Control of early plant development by light quality

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Chapter 4

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

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Abstract

For a horticultural crop such as tomato (*Solanum lycopersicum*), young plants are generally grown in the controlled environment of a growth chamber before their transfer to the greenhouse. For this initial growth phase, multi-layer systems can be applied to reduce space and energy costs. Here LEDs form the ideal lighting system, as they decouple light intensity from heating. Moreover, the spectral quality control of LEDs may be utilised 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 cultivars Moneymaker and Foundation, and *Arabidopsis thaliana* (Arabidopsis) ecotypes Columbia and Landsberg *erecta* grown in white, monochromatic red, or monochromatic blue light. Our histological and microscopic analysis revealed that the red LED condition increased cell elongation in hypocotyls and stems, whereas the blue LED condition decreased cell elongation, compared to white light-grown plants. This was observed in both species and correlated to hypocotyl length and plant height measurements from previous experiments. In seedlings, primary radial growth was affected by light quality in both species, resulting in thinner hypocotyls in the red LED condition, and thicker hypocotyls in the blue LED condition, compared to white light. However, in older plants, only Arabidopsis showed sensitivity of primary radial growth to light quality. Interestingly, the effect of red or blue light on Arabidopsis inflorescence stems was opposite to what was observed in hypocotyls. Possible effects of light quality on secondary radial growth remain to be investigated in both species. 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 summarise, LEDs can be used to regulate both apical and primary radial plant growth, but the resulting phenotypes may be plant age- or species-specific.

Keywords: LEDs, apical growth, primary radial growth, histology, Arabidopsis, tomato

Introduction

Before the production cycle of a horticultural crop, such as tomato or cucumber, in greenhouses is started, young plants are generally grown in growth chambers (nurseries) until the appearance of the first truss. During this initial phase, plants may be grown in multi-layer systems to reduce space and energy costs. LED lights decouple light intensity from heating and can therefore be applied in these systems without the risk of overheating the plants. 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). A key phytohormone for apical stem development, gibberellic acid (GA), promotes cell division in the RZ by relieving DELLA-inhibited class I TCP (TEOSINTE BRANCHED 1, CYCLOIDEA, PROLIFERATING CELL FACTOR) transcription factors (Davière et al., 2014). In addition, BRASSINAZOLE-RESISTANT 1 (BZR1) proteins physically interact with DELLAs, suggesting that GA indirectly regulates brassinosteroid (BR)-induced stem growth as well (Gallego-Bartolomé et al., 2012). Finally, interplay between auxin, abscisic acid and GA biosynthesis has been shown to regulate stem elongation (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 phytohormones, and has already been reported in several species (Zheng et al., 2019; Gawronska et al., 1995; Hisamatsu et al., 2005; Paradiso and Proietti, 2021), with the most well-known example being the shade avoidance syndrome where stem growth is increased under low light intensity or low red/far-red (R:FR) ratios. 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). Vascular stem cell proliferation and differentiation rely on the phytohormones auxin, cytokinin, BR, and their downstream signalling components. Proliferation of the vascular cambium is promoted directly through the ligand-receptor complex TRACHEARY ELEMENT DIFFERENTIATION (TDIF)-PHLOEM INTERCALATED WITH XYLEM (PXY), and indirectly through its activation of WUSCHEL-RELATED HOMEOBOX 4 (WOX4) and WOX14 transcription factors that

inhibit xylem differentiation (Suer et al., 2011; Etchells et al., 2013, 2016). Further towards the outside of the stem, HIGH CAMBIAL ACTIVITY 2 (HCA2) and LATERAL ORGAN BOUNDARIES DOMAIN 1 (LBD1) promote phloem formation, whereas VASCULAR-RELATED NAC-DOMAIN 6 (VND6) and VND7 are important for xylem production 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 (MacMillan et al., 2013; Botterweg-Paredes et al., 2020; Li-Li et al., 2020). However, the underlying mechanisms remain to be elucidated. 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 **chapter 3**, we observed that hypocotyl and stem elongation can be modulated by red or blue light in both *Arabidopsis thaliana* (Arabidopsis) and *Solanum lycopersicum* (tomato). 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 confirmed 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 young 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.

Results

Light quality regulates apical growth of Arabidopsis and tomato hypocotyls.

