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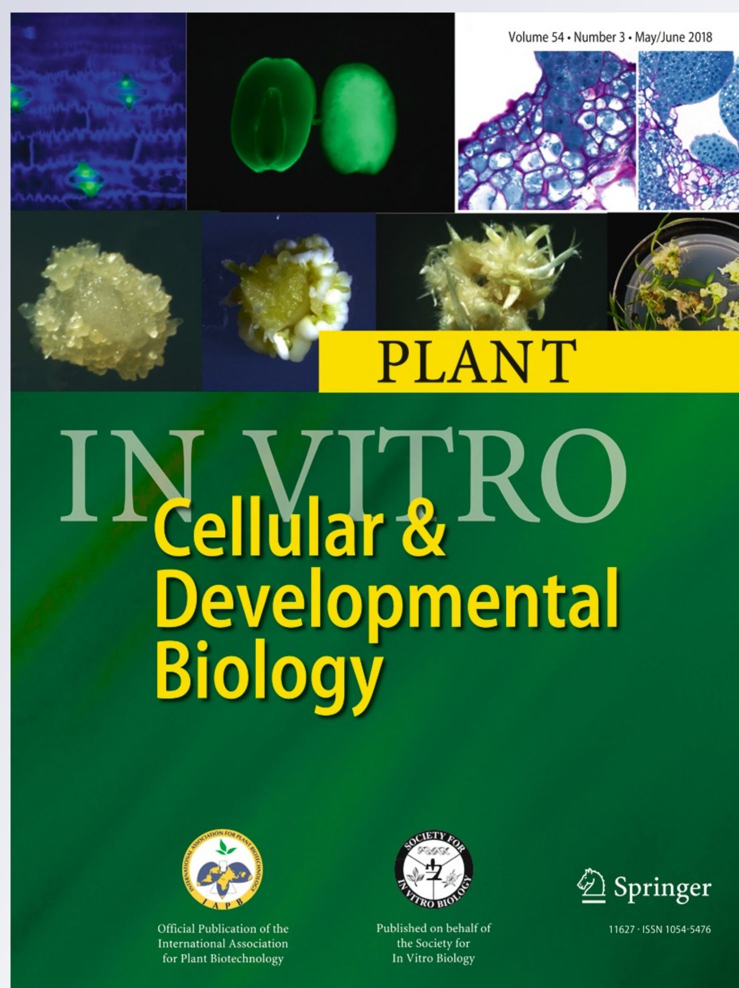

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DEVELOPMENTAL BIOLOGY

The initial hours of post-excision light are critical for adventitious root regeneration from *Arabidopsis thaliana* (L.) Heynh. cotyledon explants

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Abstract

How light exposure of excised plant tissues impacts the success of subsequent adventitious root regeneration is poorly understood. Here, exposure to high light intensity was observed to inhibit root regeneration from *Arabidopsis thaliana* (L.) Heynh. cotyledon explants. Transfers to dark followed by high-intensity light (or reciprocal) were used to define when the explants were most light-sensitive and when adventitious root formation was most inhibited. Exposure of explants to light during the first 6–48 h after excision strongly inhibited root regeneration. Mutants and chemical inhibitors were used to identify modulators of this light-induced response. During the first 48 h post excision, reduction in photoprotective xanthophylls or application of chemicals known to promote reactive oxygen species caused the cotyledon explants to become light-hypersensitive, and decreased adventitious root regeneration. Filtering out blue/ultraviolet-A wavelengths reduced the negative effects of light, while mutants defective in phytochrome A or light-activated transcription factor ELONGATED HYPOCOTYL 5 were hypersensitive to early light exposure. A mutant defective in chalcone synthase (*transparent testa 4*) showed reduced root regeneration, regardless of early light or dark exposure. Application of a polar auxin transport inhibitor, 1-N-naphthylphthalamic acid, during the first 24 h post excision reduced explant light sensitivity and increased the percentage that successfully induced adventitious roots. These results indicated a critical role for light during the initial post-excision hours on root regeneration in *Arabidopsis*. The data suggested that complex interactions between light, photoreceptor signaling, reactive oxygen species, photoprotective pigments, and auxin act upon adventitious root induction in *A. thaliana* cotyledon explants.

Keywords Adventitious root regeneration · Light · Xanthophyll · Photoperception · Auxin

Introduction

Severed plant tissues (*e.g.*, leaves or stem cuttings) must regenerate adventitious roots in order to survive (De Klerk *et al.* 1999), a process that has been studied for more than a century (Van Tieghem and Douliot 1888). Increased understanding of specific regeneration mechanisms is important for agricultural and horticultural vegetative plant propagation (Preece 2003; Ikeuchi *et al.* 2016). Studies in diverse plants (including woody, herbaceous, monocot, dicot, leguminous, and non-leguminous species), such as *Vigna radiata* (L.) R.Wilczek (mung bean), *Prunus serotina* Ehrh. (black cherry), and *Eleusine coracana* (L.) Gaertn. (finger millet), have shown that explants exposed to long periods (*i.e.*, weeks) of light or dark dramatically impact subsequent root regeneration (Jarvis and Ali 1985; Patton and Meinke 1988; Fuernkranz *et al.* 1990; Tyburski and Tretyn 2004; Xu *et al.* 2016a; Statish *et al.* 2016). However, the exact timing of post-excision light sensitivity and the underlying mechanisms coordinating light or dark exposure with adventitious root formation have not been systematically characterized.

