Entrainment of Arabidopsis roots to the light:dark cycle by light piping

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Running title: Light piping entrains roots

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Abstract

Correct operation of the plant circadian clock is crucial for optimal growth and development. Recent evidence has shown that the plant clock is tissue-specific and potentially hierarchical, implying that there are signalling mechanisms that can synchronise the clock in different tissues. Here I have addressed the mechanism that allows the shoot and root clocks to be synchronised in light:dark cycles but not in continuous light. Luciferase imaging data from two different Arabidopsis accessions with two different markers show that the period of the root clock is much less sensitive to blue light than to red light. Decapitated roots were imaged either in darkness or with the top section of root tissue exposed to light. Exposure to red light reduced the period of the root tissue maintained in darkness whereas exposure to blue light did not. The data indicate that light can be piped through root tissue to affect the circadian period of tissue in darkness. I propose that the synchronisation of shoots and roots in light:dark cycles is achieved by light piping from shoots to roots.

Summary

The plant circadian clock is tissue-specific and potentially hierarchical, implying that there are signalling mechanisms that can synchronise the clock in different tissues. This work addresses the mechanism that allows the shoot and root clocks to be synchronised in light:dark cycles but not in continuous light. The data indicate that light can be piped through root tissue to affect the circadian period of tissue in darkness. This emphasizes the physiological importance of light piping in plant biology.

Keyword index

Arabidopsis thaliana, circadian clock, entrainment, light piping, light quality, roots

Introduction

Circadian clocks have evolved in many organisms in response to the daily rotation of the earth and the resulting light:dark (L:D) cycle. By driving rhythms at the molecular and cellular levels they temporally regulate many aspects of physiology and behaviour to anticipate changes in the environment, and thus provide a fitness benefit (Green *et al*., 2002; Dodd *et al*., 2005). About one-third of the Arabidopsis genome is under circadian regulation (Michael & McClung 2003; Covington *et al*., 2008) and at the physiological level the circadian clock can control many plant processes, including photosynthesis, leaf movement, hormone responses, stem extension and stomatal opening (McClung 2006; Harmer 2009; Pruneda-Paz & Kay 2010; Greenham & McClung, 2015).

Conceptually the circadian clock requires a core oscillator with input pathways that allow entrainment to the environment and output pathways that determine the timing of physiological rhythms. Experimental studies, mainly on seedlings, and modelling have shown that the Arabidopsis core oscillator includes multiple interlocking feedback loops of gene expression, modulated by post-translational control at several levels (Harmer, 2009; Nagel & Kay, 2012; Hsu & Harmer, 2014). Key players include the morning-expressed MYB transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), the day-phased transcriptional regulators PSEUDO-RESPONSE REGULATOR 9 (PRR9) and PRR7 and the evening-phased components GIGANTEA (GI) and PRR1 (also known as TIMING OF CAB EXPRESSION 1, TOC1). The main signals that entrain the clock are thought to be light and temperature. Light can affect the clock through effects on the rates of transcription and translation, and the stabilities of mRNA and protein; the photoreceptors involved include phytochromes, cryptochromes and the LOV domain-containing blue light receptor ZEITLUPE (ZTL) (Franklin *et al*., 2014; Hsu & Harmer, 2014).

While the mammalian circadian system involves a central clock in the suprachiasmatic nuclei that can coordinate peripheral clocks (Mohawk *et al*., 2012), early work indicated that the plant circadian system was uncoupled (Thain *et al*., 2000), though subsequently some weak coupling between cells was observed (Fukuda *et al*., 2007; Para *et al*., 2007; Wenden *et al*., 2012). However it is now clear that the plant clock shows tissue specificity of expression (Endo, 2016): this raises the question of the extent to which the circadian clocks in different tissues are coupled in order to coordinate responses across the plant, and the nature of any underlying coupling mechanisms. For example James *et al*. (2008) showed that the clocks in mature shoots and roots have different free-running periods but are synchronised in L:D cycles. Endo *et al*. (2014) reported that the circadian clock in the vasculature is robust and has distinct gene expression dynamics. Moreover it has the ability to control both the clock in neighbouring mesophyll cells and the whole-plant response of flowering. Using Arabidopsis seedlings, Takahashi *et al*. (2015) noted that the clocks in cells of the shoot apex were tightly coupled and that removal of the shoot apex led to a loss of robustness in root rhythms. Data from grafting experiments indicated that signals from the shoot apex could synchronize roots but this work focussed on clock architecture rather than possible signalling mechanisms.

Much of our understanding of shoot-to-root signalling has come from studies of photomorphogenesis in seedlings (Lee *et al*., 2017). There is evidence for mobile signals such as the phytochrome-stimulated shoot to root movement of auxin (Salisbury *et al*., 2007) and the transcription factor ELONGATED HYPOCOTYL5 (HY5) which can move from shoots to roots to coordinate growth with N and C metabolism in response to light (Chen *et al*., 2016). Light itself is another potential signal because it can penetrate several cm into soil (Tester & Morris, 1987) and can be piped directly to root tissue (Mandoli & Briggs, 1984;

Sun *et al*., 2003, 2005) which expresses a wide range of photoreceptors (Mo *et al*., 2015). For example phytochrome B triggers responses to stem-piped light (Lee *et al*., 2016).

James *et al*. (2008) used indirect evidence to suggest that shoot-derived sucrose could coordinate the shoot and root clocks of mature plants in L:D cycles and this is consistent with the demonstration that pulses of sucrose can reset the phase of rhythms in seedlings (Haydon *et al*., 2013). However the nature of shoot-to-root signalling in mature plants has not been studied extensively. We recently developed a system to monitor luciferase activity and thence circadian clock function independently in mature Arabidopsis shoots and roots (Bordage *et al*., 2016). Our data showed that roots are directly sensitive to low levels of light and the differences between the shoot and root clocks can largely be explained by organ-specific light inputs; for example we found that for roots but not shoots the circadian period in constant light (LL) at fixed intensity depended on light quality. Here I have further defined the difference in the responses of shoots and roots to red and blue light and used this system to demonstrate that light affects circadian period in roots via light piping.

Materials & Methods

Plant material and growth

The CCA1:LUC+ and GI:LUC+ expressing lines in the Ws (Wassilewskija) background and the CCA1:LUC+ expressing lines in L*er* (Landsberg *erecta*) and *phyA-201*; *phyB-5* have been described previously (Doyle *et al*., 2002; Edwards *et al*., 2010; Haydon *et al*., 2013).

All seeds were surface sterilised, stratified for 4 days at 4 °C and sown on 1.2% agar in 0.5 strength Murashige and Skoog (MS) medium adjusted to pH 5.7 in 120 mm square vertical plates which were exposed to L:D cycles (12 h white light provided by fluorescent tubes, 80-100 $\mu mol.m^{-2}.s^{-1}$, 12 h dark) at 20°C. For imaging of intact plants, 10-12 days after germination seedlings (2 clusters of 3 plants per plate) were transferred to fresh plates in

which the top 3 cm had been replaced with 1.8% agar and 2% charcoal in 0.5 strength MS medium, readjusted to pH 5.7 after addition of charcoal. After a further 10-12 days plants were sprayed with luciferin and the plates were sealed with new lids containing a black barrier which separate the shoot and root compartments and prevent cross-contamination of their signals (Bordage *et al*., 2016).