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 in **chapter 3**, hypocotyls were longer in red light, and shorter in blue light, when compared to white light conditions (**Figure 1A, B**). Scanning electron microscopy analysis revealed that the hypocotyl epidermis cells were greatly affected by light quality (**Figure 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 (**Figure 1C**). Next, we used stereomicroscopy to visualise 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

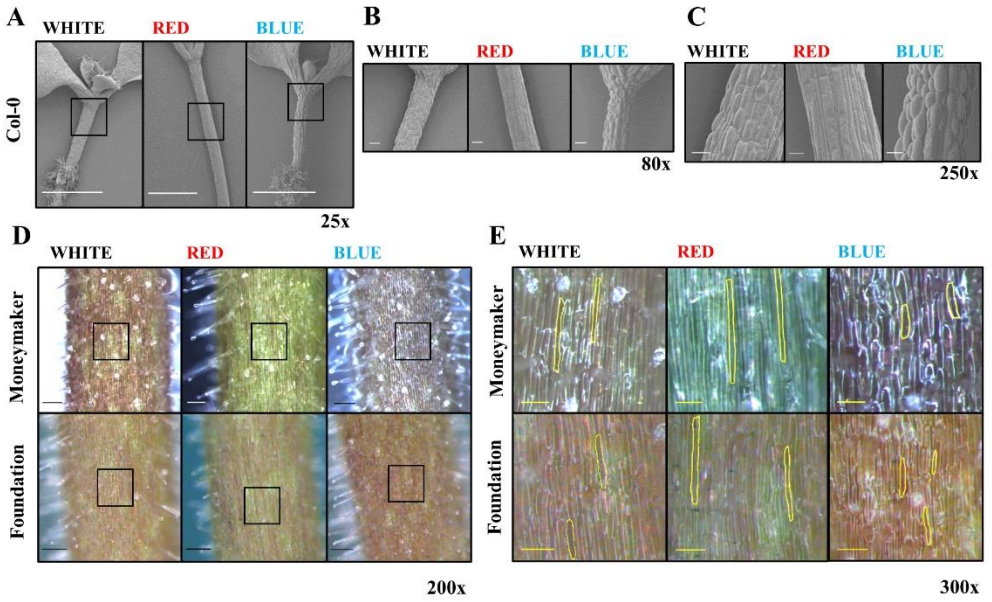
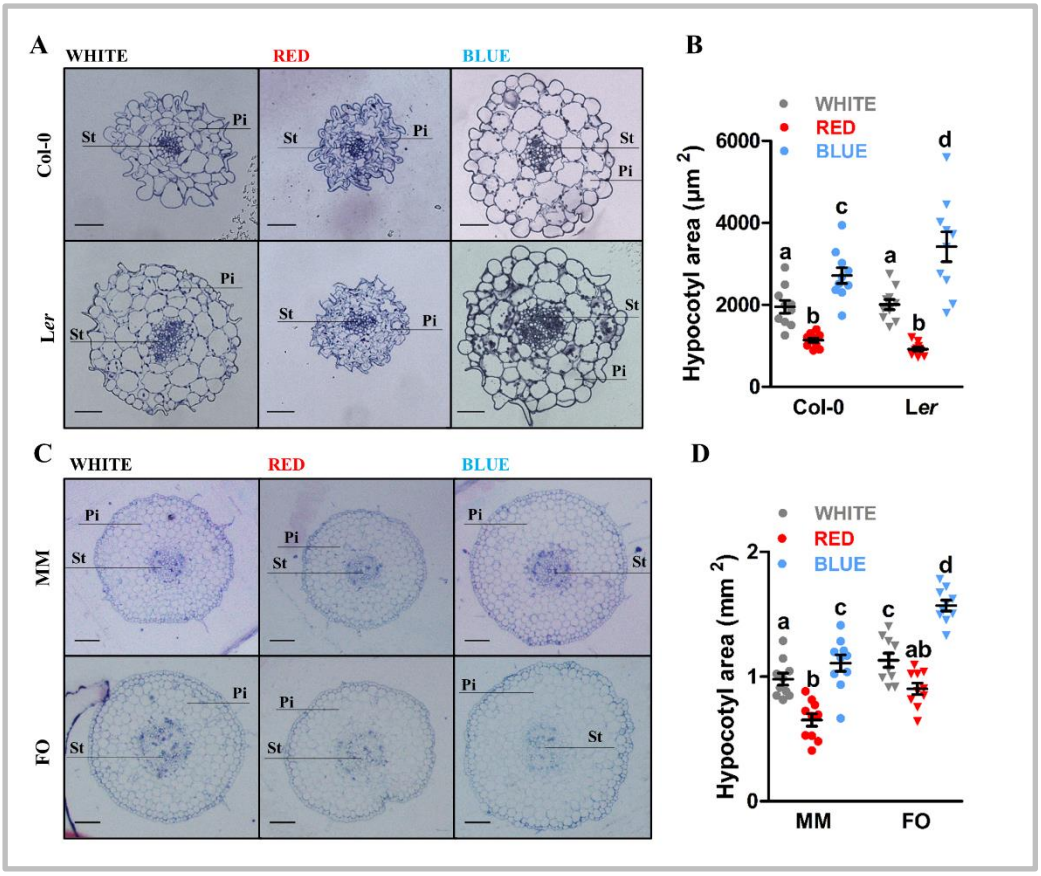