Many factors contribute to the effect of light on adventitious root regeneration. Differing wavelengths of red/far-red (R/FR) and blue/ultraviolet-A (UVA) light are known to regulate plant regeneration in a species-specific manner (Chee 1986; Fuernkranz *et al.* 1990; Rossi *et al.* 1993; Morini *et al.* 2000; Tyburski and Tretyn 2004; Chung *et al.* 2010; Gu *et al.* 2012; Nameth *et al.* 2013). For example, in *Vitis* spp. (grape) and black cherry, blue light has been shown to inhibit root regeneration (Chee 1986; Fuernkranz *et al.* 1990).

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By contrast, in *Prunus domestica* subsp. *insititia* (L.) Bonnier & Layens, white light, red light, far-red light, blue light, and darkness all promoted root regeneration, but in an naphthaleneacetic acid (NAA)-dependent manner (Rossi *et al.* 1993). In *Solanum lycopersicum* (L.) (tomato) hypocotyl explants, continuous exposure to darkness, red light, and blue light all decreased root regeneration (Tyburski and Tretyn 2004). Interestingly, in that study, several days of early exposure to white light enhanced regeneration, suggesting that the timing of light exposure was critical.

Efficient adventitious root formation in the model species *Arabidopsis thaliana* (L.) Heynh. is not a natural phenomenon and must be induced by exposure to hormones, namely, auxin (Zhao *et al.* 2002; Ludwig-Muller *et al.* 2005; Gutierrez *et al.* 2012; Welander *et al.* 2014). Nevertheless, studies have been performed in *A. thaliana* that show that the first 24 h are critical to tissue regeneration (Sena *et al.* 2009; Sena 2014; Welander *et al.* 2014). The *A. thaliana* genome encodes five phytochromes (phy) that are responsible for R/FR perception, with phyA as the main receptor for far-red light (Nagatani *et al.* 1993; Reed *et al.* 1994; Fankhauser and Christie 2015), and phyB a primary photoreceptor for red light (Koornneef *et al.* 1980; Reed *et al.* 1993). *A. thaliana* perceives blue/UVA light through at least four photoreceptors, including two cryptochromes (CRY1, 2) responsible for photomorphogenic responses (Ahmad *et al.* 1995), and two phototropins (PHOT1, 2) responsible for directional chloroplast and plant movements (Baldwin *et al.* 2002; Kagawa and Wada 2002; Fankhauser and Christie 2015). Downstream of several photoreceptors, the light-responsive transcription factor ELONGATED HYPOCOTYL 5 (HY5) is a key regulator of photomorphogenesis and is considered a master regulator of gene expression that integrates light with auxin and cytokinin signaling, anthocyanin production, and circadian rhythms (Oyama *et al.* 1997; Lee *et al.* 2007; Vandenbussche *et al.* 2007; Vanstraelen and Benkova 2012). ELONGATED HYPOCOTYL 5 can physically move between shoots and roots to enable whole plant coordination of resource supply and demand (Chen *et al.* 2016; Palme *et al.* 2016). Despite its importance, a potential role for HY5 in root regeneration has not been previously reported.

High-intensity light has been shown to indirectly lower root regeneration success by inducing reactive oxygen species (ROS), which can result in photooxidative damage (Jarvis and Ali 1985) and/or act as signaling molecules (Pasternak *et al.* 2005; Tognetti *et al.* 2012; Carmody *et al.* 2016; Dietz *et al.* 2016). Plants prevent photooxidative damage primarily through photoprotective pigments, including carotenoids and xanthophylls, which dissipate excess light energy in the chloroplast *via* non-photochemical quenching (Demmig-Adams and Adams 1992; Niyogi and Truong 2013). Another class of photoprotective pigments are anthocyanins, which absorb high-energy UV and blue and green wavelengths (Gould *et al.* 2002; Landi *et al.* 2015). Anthocyanins are flavonoid-derived purple and red pigments localized in the vacuole, which are induced by light and other stresses (Gould 2004; Landi *et al.* 2015).