For imaging of decapitated plants the same procedure was used except that seedlings were transferred to 1.2% agar plates with no charcoal and shoots were removed before spraying with luciferin. Where stated, roots were kept fully in the dark during imaging by an automated system in which a tight-fitting black cover supported on runners could be moved across the root compartments as required (Bordage *et al*., 2016). In some experiments the top of the root system projected above the cover and was illuminated in the light phase of LD cycles or in constant light (LL); the exposed and covered sections are referred to as 'exposed top' and 'covered, top exposed' respectively. In these experiments plates were sealed with lids containing black barriers as above; otherwise plates were sealed with plain lids.

Luciferase imaging

For intact plants, 3-4 week old plants were sprayed with 60 mM D-Luciferin in 0.01% triton (300 µL per plate). Decapitated roots were sprayed with 60 mM D-Luciferin in 0.01% triton (200 µL per plate). Plates were kept at 20°C and illuminated by blue and/or red light provided by LEDs (Luxeon Star 447 nm and 627 nm respectively) at 20 $\mu mol.m^{-2}.s^{-1}$ unless stated otherwise. Bioluminescence was usually detected using a Photek 225/18 Intensified CCD camera with a 16 mm lens. The camera, LEDs and covering system were controlled using Photek IFS32 software. Images (15 min) were recorded every 1.5 – 3 h in photon counting mode, without any filters. Root and shoot regions were defined and luminescence data extracted using Photek IFS32 software. In some experiments bioluminescence was detected with a Photometrics Evolve 512 camera and data was extracted using Image-Pro Plus

software. In both cases the luminescence for each time-point was normalised to the average luminescence over the corresponding time-course. Control experiments showed that the results from the two cameras after normalisation were essentially identical.

Data analysis

Normalised time-course data from imaging and qPCR experiments were analysed using Biological Rhythm Analysis Software System (BRASS) (www.millar.org), discarding the data from the first 24 h in constant conditions. Period, amplitude and relative amplitude error (RAE) were analysed using the FFT-NLLS suite of programs. The RAE is the ratio of the amplitude error to the most probable amplitude. It assesses rhythm robustness: values close to 0 and 1 indicate robust and weak (if any) rhythms respectively.

Results

Bordage *et al*. (2016) noted that for Arabidopsis roots the circadian period in constant light (LL) was strikingly longer in blue than red light whereas the shoot period was slightly shorter in blue than red light. Fig. 1 illustrates this behaviour by showing plots of period against RAE for the ecotype Ws, with two different markers, CCA1:LUC and GI:LUC, at a light intensity of 15 $\mu mol.m^{-2}.s^{-1}$; typical luminescence traces for GI:LUC are shown in Fig. S1 and periods are given in Table S1. Similar data for CCA1:LUC in L*er* are shown in Fig. S2 and Table S1. To extend this study the free-running period of shoots and roots was monitored over a range of light intensities. Fig. 2 shows that, for plants expressing GI:LUC, both red and blue light in the range 0-35 $\mu mol.m^{-2}.s^{-1}$ decrease the shoot period as expected from Aschoff's rule, which holds that in diurnal organisms clock period decreases with increasing light intensity (Aschoff, 1960). In contrast, while the period of roots in LL is decreased by increasing intensities of red light, it is only marginally affected by blue light intensity. Rhythms of GI:LUC plants in constant dark (DD) were variable and of low amplitude (Bordage et al.,

2016) but there was no significant difference in period between shoots and roots. Fig. S3 shows a similar experiment with plants expressing CCA1:LUC. With this marker rhythmicity could not be detected in DD, as reported previously for seedlings (Dalchau *et al*., 2011); however the data confirm that, over the range 5-25 $\mu mol.m^{-2}.s^{-1}$, the root period is appreciably higher in blue light than in red light.

While blue light has little effect on the root period, it may be able to entrain roots. To investigate this, decapitated L*er* plants expressing CCA1:LUC were released into DD in the presence of 1% sucrose, which supports robust root rhythms (Bordage *et al*., 2016), and subjected to short pulses of either red or blue light after 35 h in DD. Fig. 3 shows that 60 min pulses of red or blue light at 15 $\mu mol.m^{-2}.s^{-1}$ are equally effective in setting the phase of the subsequent rhythm. A similar experiment with plants expressing GI:LUC is shown in Fig. S4 and leads to the same conclusion. Thus blue light can entrain roots at an intensity which does not affect period, showing that its effects on period and phase are clearly distinguishable.

To assess the photoreceptor(s) responsible for the effects of red light on roots, the luminescence of CCA1:LUC in *phyAB* plants was examined. The plots of period against RAE in Fig. 4 and the period data in Table S2 show that the usual difference in period between shoots and roots is observed in this mutant in blue light but not in red light. The root data are re-plotted in Fig. S5 to show that, unlike the wild-type, there is no difference in root period between red and blue light for *phyAB* plants. These data indicate that roots of the *phyAB* mutant are essentially 'blind' to red light as regards period.

Decapitated roots are directly sensitive to red and blue light (Bordage *et al*., 2016), but how do roots perceive light in the natural environment? Light could affect roots by its limited penetration into soil (Tester & Morris, 1987) and/or by light piping from above-ground tissue (Mandoli & Briggs, 1984; Sun *et al*., 2003, 2005; Lee *et al*., 2016). This issue was investigated by making use of the observation (above) that the period of root rhythms is

markedly reduced by increasing red light intensity but only marginally affected by blue light intensity. Col-0 plants expressing GI:LUC were used because root periods with this marker are more tightly clustered than those with CCA1:LUC (Fig. 1). Plants were decapitated and the root portion of imaging plates was covered with an automatic system as described in Bordage *et al*. (2016) so as to leave either some or no root material directly exposed to light during imaging experiments, as illustrated in Fig. 5a and Fig. S6. If light can be piped down root tissue the period of the covered portion of roots with some root exposed to red light should be lower than the period of the covered portion of roots with no exposed root, while with blue light there should be very little or no difference. This argument does not require that there be absolutely no light leakage into the root compartment; instead it assumes that the extent of light leakage, if any, would be the same irrespective of whether the top part of the root was exposed or not. The location of plates under the automated cover was therefore randomised to eliminate any equipment bias. When these plants were imaged in LL with red light at 20 $\mu mol.m^{-2}.s^{-1}$, roots with no part exposed to the light showed a significantly longer period than roots with part of the tissue exposed to light (Fig. 5b, Fig. S7, Table 1). The period of the section of roots directly exposed to light was shorter than those of either of the covered portions. However when blue light was used the root period was not affected by whether some of the root was exposed to light (Fig. 6, Table 1). This eliminates the possibility that the difference in period between fully covered roots and roots with exposed tops is due to the difference in position of decapitation (Fig. 5a). The period of fully covered roots was slightly shorter in red light than in blue light (Table 1), suggesting that there is a small amount of light leakage into the root compartment, but as noted above this does not affect the argument. These data are therefore consistent with the hypothesis that light can be transmitted through root tissue by piping to influence circadian period.