Figure 1: Light quality regulates epidermal cell elongation in Arabidopsis and tomato hypocotyls.

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.

to white light (**Figure 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 (**Figure 1E**). Together, these results show that hypocotyl elongation of both Arabidopsis and tomato seedlings can be modulated by light quality.

Light quality regulates primary radial growth of Arabidopsis and tomato hypocotyls.

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

Figure 2: Light quality regulates primary radial growth in Arabidopsis and tomato hypocotyls.
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.

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 (**Figures 2A, B**). This difference in hypocotyl thickness appears to rely on cell size rather than cell number (**Figure 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 (**Figures 2C, D**). Furthermore,

as in Arabidopsis, primary radial growth of tomato hypocotyls appeared to rely mostly on cell size (**Figure 2C**). Secondary growth was not observed in any of the Arabidopsis or tomato hypocotyls. To summarise, these results show that radial hypocotyl growth is affected by light quality in both Arabidopsis and tomato seedlings.

Light quality regulates apical growth of Arabidopsis and young tomato stems.

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 (**Figure 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 (**Figure 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 (**Figure 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 (**Figure 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.

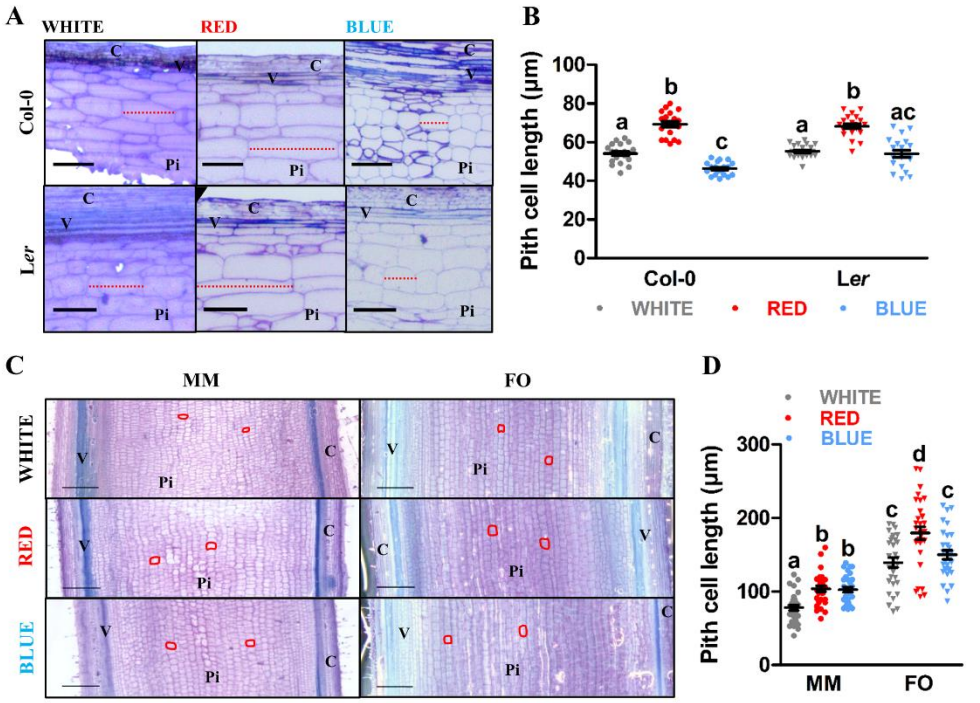


Figure 3: Light quality regulates pith cell elongation in Arabidopsis and tomato stems.

