm photoreceptor functions in white light, double and triple mutants should be studied to identify putative interactions between PHY and CRY signalling. LED conditions and plant lines were compared using a one-way ANOVA followed by a Tukey's test (letters a, b, c, d, e, and f indicate statistically significant differences, $p<0.05$) in A–C. Error bars represent standard error from mean in A-C ($n=20$). Similar results were obtained from two independent experiments.

flaccid. In contrast, blue light-grown seedlings had small and turgid epidermal cells. Although the cell elongation phenotype was clear, the microscopic analysis was insufficient to observe changes in cell division. In addition, cell layers other than the epidermis remain to be investigated. Since most hypocotyl cells are produced by cell divisions during embryogenesis, cell elongation is generally thought to be the driving force of hypocotyl growth (Gendreau et al., 1997). Stereomicroscopy images of tomato seedlings revealed a similar hypocotyl phenotype, suggesting that apical growth responses to light are conserved, which is in line with studies performed in lettuce and chili peppers (Volmaro et al., 1998; Liu et al., 2019). In contrast, cucumber seedlings have long hypocotyls in blue light, thus indicating some species-specificity (Hernández and Kubota, 2016). Longitudinal sections of the primary inflorescence stem showed a similar response to light quality in reproductive Arabidopsis plants, suggesting that apical growth of Arabidopsis is promoted by red wavelengths and inhibited by blue wavelengths throughout its life cycle. While similar effects on the plant height of wheat and chili peppers have been reported, these studies did not include any genetic or cell biological analyses (Gangadhar et al., 2012; Monostori et al., 2018). Our analysis showed that apical growth of the *cry* photoreceptor mutants was significantly increased compared to wild type, suggesting that CRY1 and CRY2 photoreceptors inhibit respectively hypocotyl and inflorescence stem elongation in spectra that contain blue light. CRY1 has been reported to inhibit epidermal cell elongation in Arabidopsis hypocotyls grown during thermomorphogenesis (Ma et al., 2015), but this function has not been correlated to light quality yet. Similarly, we showed that apical growth of *phyB* and *phyC* mutants was significantly increased, while *phyD* mutants were significantly shorter than wild type. This suggests that, in spectra that contain red light, PHYB and PHYC photoreceptors inhibit apical growth as well, while PHYD promotes apical growth. Although PHYB has been reported to inhibit hypocotyl epidermal cell elongation (Allen et al., 2019), its role in apical stem growth has until now been limited to plant height studies without further histological or microscopic analysis (Weller et al., 2000; Cortés et al., 2016). Moreover, no roles for PHYC and PHYD in apical cell elongation have yet been reported. To conclude: in monochromatic red light, there is no CRY inhibition of apical growth, but instead there is PHYD promotion of apical growth, resulting in taller plants. In contrast, in the blue LED condition, PHYD cannot promote, while CRYs actively inhibit apical growth, resulting in shorter plants (Fig. 6D). In tomato, apical growth responses to light quality seem to change as the plant ages. While red or blue wavelengths respectively promote or inhibit apical growth in seedlings and young plants, the growth responses turn opposite in older, flowering plants (Yang et al., 2018). We expect this to be a gradual change over time, and that our 30-day-old plants that had only just started to flower were undergoing that transition at the time of measurements, thus explaining the increased pith cell length in the blue LED condition. To summarize, we clearly show that red and blue light affect apical growth through cell elongation. During cell elongation, loosening of the normally rigid cell wall is required, while turgor pressure inside the vacuole drives expansion (Kaiser and Scheuring, 2020). We hypothesize that in monochromatic red light epidermal cell walls are loose, allowing the cells to grow rapidly while water uptake lags behind. In contrast, the

blue LED condition might cause extremely rigid cell walls, causing vacuolar pressure to build. Therefore we suggest that light quality affects both auxin-dependent cell wall acidification for cell wall loosening, and auxin-mediated vacuolar osmosis to regulate apical growth (Fendrych et al., 2016; Scheuring et al., 2016).