Discussion

The growth, development and behaviour of roots are affected by many environmental factors including light (Kiss *et al*., 2003; Dyachok *et al*., 2011; Warnasooriya *et al*., 2011; Sabthai *et al*., 2015; Lee *et al*., 2016), and this has prompted interest in the nature and roles of shoot-to-root signals. Several systems have been described to investigate this signalling under conditions that mimic the natural environment in the sense that roots can be maintained in the dark (James *et al*., 2008; Xu *et al*., 2013; Silva-Navas *et al*., 2015; Bordage *et al*., 2016; Lee *et al*., 2016). In this work our discovery that the circadian clock in roots is synchronised with shoots in L:D cycles (James *et al*., 2008) was investigated in the light of our observation that the main difference between the circadian clocks of the two organs lies in light inputs (Bordage *et al*., 2016). Two notable points emerge from the results.

First, the data presented here extend our earlier work (Bordage *et al*., 2016) by showing that the period of the root circadian clock is only slightly affected by blue light intensity over the range 0-35 $\mu mol.m^{-2}.s^{-1}$, whereas red light markedly reduces the period (Fig. 2, see also Fig. S3); this covers the range of intensity over which most of the reduction in period occurs (Devlin & Kay, 2000; Covington *et al*., 2001). It is possible that stimulation of blue light photoreceptors may have a slight effect on root period; alternatively the very weak effect of blue light on period may be due to slight excitation of phytochromes by the blue LEDs used in this work. In contrast to period, blue light is as effective as red light in re-setting the phase of the root circadian clock (Figs. 3 and S4). While much is known about the photoreceptors that feed in to the clock (Devlin & Kay, 2000; Fankhauser & Staiger, 2002; Mas *et al*., 2003), the mechanisms that underlie effects of light on period and phase are still poorly understood. This work shows that the mechanisms responsible for the effect of light on period and phase are distinguishable, at least for blue light.

Secondly, the data point strongly to the significance of light piping down root tissue. The circadian period of decapitated darkened roots is lower when a small part of the root tissue is exposed to red light than when it is not (Fig. 5), showing that a light-dependent signal is transmitted from the exposed part of the root to the darkened tissue. This is clearly unrelated to any sucrose signalling because all green photosynthetic tissue had been removed. It is very unlikely that it represents translocation of HY5 because blue light, like red light, causes accumulation of HY5 (Osterlund *et al*., 2000) whereas exposure of a part of the root to blue light does not affect the period of the darkened tissue (Fig. 6). Similar reasoning argues against involvement of auxin. Signalling via jasmonate as a light-induced mobile messenger is also unlikely because red light does not induce jasmonate-responsive genes such as MYC2 and PDF1.2 in roots (Molas *et al*., 2006). Light piping down sections of root exposed to lateral illumination has already been demonstrated (Sun *et al*., 2005). Thus, while the involvement of some other unknown transmissable signal cannot be excluded, the data presented here suggest that light piping makes at least a significant contribution to the synchronisation of roots with shoots in LD cycles (James *et al*., 2008). Another potential factor, the unidentified signal between the seedling shoot apex and roots (Takahashi *et al*., 2015), seems to affect mainly the detectability and robustness of root rhythms rather than their phase.

While application of sucrose can affect both period and phase in seedlings (Haydon *et al*., 2013), it is clear that translocation of sucrose from shoots to roots cannot be responsible for the different effects of red and blue light on the period of the root clock because the rate of photosynthesis at a fixed light intensity would not be expected to differ between red and blue light. Instead the effects of red light on the root clock are mediated by phytochrome A and/or phytochrome B; both have previously been implicated in the response of roots to red light (Kiss *et al*., 2003; Salisbury *et al*., 2007; Lee *et al*., 2016).

We have already noted that aspects of the root circadian clock, low-amplitude rhythms of clock genes and a reduced number of rhythmic output genes (James *et al*., 2008; Bordage *et al*., 2016) are reminiscent of the shoot clock under FR light (Wenden *et al*., 2011). This might suggest a molecular explanation of why the difference in light inputs between the shoot and root clocks (Bordage *et al*., 2016) leads to the longer period in roots. Analysis of multiple *phy* mutants led Hu *et al*. (2013) to suggest that while the Pfr form could speed up the circadian oscillator, the Pr form could slow it down. Piped light is enriched in the far red (FR) region of the spectrum (Sun *et al*., 2005; Lee *et al*., 2016). Hence the higher Pr/Pfr ratio expected in roots sensing piped light would lead to a long period.

This work thus suggests that in mature roots the circadian clock is sensitive to piped light, the red component of which is sensed by PHYA and/or PHYB. Hence control of the circadian clock can be added to root architecture and gravitropism as physiological responses to light piping mediated by PHYs (Lee *et al*., 2016). Given the wide range of features that depend on the circadian clock, this emphasizes the physiological importance of light piping in plant biology.

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References

Aschoff J. (1960) Exogenous and Endogenous Components in Circadian Rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25, 11-28.

Bordage S., Sullivan S., Laird J., Millar A.J. & Nimmo H.G. (2016) Organ spcificity in the plant circadian clock is explained by different light inputs to the shoot and root clocks. *New Phytologist* 212, 136-149.

Chen X., Yao Q., Gao X., Jiang C., Harberd N.P. & Fu X. (2016) Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. *Current Biology* 26, 640-646.

Covington M.F., Panda S., Liu X.L., Strayer C.A., Wagner D.R. & Kay S.A. (2001) ELF3 modulates resetting of the circadian clock in Arabidopsis. *The Plant Cell* 13, 1305-1315.

Covington M.F., Maloof J.N., Straume M., Kay S.A. & Harmer S.L. (2008) Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. *Genome Biology* 9, R130.

Dalchau N., Baek S.J., Briggs H.M., Robertson F.C., Dodd A.N., Gardner M.J., Stancombe M.A., Haydon M.J., Stan G.B., Goncalves J.M. & Webb A.A.R. (2011) The circadian oscillator gene GIGANTEA mediates a long-term response of the Arabidopsis thaliana circadian clock to sucrose. *Proceedings of the National Academy of Sciences of the United States of America* 108, 5104-5109.

Devlin P.F. & Kay S.A. (2000) Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. *The Plant Cell* 12, 2499-2509.

Dodd A.N., Salathia N., Hall A,. Kevei E., Toth R., Nagy F., Hibberd J.M., Millar A.J. & Webb A.A.R. (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* 309, 630-633.

Doyle M.R., Davis S.J., Bastow R.M., McWatters H.G., Kozma-Bognar L., Nagy F., Millar A.J. & Amasino R.M. (2002) The ELF4 gene controls circadian rhythms and flowering time in Arabidopsis thaliana. *Nature* 419, 74-77.

Dyachok J., Zhu L., Liao F., He J., Huq E. & Blancaflor E.B. (2011) SCAR Mediates Light-Induced Root Elongation in *Arabidopsis* through Photoreceptors and Proteasomes. *The Plant Cell* 23, 3610-3626.

Edwards K.D., Akman O.E., Knox K., Lumsden P.J., Thomson A.W., Brown P.E., Pokhilko A., Kozma-Bognar L., Nagy F., Rand D.A. & Millar A.J. (2010) Quantitative analysis of regulatory flexibility under changing environmental conditions. *Molecular Systems Biology* 6, 424.