In addition to environmental factors, the plant hormone auxin is widely known to stimulate adventitious root regeneration (Jarvis and Ali 1985; Fett-Neto *et al.* 2001; Tyburski and Tretyn 2004; Correa *et al.* 2012; Mironova *et al.* 2012). Auxin is transported directionally by polarly localized influx and efflux carriers, which are able to re-orient to alter the direction of auxin flow (Jenik and Barton 2005; Cho and Cho 2013; Robert *et al.* 2015). Both magnitude and direction of auxin transport are important, because auxin acts as a concentration-dependent morphogen to regulate stem cells, vascular tissue differentiation, and organ initiation (Jenik and Barton 2005; Robert *et al.* 2015). In *A. thaliana*, addition of 1-N-naphthylphthalamic acid (NPA), an inhibitor of polar auxin transport, suppresses root meristem regeneration when applied to severed root tips during the initial hours after wounding (Sena *et al.* 2009), and during lateral root initiation in whole roots (Casimiro *et al.* 2001). Along with other results (Efroni *et al.* 2016), this suggests that early, post-excision auxin signaling is essential for root regeneration.

Here, the effects of exposing *A. thaliana* cotyledon explants to different light and dark treatments are reported in order to elucidate the temporal window of explant light sensitivity with respect to root regeneration success rate. Mutants, chemical inhibitors, and elicitors were utilized to study genetic factors and physiological mechanisms underlying this period of light hypersensitivity. Together with a previously published paper (Nameth *et al.* 2013), in which light signaling effects on shoot regeneration of *A. thaliana* cotyledon explants were reported, this research provides optimized conditions for full plant regeneration from cotyledon explants.

Materials and methods

***Arabidopsis* seed sources** For the evaluation of natural variation and light and dark exposure experiments (Figs. 1, 2, 3, and 4), *A. thaliana* wild-type (WT) ecotypes were acquired from Lehle Seeds (Round Rock, TX): Columbia-0 (Col-0; WT-2), Dijon G (Dij-G; WT-10), Nossen-0 (No-0; WT-9), Estland 1 (Est-1; WT-6A), and Landsberg *erecta*-0 (L*er*-0; WT-4). As controls for inhibitor and mutant experiments (Figs. 5, 6, 7, and 8), wild-type control seeds were acquired from the *Arabidopsis* Biological Resource Center (ABRC, The Ohio State University, Columbus, OH): Wassilewskija 2 (Ws2; CS2360/CS22659), L*er*-0 (CS20), and C24 (CS906). The quadruple blue mutant [*cry1cry2phot1phot2*, line 210] (Ohgishi *et al.* 2004) was acquired in a hybrid L*er*-0 (CS20)/ Ws2 background (CS2360/CS22659; Sakai Lab, Yokohama,

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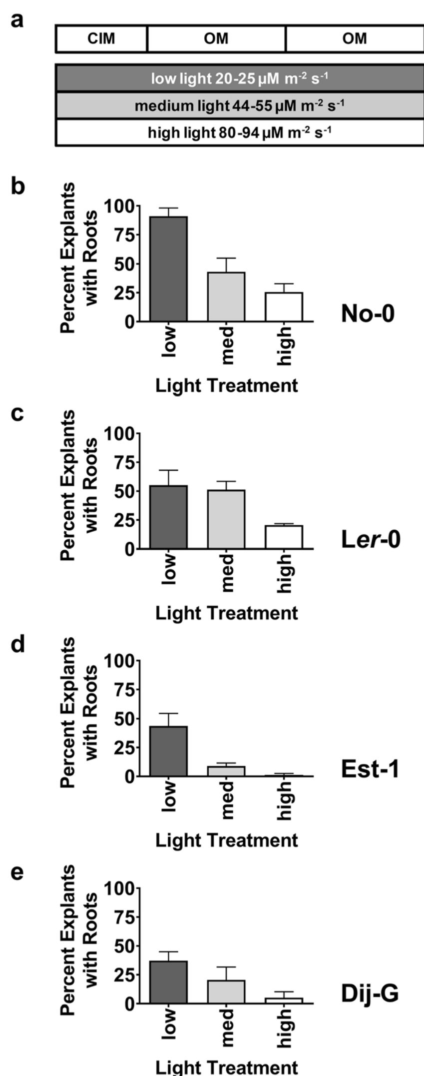

Fig. 1. An increase in light exposure decreased adventitious root regeneration from cotyledons of diverse *Arabidopsis thaliana* ecotypes. (*a*) Treatment summary. *CIM* callus induction medium, *OM* organogenesis medium. (*b*–*e*) The percentage of cotyledon explants that regenerated roots 4 wk. after excision for the respective ecotypes: (*b*) No-0; (*c*) L*er*-0 (Lehle); (*d*) Est-1; and (*e*) Dij-G. *Error bars* are the standard errors of the means (SEM) of three replicate Petri dishes ($n = 26$ cotyledons per replicate).

Japan). All other mutant stocks were acquired from ABRC in a L*er*-0 (CS20) background: *hy1-1* (CS67), *hy5-1* (CS71), *non-photochemical quenching 1* (*npq1-2*; CS3771), *phyA-201* (CS6219), *phyB-1* (CS6211), and *transparent testa* 4 (*tt4-1*; CS85). Descriptions of wild-type and mutant lines are provided in Tables 1 and 2.