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.

Red and blue light regulate primary radial growth of Arabidopsis, but not of tomato stems.

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 (**Figure 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 (**Figure 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 (**Figure 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 (**Figures 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 analysed cross 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 (**Figure 5A, B**). In addition, we did not observe any differences in stem tissues (**Figure 5C**), xylem width or vascular bundle number (**Figure 5D, E**). These results indicate that primary

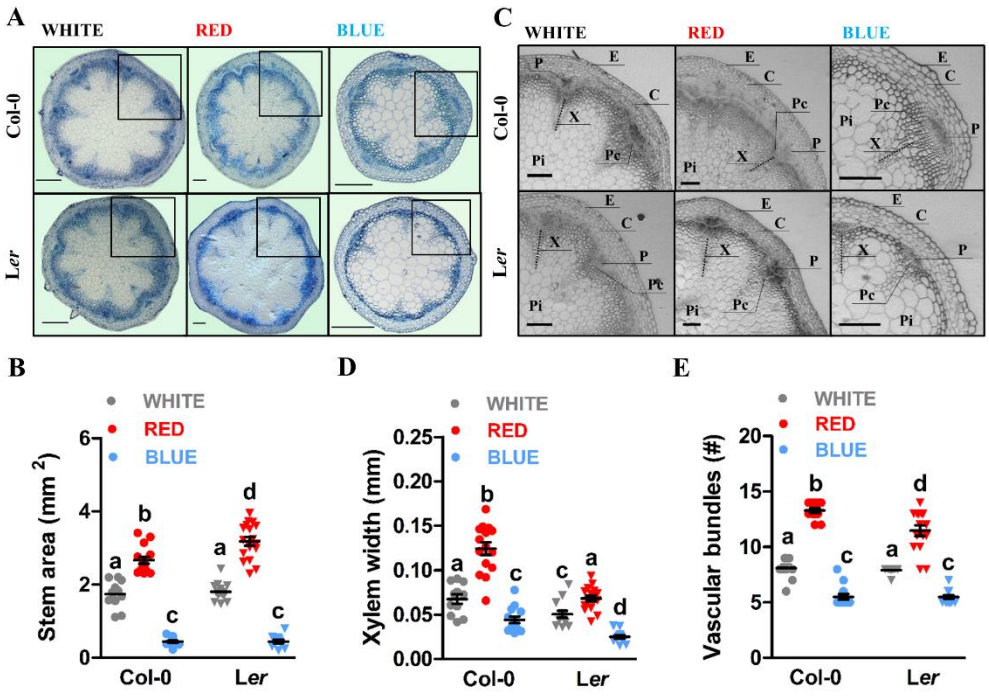


Figure 4: Light quality regulates primary radial growth of Arabidopsis stems. **A.** Stereomicroscopy images of toluidine blue stained cross sections from the most basal internode of the primary inflorescence of Arabidopsis plants (ecotypes Columbia (Col-0) and Landsberg *erecta* (L*er*)) grown in white, 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.