Endo M., Shimizu H., Nohales M.A., Araki T. & Kay S.A. (2014) Tissue-specific clocks in Arabidopsis show asymmetric coupling. *Nature* 515, 419-422.

Endo M. (2016) Tissue-specific circadian clocks in plants. *Current Opinion in Plant Biology* 29, 44-49.

Fankhauser C. & Staiger D. (2002) Photoreceptors in *Arabidopsis thaliana*: light perception, signal transduction and entrainment of the endogenous clock. *Planta* 216, 1-16.

Franklin K.A., Toledo-Ortiz G., Pyott D.E. & Halliday K.J. (2014) Interaction of light and temperature signalling. *Journal of Experimental Botany* 65, 2859-2871.

Fukuda H., Nakamichi N., Hisatsune M., Murase H. & Mizuno T. (2007) Synchronization of plant circadian oscillators with a phase delay effect of the vein network. *Physical Review Letters* 99, 098102.

Green R.M., Tingay S., Wang Z.Y. & Tobin E.M. (2002) Circadian rhythms confer a higher level of fitness to Arabidopsis plants. *Plant Physiology* 129, 576-584.

Greenham K. & McClung C.R. (2015) Integrating circadian dynamics with physiological processes in plants. *Nature Reviews Genetics* 16, 598-610.

Harmer S. (2009) The circadian system in higher plants. *Annual Review of Plant Biology* 60, 357-377.

Haydon M.J., Mielczarek O., Robertson F.C., Hubbard K.E. & Webb A.A.R. (2013) Photosynthetic entrainment of the Arabidopsis thaliana circadian clock. *Nature* 502, 689-692.

Hsu P.Y. & Harmer S.L. (2014) Wheels within wheels: the plant circadian system. *Trends in Plant Science* 19, 240-249.

Hu W., Franklin K.A., Sharrock R.A., Jones M.A., Harmer S.C. & Lagarias J.C. (2013) Unanticipated regulatory roles for *Arabidopsis* phytochromes revealed by null mutant analysis. *Proceedings of the National Academy of Sciences of the United States of America* 110, 1542-1547.

James A.B., Monreal J.A., Nimmo G.A., Kelly C.L., Herzyk P., Jenkins G.I. & Nimmo H.G. (2008) The circadian clock in Arabidopsis roots is a simplified slave version of the clock in shoots. *Science* 322, 1832-1835.

Kiss J.Z., Mullen J.L., Correll M.J. & Hangarter R.P. (2003) Phytochromes A and B mediate red-light-induced positive phototropism in roots. *Plant Physiology* 131, 1411-1417.

Lee H.J., Ha J.H., Kim S.G., Choi H.K., Kim Z.H., Han Y.J., Kim J.I., Oh Y., Fragoso K., Shin K., Hyeon T., Choi H.G., Oh K.H., Baldwin I.T. & Park C.M. (2016) Stem-piped light activates phytochrome B to trigger light responses in *Arabidopsis thaliana* roots. Science Signaling 9, ra106.

Lee H.J., Park Y.J., Baldwin I.T & Park C.M (2017) Multiple routes of light signaling during root photomorphogenesis. *Trends in Plant Science* 22, 803-812.

Mandoli D.F. & Briggs W.R. (1984) Fiber-Optic Plant-Tissues - Spectral Dependence in Dark-Grown and Green Tissues. *Photochemistry and Photobiology* 39, 419-424.

Mas P., Kim W.Y., Somers D.E & Kay S.A. (2003) Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. Nature 426, 567-570.

Mo M., Yokawa K., Wan Y. & Baluska F. (2015) How and why do root apices sense light under the soil surface? Frontiers in Plant Science 6, 775.

Mohawk J.A., Green C.A. & Takahashi J. (2012) Central and peripheral circadian clocks in mammals. *Annual Review of Neuroscience* 35, 445-482.

Molas M.L., Kiss J.Z. & Correll M.J. (2006) Gene profiling of the red light signalling pathway in roots. *Journal of Experimental Botany* 57, 3217-3229.

McClung C.R. (2006) Plant Circadian Rhythms. *The Plant Cell* 18, 792-803.

Michael T.P. & McClung C.R. (2003) Enhancer trapping reveals widespread circadian clock transcriptional control in Arabidopsis. *Plant Physiology* 132, 629-639.

Nagel D.H, & Kay S.A. (2012) Complexity in the Wiring and Regulation of Plant Circadian Networks. *Current Biology* 22, R648-R657.

Osterlund M.T., Hardtke C.S. & Deng X.W. (2000) Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* 405, 462-466.

Para A., Farre E.M., Imaizumi T., Pruneda-Paz J.L., Harmon F.G. & Kay S.A. (2007) PRR3 is a vascular regulator of TOC1 stabiity in the Arabidopsis circadian clock. *The Plant Cell* 19, 3462-3473.

Pruneda-Paz J.L. & Kay S.A. (2010) An expanding universe of circadian networks in higher plants. *Trends in Plant Science* 15, 259-265.

Salisbury F., Hall A., Grierson C.S. & Halliday K.J. (2007) Phytochrome coordinates Arabidopsis shoot and root development. *The Plant Journal* 50, 429-438.

Sabthai SB, Ristova D. & Busch W. (2015) Underground tuning: quantitative regulation of root growth. *Journal of Experimental Botany* 66, 1099-1112.

Silva-Navas J., Moreno-Risuena M.A., Manzano C., Pallero-Baeno M., Navarro-Neila S., Tellez-Robledo B., Garcia-Mina J.M., Baigorri R., Gallego F.J. & del Pozo J.C. (2015) D-Root: a system for cultivating plants with the roots in darkness or under different light conditions. *The Plant Journal* 84, 244-255.

Sun Q., Yoda K. & Suzuki H. (2005) Internal axial light conduction in the stems and roots of herbaceous plants. *Journal of Experimental Botany* 56, 191-203.

Sun Q,, Yoda K,, Suzuki M, & Suzuki H. (2003) Vascular tissue in the stem and roots of woody plants can conduct light. *Journal of Experimental Botany* 54, 1627-1635.

Takahashi N., Hirata Y., Aihara K. & Mas P. (2015) A Hierarchical Multi-oscillator Network Orchestrates the Arabidopsis Circadian System. Cell 163, 148-159.

Tester M. & Morris C. (1987) The Penetration of Light Through Soil. *Plant Cell and Environment* 10, 281-286.

Thain S.C., Hall A. & Millar A.J. (2000) Functional independence of circadian clocks that regulate giant gene expression. *Current Biology* 10, 951-956.

Warnasooriya S.N., Porter K.J. & Montgomery B.L. (2011) Tissue- and isoform-specific phytochrome regulation of light-dependent anthocyanin accumulation in Arabidopsis thaliana. *Plant Signaling and Behavior* 6, 624-631.

Wenden B., Kozma-Bognar L., Edwards K.D., Hall A.J.W., Locke J.C.W. & Millar A.J. (2011) Light inputs shape the Arabidopsis circadian system. *Plant Journal* 66, 480-491.

Wenden B., Toner D.L.K., Hodge S.K., Grima R. & Millar A.J. (2012) Spontaneous spatioteemporal waves of genes expression from biological clocks in the leaf. *Proceedings of the National Academy of Sciences of the United States of America* 109, 6757-6762.