Light measurements Photosynthetically active radiation (PAR) with a wavelength range of 400–700 nm was quantified using a BQM-01 meter (Apogee Instruments, Logan, UT). All experiments employed cool white fluorescent lamps (F72T12CW/VHO, Osram Sylvania, Wilmington, MA). Unless otherwise noted, detached tissues were exposed to 24 or 48 h of darkness, or continuous high light (90–120 μmol m^{-2} s^{-1}), followed by 4–5 wk. of continuous high light.

Germination conditions Seeds were surface sterilized, soaked in sterile water for 2–7 d (ecotype-dependent), and then germinated on Murashige and Skoog (Murashige and Skoog 1962) medium with Gamborg's Vitamins (M0404, Sigma-Aldrich®, St. Louis, MO) in 100-mm-diameter × 25-mm-deep Petri dishes, as previously described in Nameth *et al.* (2013). Twenty-six seeds were evenly distributed on the surface of each plate, and the plates were sealed with Micropore™ surgical tape (1530-1, 3M®, London, Canada). The seeds were germinated in 24-h continuous light (cool white fluorescent lamps at 50–80 μmol m^{-2} s^{-1}) at 25°C for 6–7 d in a room devoid of background sunlight.

Root regeneration assay Individual cotyledons were excised 6–7 d post germination at the base of each blade (excluding petiole). Uniformly sized, healthy cotyledons were selected as explant sources. Twenty-six explants were evenly distributed onto callus-inducing medium (CIM) pretreatment (Zhao *et al.* 2002) or directly onto shoot-inducing medium (referred to as organogenesis medium, OM, to prevent confusion) in 100-mm-diameter × 25-mm-deep Petri dishes. Both media consisted of Gamborg's B5 (Gamborg *et al.* 1968) Basal Medium with Minimal Organics (G5893, Sigma-Aldrich®), with 0.5 g L^{-1} 2-(N-morpholino)ethanesulfonic acid (MES; Sigma-Aldrich® MES2933), 20 g L^{-1} glucose (G8270, Sigma-Aldrich®), and 3 g L^{-1} Phytagel™ (P8169, Sigma-Aldrich®), and adjusted with KOH (1 N) to pH 5.8 prior to autoclaving at 121°C for 30 min. For CIM, 0.1 mg L^{-1} kinetin (K1885, Sigma-Aldrich®) and 0.5 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D; D6679, Sigma-Aldrich®) were filter-sterilized (0.22 μm; 09-720-000, Thermo Fisher Scientific®, Ottawa, Canada) and added after autoclaving. For OM, 0.9 mg L^{-1} N^6-(Δ^2-isopentenyl)adenine (2-iP; D7674, Sigma-Aldrich®) and 0.1 mg L^{-1} NAA (N0640, Sigma-Aldrich®) were filter-sterilized (0.22 μm; 09-720-000, Thermo Fisher Scientific®) and added following autoclaving. The default parameters for regeneration were: 5 d on CIM, followed by 10 d on OM, and then a transfer to fresh OM for ~3 wk. Explant regeneration occurred in a growth chamber (Conviron, Winnipeg, Canada) under 24-h continuous fluorescent lights (CW/VHO, Sylvania, Mississauga, Canada), at 23°C continuous temperature and 50% relative humidity. Petri dishes were sealed with Micropore™ tape, randomized continuously, and never exposed to sunlight.

Scoring for root regeneration occurred ~4 wk. post excision. The roots were dissected from callus, and dried on Kimwipes™ (Kimberly-Clark®, Irving, TX) to remove residual medium and condensation, before weighing. Besides fresh adventitious root weight, the number of explants with