4.2. Regulation of primary radial growth by light quality

Cross sections of Arabidopsis and tomato hypocotyls revealed that primary radial growth was decreased in monochromatic red light, and increased in monochromatic blue light, when compared to white light. Microscopic analysis showed that in both species this phenotype relied on cell size, rather than cell division. Similar hypocotyl phenotypes have been observed in radish (Samuoliene et al., 2011). We did not observe any secondary growth in Arabidopsis or tomato hypocotyls, therefore we cannot exclude additional light responses during later stages of radial hypocotyl growth. The hypocotyl cross sections of both species showed small flaccid pith cells in the red LED condition, and big, turgid pith cells in the blue LED condition, in line with our scanning electron microscopy observations of epidermis cells. This suggests that the effects of light quality on apical and primary radial growth are interconnected during the seedling stage. Members of the *ERECTA* receptor kinase family have been shown to inhibit radial hypocotyl growth, while promoting apical hypocotyl growth (Ikematsu et al., 2017; Qu et al., 2017), and thus may be key factors in this interconnection. Interestingly, the radial growth phenotypes changed throughout their development. In the stems of 30-day-old tomato plants, the primary radial growth was completely indifferent to red or blue light. This may be explained by the possibility that plants were in a transitional state at the time of measurements. Perhaps older tomato plants will show light-induced changes in primary radial growth, or even in secondary growth. On the other hand, we have observed a similar indifference of tomato leaf production and flowering to red or blue light, suggesting this may result from fundamental differences in plant architecture and life cycle between species (Spaninks et al., 2020). Although radial stem growth has been correlated to light intensity in several species (Feng et al., 2019; Chung et al., 2020; Hamedalla et al., 2022), the effect of light quality has yet to be studied in most species. In Arabidopsis, we observed that the radial growth phenotypes turned opposite throughout their life cycle. In reproductive Arabidopsis plants, stems were thicker in monochromatic red light, and thinner in monochromatic blue light, when compared to white light. Since secondary xylem and phloem were not visible in any of the Arabidopsis stems, the differential stem thickness was caused by changes in primary radial growth such as pith cell size and number, primary xylem width, and the number of vascular bundles. Thinner stems of *phyB* and *phyD* mutants, compared to wild type, suggested that the photoreceptors PHYB and PHYD promote radial growth in spectra that contain red light. In line with our observations, PHYB has been reported to promote radial stem growth in maize (Wies et al., 2019). The stems of *cry* mutants were also thin compared to wild-type seedlings, however, since this phenotype was also observed in the red LED condition, we expect it to be light-independent, or to require CRY-PHY interactions. To conclude: in monochromatic red light, PHYB and PHYD promote primary radial growth, resulting in thick stems. In contrast, in the blue LED condition, PHYB and PHYD are inactive, thus resulting in thinner stems (Fig. 6D). Our histological analysis of Arabidopsis stems revealed changes in primary xylem width and in the number and size of pith cells in the different LED conditions, that correlated to the stem thickness. In addition, the changes in vascular bundle numbers correlated to more branching in monochromatic red light, and reduced branching in the blue LED condition (Spaninks et al., 2020). Therefore we hypothesize that light quality regulates primary radial growth both through auxin-mediated vascular patterning, lateral organ formation, and primary xylem differentiation (Baima et al., 2001; Fàbregas et al., 2015). To summarize, the use of LEDs opens up new possibilities to steer primary radial growth in young plants, but additional experiments in different species, and at different developmental stages are required. Moreover, the appearance of an interfascicular cambium in stems of Arabidopsis plants grown in red light, but not in stems of plants grown in white or blue light, suggests the possibility for light quality to steer secondary growth in older plants. For tomato, optimization of the red : blue ratio might be required to obtain sturdy plants by enhancing primary radial growth while reducing apical growth. Fortunately, the red : blue ratio is unlikely to affect plastochron nor flowering time, two other important traits during the initial growth phase of tomato plants (Spaninks et al., 2020).

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CRediT authorship contribution statement

Kiki Spaninks: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Gerda Lamers:** Formal analysis, Methodology, Writing – review & editing. **Jelmer van Lieshout:** Methodology, Investigation, Writing – review & editing. **Remko Offringa:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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Data availability

The raw data that support the findings presented in this study are available from the corresponding author (R.O.) on request.

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Supplementary materials

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