Xu W., Ding G., Yakawa K., Baluska F., Li Q.F, Liu Y., Shi W., Liang J. & Zhang J. (2013) An improved agar-plate method for studying root growth and response of *Arabidopsis thaliana*. *Scientific Reports* 3, 1273.

Table 1

Periods of root tissue

Plants were imaged under LL at 20 µmol.m^{-2}.s^{-1}. Periods were estimated over the times 48 – 120 h in Figs 5 and 6. *, different from the covered, top exposed value by Student's t-test ($P < 0.001$).

Light	**Root tissue imaged**	**Period** (h)	**n**
Red	Exposed top	26.26 ± 0.18	8
Red	Covered, top exposed	27.53 ± 0.15	24
Red	Fully covered	28.87 ± 0.14*	24
Blue	Covered, top exposed	30.19 + 0.14	8
Blue	Fully covered	30.35 + 0.14	8

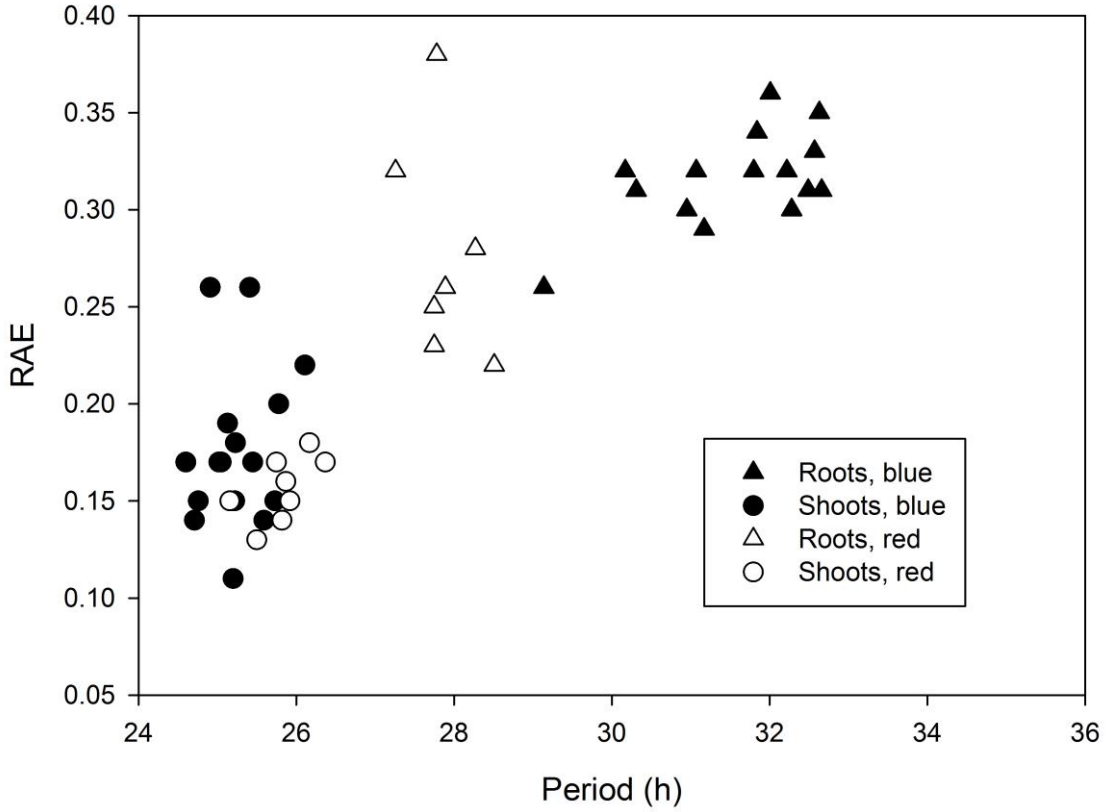

Figure 1a

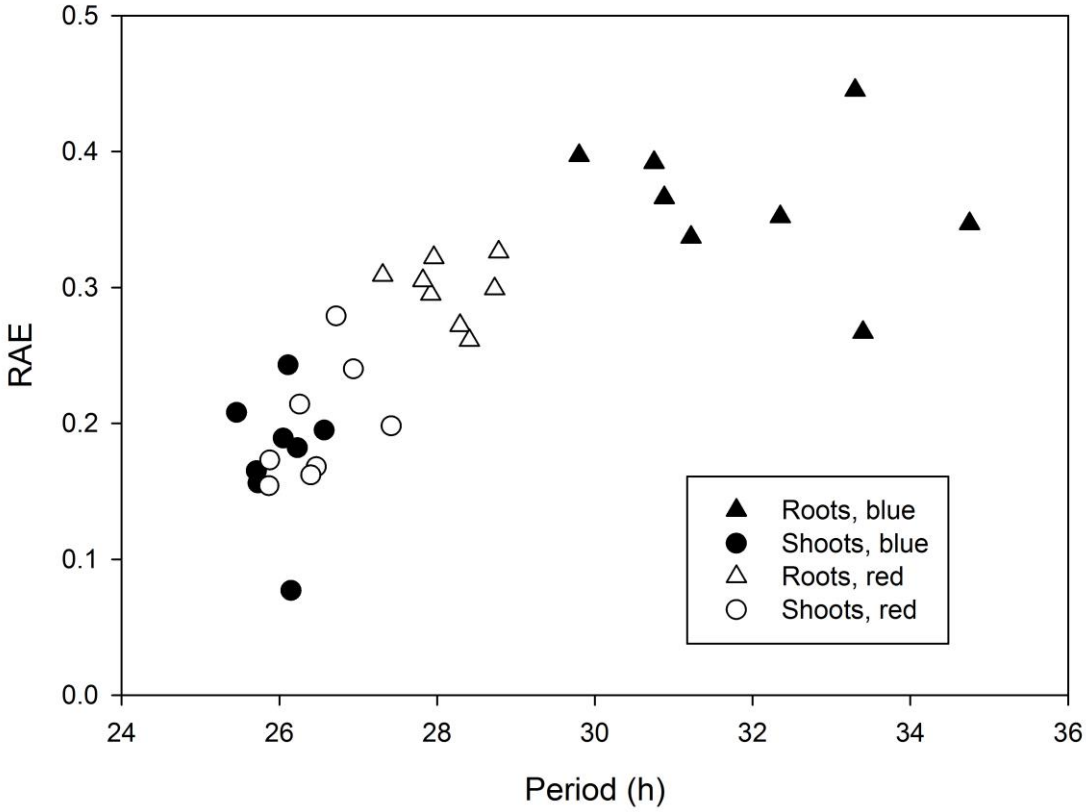

Figure 1b

Figure 1 The period of the root clock is longer in blue than red light

Intact plants were imaged in LL at 15 µmol.m^{-2}.s^{-1} of red or blue light. Period and RAE values were estimated over 48-120 h in LL. (a), GI:LUC in Ws, (b), CCA1:LUC in Ws