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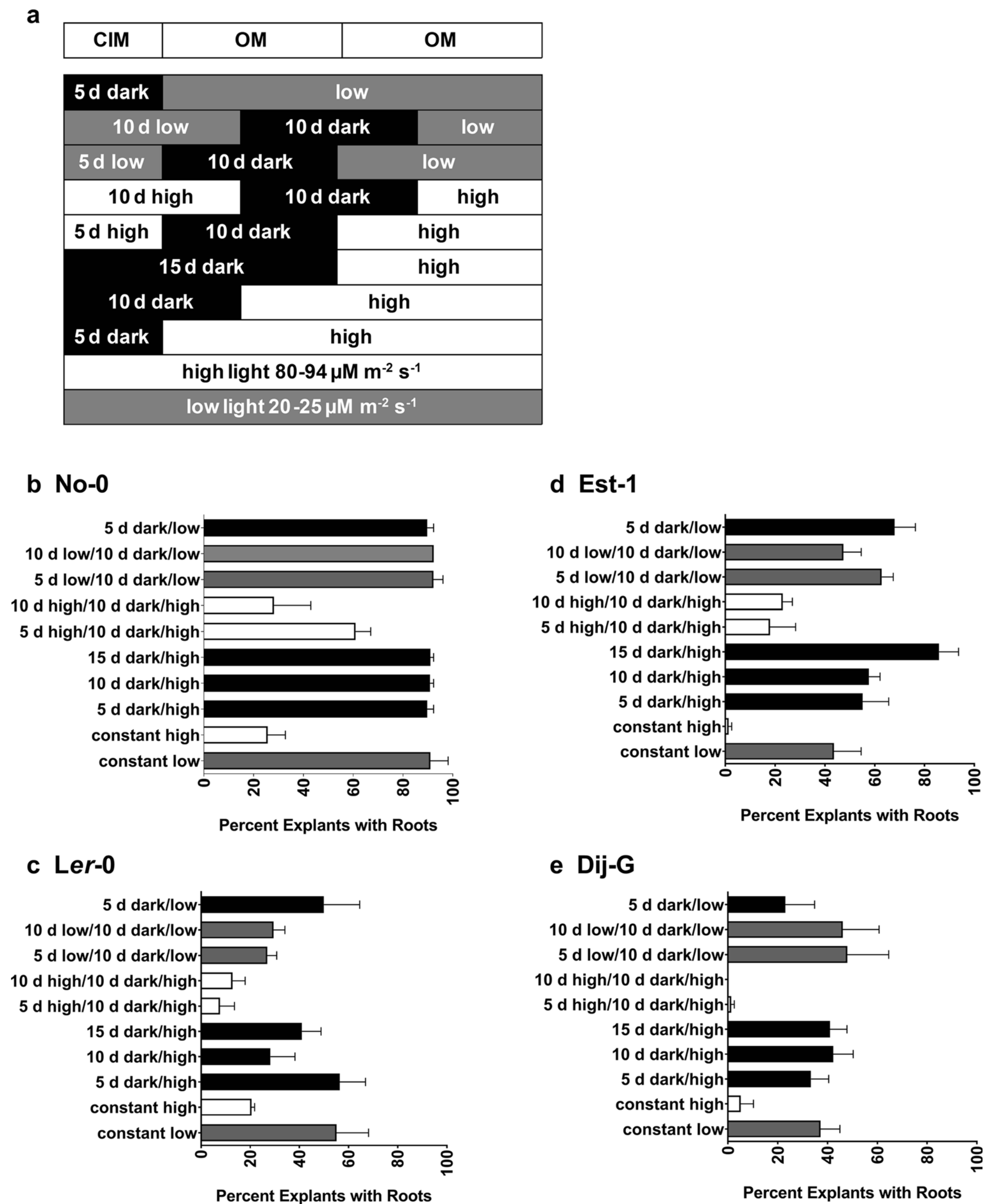

Fig. 2. Light intensity during the initial 5 d after cotyledon excision modulated the rate of root regeneration evaluated 4 wk. post excision. (*a*) Treatment summary. *CIM* callus induction medium, *OM* organogenesis medium. (*b*–*e*) The percentage of cotyledon explants that regenerated roots for the respective ecotypes: (*b*) No-0; (*c*) L*er*-0 (Lehle); (*d*) Est-1; and (*e*) Dij-G. *Error bars* are the standard errors of the means (SEM) of three replicate Petri dishes ($n = 26$ cotyledons per replicate).

minimally one root visible under a light microscope at up to ×32 magnification (Stemi DV4 stereo microscope, Zeiss®, Jena, Germany) was also recorded. Statistical significance was calculated using Mann-Whitney tests (InStat 3.0 for Mac, GraphPad Software Inc., La Jolla, CA).

Chemical treatments The treatments and corresponding solvents used for each chemical evaluation were as follows: 12.5, 25, or 50 μM of NPA (PS-343, Chem Service, West Chester, PA) in dimethyl sulfoxide (DMSO; D8418, Sigma-Aldrich®); 75 μM Norflurazon (NF; SAN9789A, Syngenta Crop Protection Inc., Greensboro, NC) in ethanol (16368, Sigma-Aldrich®); 0.5 mM dithiothreitol (DTT; BPI 172-5, Thermo Fisher Scientific®) in doubly distilled water (ddH_2O); and 50 nM Paraquat (PQ; M-2254, Sigma-Aldrich®, active ingredient methyl viologen) in ddH_2O. Filter-sterilized (0.22 μm; 09-720-000, Thermo Fisher Scientific®) inhibitors were added to CIM after autoclaving. Explants of 6-d-old *A. thaliana* seedlings were placed onto CIM containing the chemical agent or respective solvent control for 24–48 h (as indicated) and placed under 24–48 h of darkness or continuous fluorescent high-intensity light (90–120 μmol m^{-2} s^{-1}). Afterwards, the explants were moved onto the basic CIM (lacking chemical agents) for the remaining 72 h, to be further incubated on OM for 10 d, followed by fresh OM for 3 wk., all under continuous fluorescent light (90–120 μmol m^{-2} s^{-1}, unless noted otherwise).