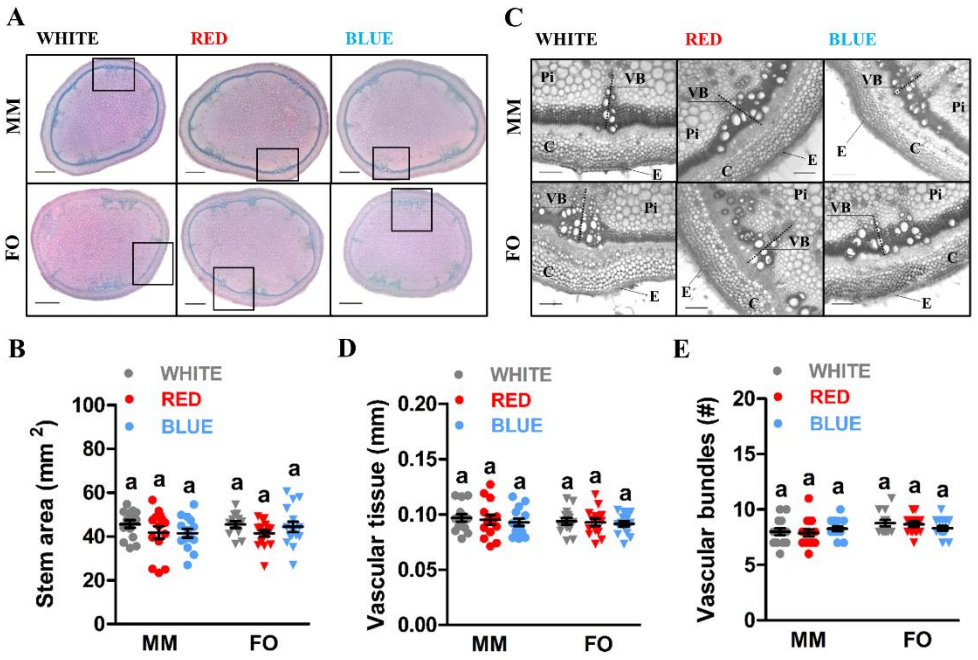


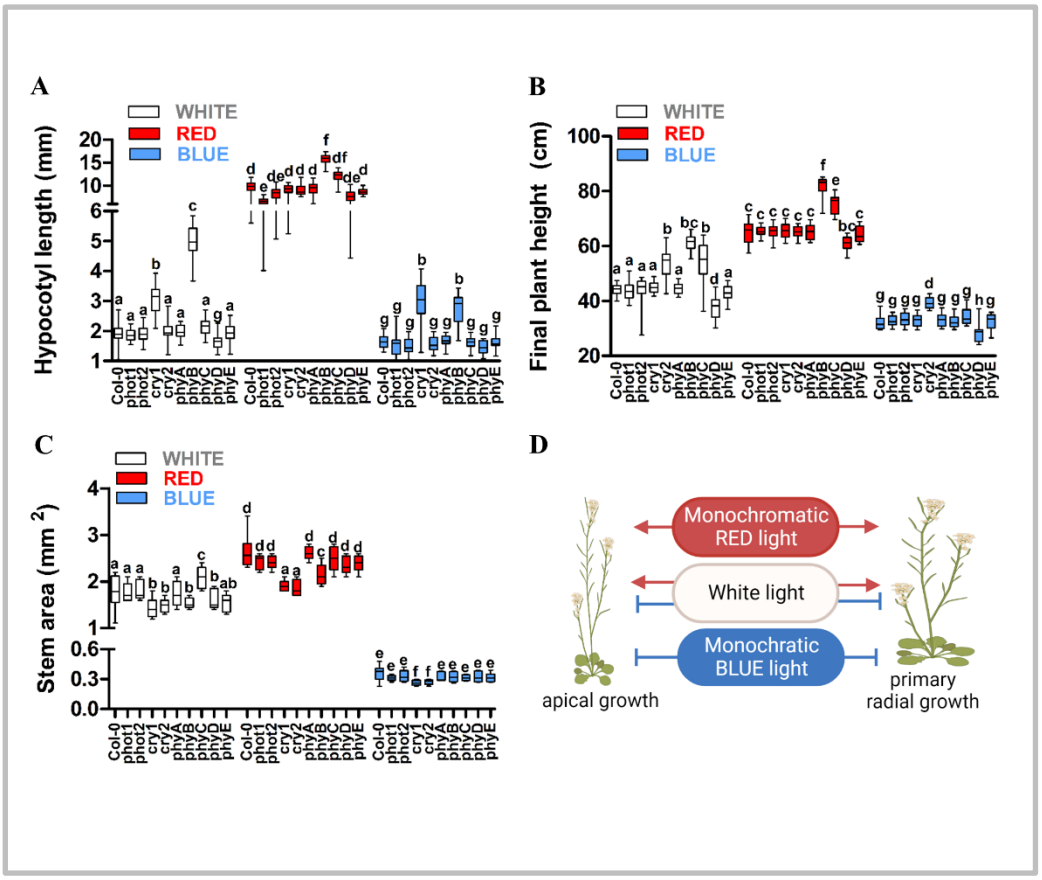
Figure 5: Primary radial growth of young tomato stems is indifferent to light quality.