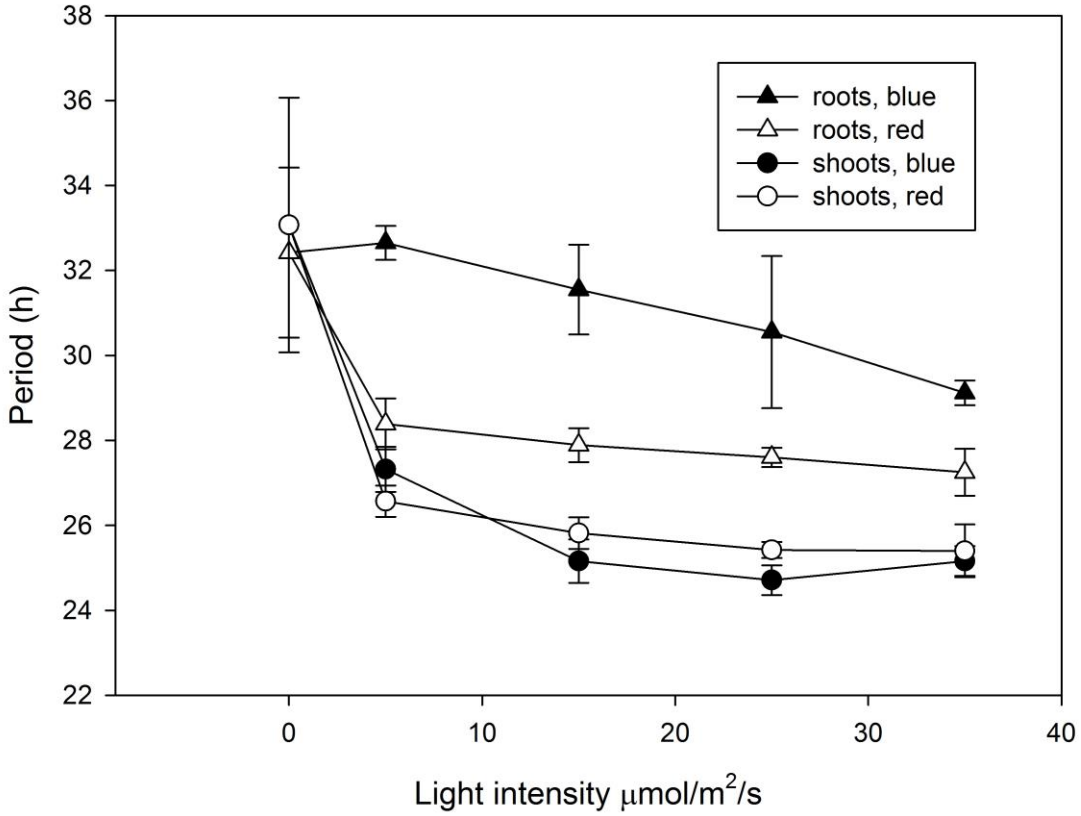

Figure 2 The period of the root clock is less sensitive to blue than red light

Intact plants expressing GI:LUC were imaged in LL at the indicated light intensity. Period values were estimated using data from 24-96 h in LL. Data are means ± SD for n = 8-16 clusters of plants in 2-4 biological repeats.

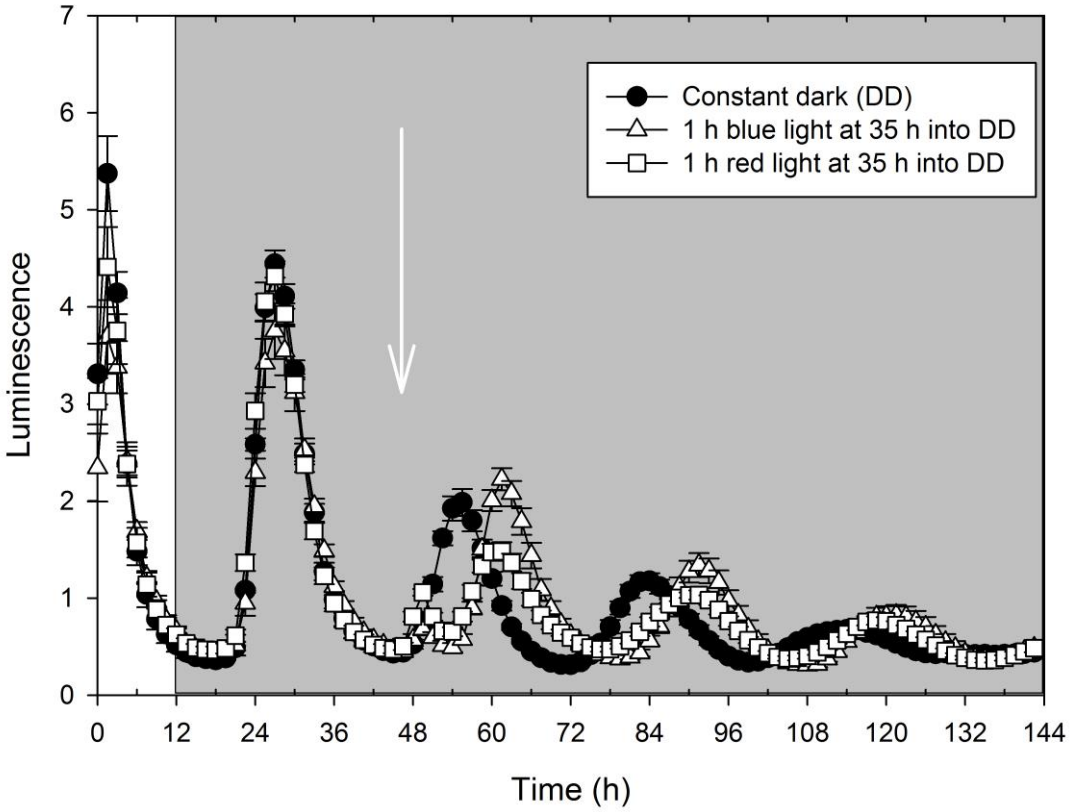

Figure 3 Both red and blue light can reset the phase of rhythms in decapitated roots

Decapitated L*er* plants expressing CCA1:LUC were imaged on plates containing 1% sucrose. The figure shows data from the last 12 h light period followed by 132 h of DD. Some plants were illuminated with red or blue light for 1 h at the point indicated by the arrow; control plants were maintained in DD. Data are means ± SD for n = 8 clusters of plants in 3 biological repeats.