Blue/UVA deficiency light filter assay For the blue/UVA deficiency filter (BDF) treatment, a yellow acetate light filter

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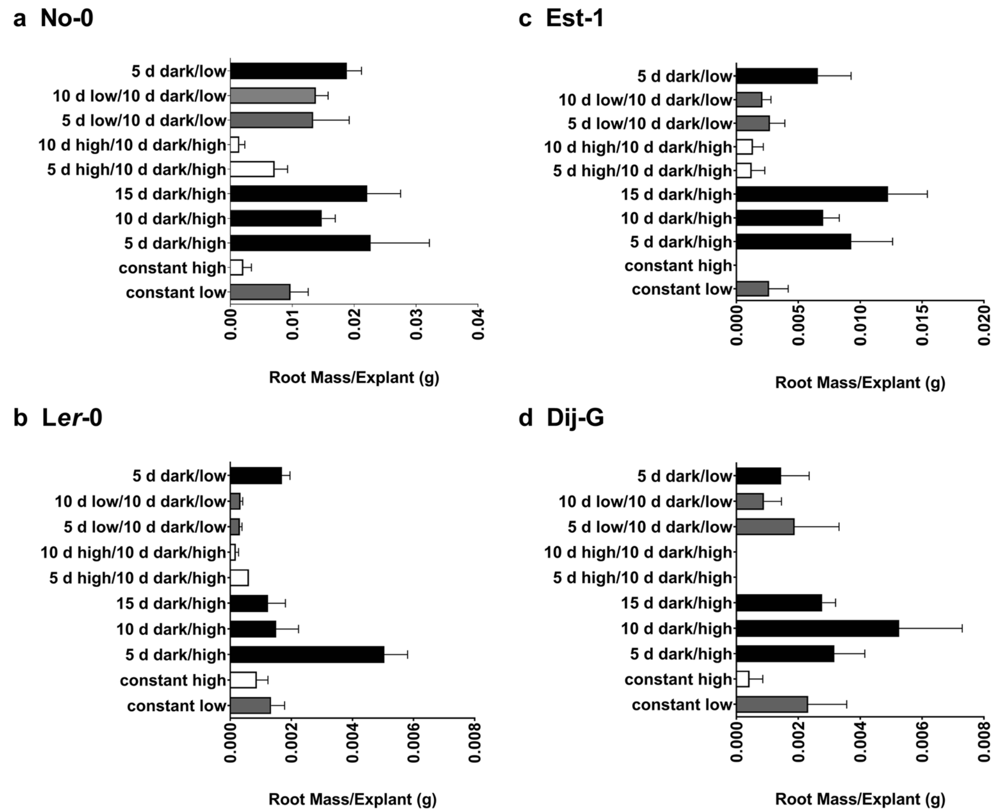

Fig. 3. Light intensity during the first 5 d after excision determined the mass of regenerating roots in diverse *Arabidopsis thaliana* ecotypes. Shown are the root masses per explant at 4 wk. after excision for the following ecotypes: (*a*) *No-0*; (*b*) *Ler-0* (Lehle); (*c*) *Est-1*; and (*d*) *Dij-G*. These data are additional to the data presented in Fig. 2. The *histograms* have *shades* that correspond to the intensity of light experienced by the explants during the initial 5 d post excision. *Error bars* represent the standard error of the mean (SEM) of three replicates ($n = 26$ cotyledons per replicate).

(LEE 101, LEE Filters, Burbank, CA) was inserted between the fluorescent bulbs (CW/VHO, Sylvania) and the explant plates. The filter was reported by the manufacturer to deplete nearly all light < 450 nm and also significantly deplete photons in the 450–530-nm wavelength range (LEE 101, LEE Filters). To control for loss of photons that were blocked by the filter, the light intensity below the filter (400–700-nm range) was normalized to 60 or 100 μmol m^{-2} s^{-1} (as noted), as measured at plant level using the BQM-01 light meter. Five light treatments were tested, each proceeded by continuous high (white) light (100 μmol m^{-2} s^{-1}). The treatments were as follows: 5 d of dark exposure; 5 d of high-intensity light exposure; 5 d of medium light exposure (60 μmol m^{-2} s^{-1}); 5 d of blue/UVA-deficient high-intensity light (BDF high); and 5 d of blue/UVA-deficient medium-intensity light (BDF medium). Control explants were exposed to unfiltered light of the CW/VHO lamps. Following 5 d of incubation under BDF or control light, the explants were exposed to CW/VHO fluorescent light for the remainder of the experiment.