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.

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.

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 (**Figure 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 (**Figure 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. (**Figure 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. (**Figure 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) (**Figure 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.

Figure 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.

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. In **chapter 3** 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. These results were consistent with other LED studies on Arabidopsis,

and a greenhouse tomato study that used LEDs as supplemental lighting (Chen et al., 2020; Dieleman et al., 2019). Here we performed histological and microscopic analyses to further investigate apical and radial growth of stems and hypocotyls under different LED conditions.

Regulation of apical growth by light quality.

First, we further investigated apical growth of Arabidopsis and tomato hypocotyls and stems. 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 flaccid. In contrast, blue light-grown seedlings had small and turgid epidermal cells. Although the effects of light quality on the epidermal cell elongation were clear, the microscopic analysis was insufficient to exclude an additional effect of light quality on cell division. Moreover, our analysis only allowed visualisation of the epidermis, thus other cell layers 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 in these species. Furthermore, similar hypocotyl phenotypes have been reported in lettuce and chili peppers (Volmaro et al., 1998; Liu et al., 2019), while the blue light response of cucumber seedlings resulted in longer hypocotyls, thus indicating some species-specificity (Hernández and Kubota, 2016). Longitudinal sections of the primary inflorescence stems of Arabidopsis plants showed a similar increase in cell size in the red LED condition and decrease in the blue LED

condition. This suggests that apical growth of Arabidopsis is promoted by red wavelengths and inhibited by blue wavelengths throughout its life cycle. While similar effects of red and blue light 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). In line with these findings, our mutant analysis suggested that the blue light receptors CRY1 and CRY2 inhibit respectively hypocotyl and inflorescence stem elongation. Also, CRY1 has been reported to inhibit epidermal cell elongation in Arabidopsis hypocotyls grown during thermomorphogenesis (Ma et al., 2015). The role of the red light photoreceptors appeared to be more diverse with PHYB and PHYC inhibiting apical growth and PHYD promoting hypocotyl and stem elongation. PHYB has been reported to inhibit epidermal cell elongation in hypocotyls (Allen et al., 2019), however, its role in inhibiting 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. Our results suggest that the enhanced plant height in monochromatic red light, is partially mediated by PHYD, but most likely results mainly from CRY inactivation. Reduced apical growth in monochromatic blue light relies heavily on CRY inhibition of stem and hypocotyl elongation, and partially on PHYD inactivation (**Figure 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 summarise, we clearly showed that red and blue light affect apical growth through cell elongation, although cell division remains to be investigated. During cell elongation, loosening of the normally rigid cell wall is required, while turgor pressure inside the vacuole drives expansion (Kaiser and Scheuring, 2020). Because our microscopic analysis revealed long and flaccid cells in the red LED condition, we hypothesise that these 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, thus resulting in the observed small and turgid cells. 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).

Regulation of primary radial growth by light quality.

We also investigated if, and how, light quality affects radial growth. 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 Arabidopsis and tomato seedlings this phenotype relied on cell size, rather than cell division. Similar hypocotyl phenotypes were observed in radish (Samuoliene et al., 2011). Moreover, since we did not observe any secondary growth in Arabidopsis or tomato hypocotyls, 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 of both Arabidopsis and tomato 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. A possible explanation for this may be that, as described above for apical growth, 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, in **chapter 3** we 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. 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, stem thickness increased in monochromatic red light, and decreased in monochromatic blue light, when compared to white light. Since secondary xylem and phloem were not yet 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. This is consistent with a radial growth-promoting role of the red light photoreceptors PHYB and PHYD that was revealed by our mutant analysis (**Figure 6D**). Surprisingly, *cry* mutants also showed a reduced stem thickness, compared to wild-type seedlings, thus suggesting radial-growth promoting functions for these blue light photoreceptors as well. However, since *cry* stems were also significantly shorter in the red LED condition, we expect that these phenotypes are either not light-dependent at all, or that they require CRY interaction with phytochromes. In line with our observations, PHYB has been reported to promote radial stem growth in maize (Wies et al., 2019). 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 (**chapter 3**). Therefore we hypothesise 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 summarise, 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 addition to primary radial growth, in older plants. For tomato, optimisation of the red : blue ratio might be required to obtain sturdy plants by enhancing primary radial growth and reducing apical growth. From **chapter 3** we know that this will not affect

plastochron nor flowering time, two other important traits during the initial growth phase of tomato plants.