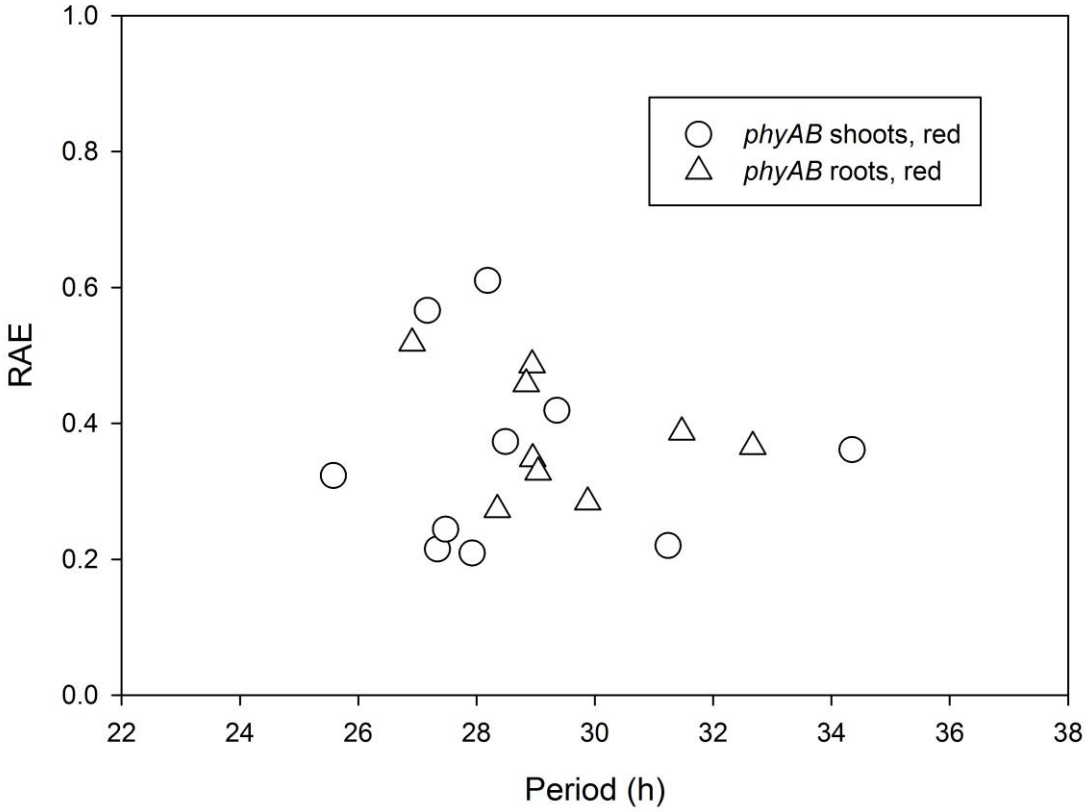

Figure 4a

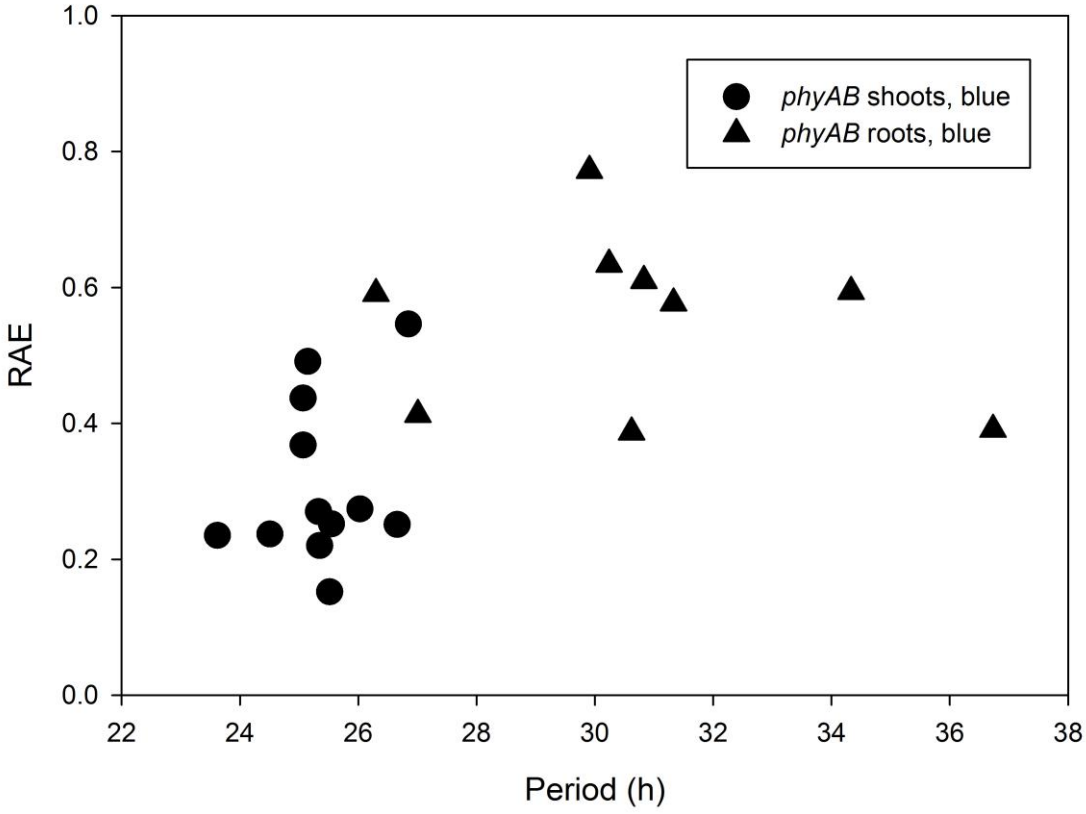

Figure 4b

Figure 4 The *phyAB* mutant is blind to red light

Intact *phyA-201*; *phyB-5* plants expressing CCA1:LUC were imaged in LL at 20 µmol.m^{-2}.s^{-1} of red or blue light. Period and RAE values were estimated over 48-120 h in LL. (a) red light, (b) blue light. Root data are replotted in Fig. S5.