Results

Effect of light intensity on root regeneration in different *A. thaliana* ecotypes To define the effect of light intensity on root regeneration in *A. thaliana* cotyledon explants, four ecotypes (No-0, L*er*-0, Est-1, and Dij-G) with diverse regeneration responses were exposed to increasing intensity levels of continuous cool white fluorescent light (Fig. 1). All four ecotypes showed a significantly decreased frequency of explants with successful root regeneration when treated with 100 μmol m^{-2} s^{-1} of light after excision ("high-intensity light"), in comparison to explants exposed to 20–30 μmol m^{-2} s^{-1} light ("low-intensity light"; Fig. 1). These light levels were used for all further experiments.

Defining explant sensitivity to light using light-dark transfers To evaluate light sensitivity of explants during the 4-wk. tissue culture period, an experiment with 16 different light and dark incubations was designed. Continuous light exposure (either in low- or high-intensity light) was compared to different combinations of a 5-, 10-, or 15-d dark, either initially starting at the time of excision or delayed by 5 or 10 d of low- or high-intensity light. Explants were subjected to 16 different light and dark transfer treatments, but only the most informative conditions were presented in Figs. 2 and 3. For the four ecotypes tested, the root regeneration frequency 4 wk. after excision was determined by the light intensity within the initial 5 d post excision (Figs. 2 and 3). Early exposure to high light inhibited root regeneration in the four ecotypes evaluated, whereas early exposure to low light or darkness promoted root regeneration. In the No-0 ecotype in general, the highest adventitious rooting percentages were observed and this ecotype was shown to be very responsive to the low light

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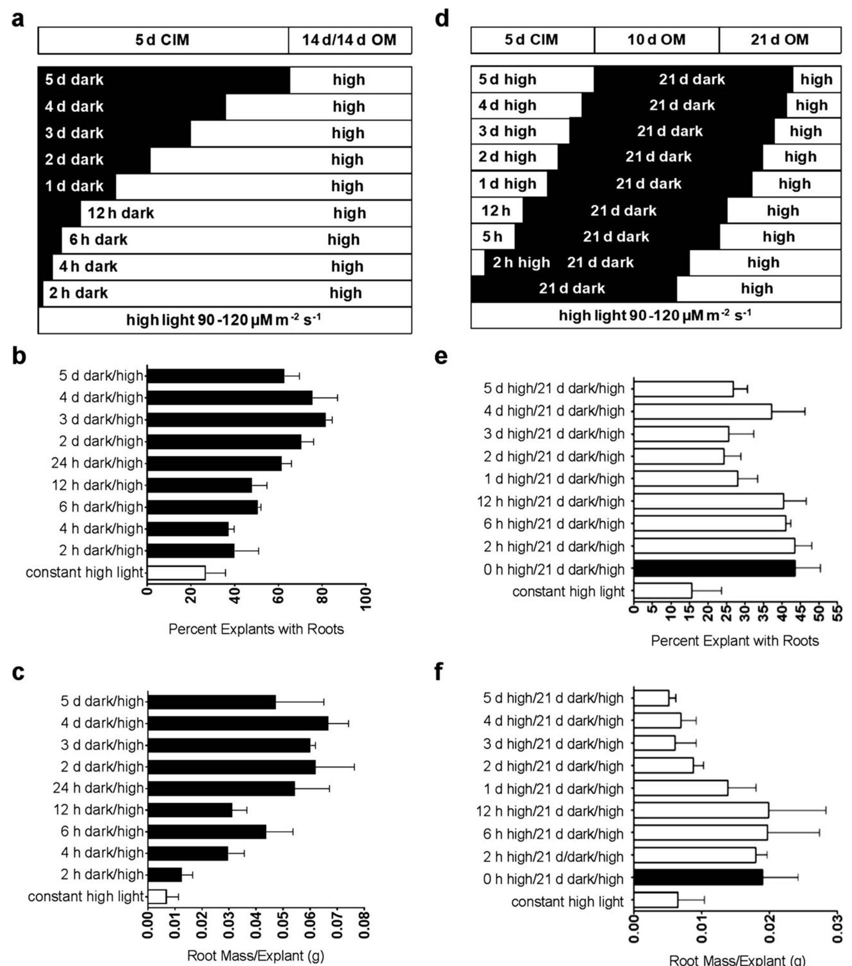

Fig. 4. Exposure to light or darkness within the initial 6–48 h after cotyledon excision modulated root regeneration in *Arabidopsis thaliana* L*er*-0 (Lehle) ecotype. (*a*, *d*) Treatment summaries. *CIM* callus induction medium, *OM* organogenesis medium. (*b*, *e*) Percentage of cotyledon explants that regenerated roots, scored at 4 wk. post excision. (*c*, *f*) the corresponding fresh weights of dissected roots. *Error bars* are the standard errors of the means (SEM) of three replicate Petri dishes ($n = 26$ cotyledons per replicate).