Materials and Methods

Growth conditions and LED treatments.

In all experiments, plants were grown at a 16 h photoperiod, under white, deep red, or blue 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** of **chapter 3**. 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.

Plant lines and seed germination.

Arabidopsis thaliana (Arabidopsis) ecotypes Columbia (Col-0) and Landsberg *erecta* (L*er*), 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 S1**. All Arabidopsis mutants (T-DNA insertion lines in Col-0 background) were genotyped with the primers listed in **Table S2**. 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

(**Chapter 3, Figure S1A**). 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 (**Chapter 3, Figure S1B**). 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).

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 hours 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 pressurised argon in a low vacuum environment, and coated samples were kept under dry vacuum conditions. Samples were imaged with a JEOL SEM 6400 scanning electron microscope 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 (#16520050, Thermo Scientific^{TM}) and imaged with a Leica MZ16FA equipped with a Leica DFC420C camera.

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 hours 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 moulds that dried overnight at 60°C, and were stored at 21°C. At 45 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 hours 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.

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 Leica MZ16FA equipped with a Leica DFC420C camera, and all hypocotyl sections were imaged with Zeiss Axioscope A1 equipped with a Zeiss AxioCam MRc5 camera.

Plant phenotyping.

To measure hypocotyl length, Arabidopsis and tomato seedlings were grown in the LED conditions and photographed at 7 DAG and 5 DAS, respectively. Plant height was measured with a tape-measure in Arabidopsis plants after termination of the primary inflorescence meristem. 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 ImageJ (Fiji) software (Schindelin et al., 2012).

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 analysed using a one-way ANOVA followed by a Tukey's honestly significant different (HSD) post hoc test and plotted into graphs using GraphPad Prism 5 software. In the graphs, the colours of the dots and lines indicate white, red, and blue LED conditions. Microscopic images were edited in ImageJ (Fiji). Schematic models were generated with BioRender software. Final figures were assembled using Microsoft PowerPoint.

Author contributions

KS and RO conceived and designed the experiments. KS and JvL performed the experiments. KS and GL performed the microscopic analysis, and KS performed the statistical analysis. KS and RO analysed the results and wrote the manuscript. All authors contributed to manuscript revision.

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Supplementary Material (Tables S1-S2 and Figure S1)

Table S1: Plant lines used in this study.

Arabidopsis and tomato seeds were obtained from Nottingham Arabidopsis Stock Centre (NASC), or a kind gift from Nunhems Netherlands BV.

PLANT LINE	DESCRIPTION	SOURCE	REFERENCE
Columbia (Col-0)	Natural Arabidopsis accession	NASC	-
Landsberg *erecta* (L*er*)	Natural Arabidopsis accession	NASC	-
Moneymaker (MM)	Non-hybrid tomato cultivar	Nunhems BV	-
Foundation (FO)	Hybrid tomato cultivar	Nunhems BV	-
phyA (SALK_014575)	T-DNA insertion in At1g09570	NASC	Ruckle et al., 2007
phyB (SALK_ 022035)	T-DNA insertion in At2g18790	NASC	Mayfield et al., 2007
phyC (phyC-3)	3 kbp deletion in At5g35840	NASC	Monte et al., 2003
phyD (SALK_027956)	T-DNA insertion in At4g16250	NASC	Christians et al., 2012
phyE (SALK_092529)	T-DNA insertion in At4g18130	NASC	Warnasooriya et al., 2011
cry1 (SALK_069292)	T-DNA insertion in At4g08920	NASC	Ruckle et al., 2007
cry2 (cry2-1)	2 kbp deletion in At1g04400	NASC	Guo et al., 1998
phot1(SAIL_1232_C01)	T-DNA insertion in At3g45780	NASC	McElver et al., 2001
phot2 (SALK_142275)	T-DNA insertion in At5g58140	NASC	Ruckle et al., 2007

Table S2: Primers used in this study.

For genotyping of SAIL and SALK T-DNA insertion lines, gene-specific primers were combined with the LB1 or the LBb1.3 forward primer, respectively.