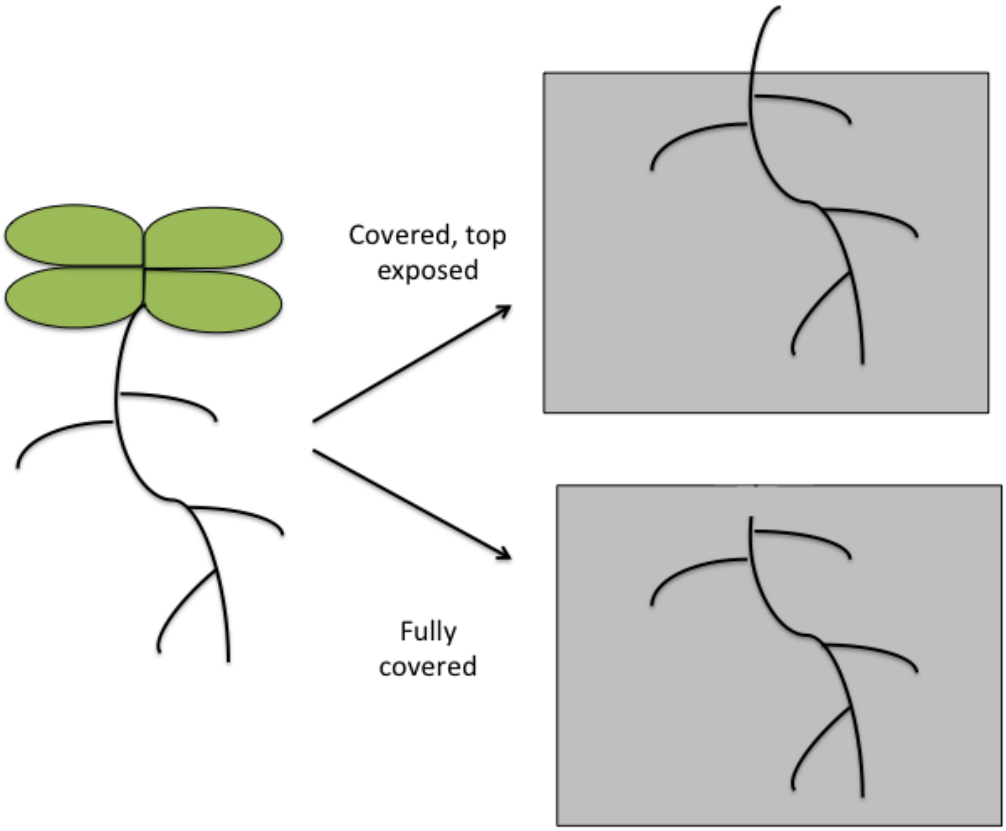

Figure 5a

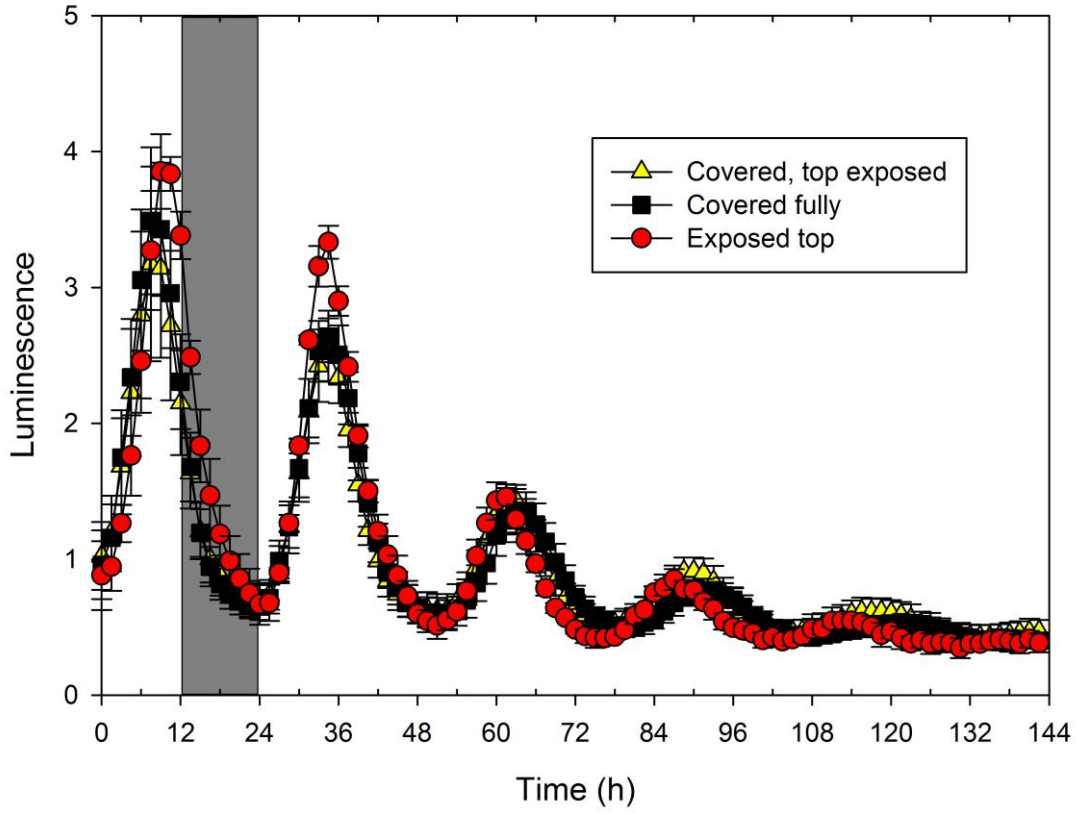

Figure 5b

Figure 5 Exposure of the top of decapitated roots to red light reduces the period of the root section maintained in darkness.

Plants expressing GI:LUC were decapitated and imaged with the root compartment covered except during imaging. (a) Sketch of the procedure, (b) Time courses showing 1 LD cycle followed by 120 h in LL; data for fully covered roots and roots with exposed tops are means ± SD for n = 24 clusters of roots in 6 separate biological repeats; data for exposed tops are means ± SD for n = 8 clusters of plants in 2 separate biological repeats. Representative images of roots either fully covered or with their tops exposed are shown in Fig. S6. An expanded view of the section 72-108 h is shown in Fig. S7.

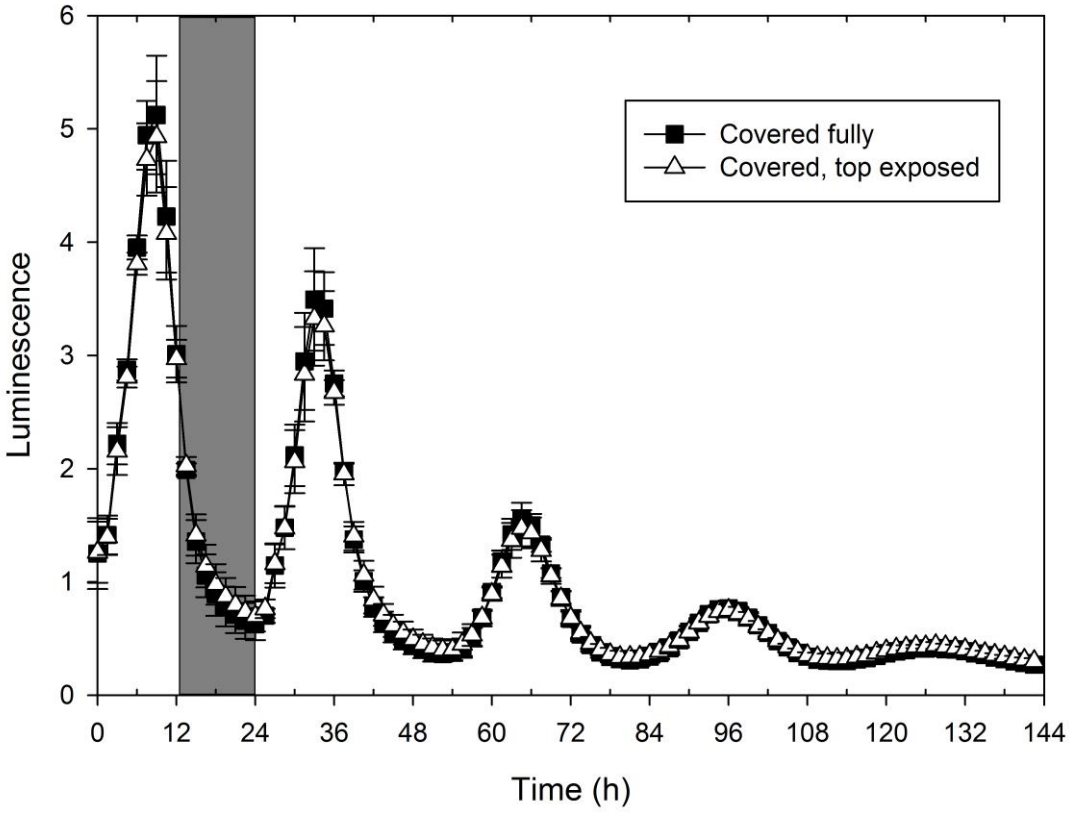

Figure 6 Exposure of the top of decapitated roots to blue light does not affect the period of the root section maintained in darkness.

The graph presents time courses showing 1 LD cycle followed by 120 h in LL, means ± SD for n = 8 clusters of roots in 2 separate biological repeats.