PRIMER NAME	TARGET GENE	SEQUENCE 5'→ 3'	EXPERIMENT
LB1	N/A	GCCTTTTCAGAAATGGATAAATA	Genotyping
LBb1.3	N/A	ATTTTGCCGATTTCGGAAC	Genotyping
phyA FW	At1g09570	CCAGTCAGCTCAGCAATTTTC	Genotyping
phyA RV	At1g09570	AATGCAAAACATGCTAGGGTG	Genotyping
phyB FW	At2g18790	CATCATCAGCATCATGTCACC	Genotyping
phyB RV	At2g18790	TTCACGAAGGCAAAAGAGTTG	Genotyping
phyC FW	At5g35840	ATGTCATCGAACACTTCACG	Genotyping
phyC RV	At5g35840	TCAAATCAAGGGAAATTCTG	Genotyping
phyD FW	At4g16250	AACCCGGTAGAATCAGAATGG	Genotyping
phyD RV	At4g16250	ATCGGTTACAGTGAAAATGCG	Genotyping
phyE FW	At4g18130	AAAGAGGCGGTCTAGTTCAGC	Genotyping
phyE RV	At4g18130	TATCAGTGGTTAAACCCGTCG	Genotyping
cry1 FW	At4g08920	TTCATGCCACTTGGTTAGACC	Genotyping
cry1 RV	At4g08920	TCCCGACAGACTGGATACATC	Genotyping
cry2 FW	At1g04400	ATGAAGATGGACAAAAAGAC	Genotyping
cry2 RV	At1g04400	TCATTTGCAACCATTTTTTC	Genotyping
phot1 FW	At3g45780	ACATAGGATGCAGCAGAAACG	Genotyping
phot1 RV	At3g45780	CAGTAGACTGGTGGGCTCTTG	Genotyping
phot2 FW	At5g58140	TCCATCTCCTTTGAATGATGC	Genotyping
phot2 RV	At5g58140	AGTGTCATTGCTCACGGATTC	Genotyping

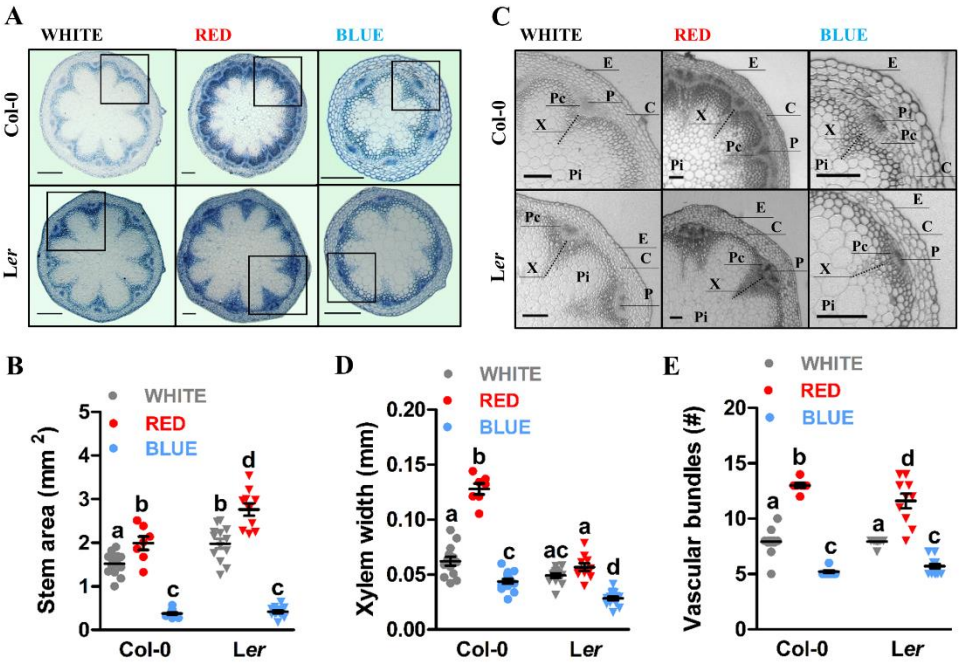

Figure S1: Light quality affects primary radial growth of Arabidopsis stems already at 1 week after bolting.

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 1 week 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). Several magnifications were reoriented for easy comparison. **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.