intensity and dark exposure treatments (Figs. 2*b* and 3*a*). With respect to the sensitivity of each ecotype to high light, No-0 was the least sensitive, followed by Est-1, then L*er*-0, and Dij-G (Figs. 2 and 3). As considerable genetic mutant resources exist for L*er*-0, this ecotype was chosen for additional experiments. First, L*er*-0 explants were exposed to an additional series of different durations of dark and light incubation following the explant excision (Fig. 4*a*–*c*). As few as 6 h of dark exposure immediately after excision resulted in a nearly 2-fold increase in root regeneration frequency (Fig. 4*b*), despite the subsequent transfer of the explants to high light for the remaining 4 wk. Incrementally extending the early dark period from 2 to 72 h increased the number of rooted explants, as well as the root mass per explant (Fig. 4*b*, *c*). In a reciprocal experiment, excised cotyledons were incubated for different durations of high light intensity followed by extended darkness, which was subsequently followed again by the required duration of high-intensity light (Fig. 4*d*). Initial exposure to ≥ 24 h of high-intensity light prior to darkness suppressed root regeneration, compared to the root induction success in cotyledon explants that were immediately transferred into the dark (Fig. 4*e*, *f*).

Interaction between photooxidative stress pathways and early light exposure Assays of root regeneration percentages were performed on cotyledon explants of *non-photochemical quenching 1* (*npq1-2*), a mutant with reduced photoprotective zeaxanthin chloroplast pigment and reduced ROS quenching (Niyogi *et al.* 1998). Compared to wild-type L*er*-0 explants, *npq1-2* explants with reduced zeaxanthin showed an 86 and 92% reduction in root regeneration, respectively, under post-excision 1-d darkness or in continuous high-intensity light, and correspondingly low root mass per explant (Fig. 5*a*, *b*; Table 3).

Npq1 has been implicated in ROS quenching. To determine the importance of photooxidative stress during the initial day(s) after excision on the subsequent adventitious root regeneration, an experiment using chemical inhibitors (DTT, NF, PQ) and stimulators of ROS production was conducted. NF is an inhibitor of the beta-carotene pathway which targets phytoene desaturase (Bramley and Britton 1993; Jung 2004); DTT inhibits NPQ1 and reduces xanthophyll (Yamamoto and Kamite 1972); and PQ is an inhibitor of photosynthesis that promotes ROS accumulation (Dodge *et al.* 1970). Explants were incubated for the initial 48 h on medium that contained chemical

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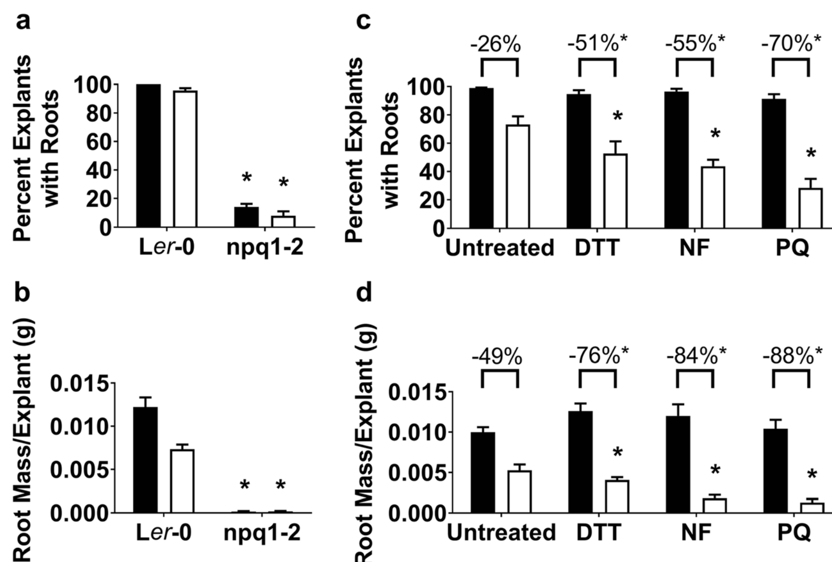

Fig. 5. Inhibition of root regeneration by suppression of photoprotective pigments or by accumulation of reactive oxygen species (ROS). (*a*, *b*) Effect of a mutant, *non-photochemical quenching 1* (*npq1-2*), on (*a*) percent explants with roots, and (*b*) root mass per explant, scored 4 wk. post excision (*Ler-0* control). Cotyledons were incubated in the dark (*black bars*) or in high-intensity light (~ 100 µmol m^{-2} s^{-1}; *white bars*) for 24 h immediately after excision, then exposed to 4 wk. of continuous high-intensity light. (*c*, *d*) Effects of chemical elicitors or inhibitors of the photooxidative stress pathway on (*c*) percent explants with roots, and (*d*) root mass per explant, scored 4 wk. after excision in ecotype L*er*-0 (*Untreated*). The inhibitors were dithiothreitol (*DTT*), Norflurazon (*NF*), and paraquat (*PQ*). Concentrations were selected based on the literature and pilot experiments (data not shown). The chemical agents in (*c*, *d*) were applied for a period of 48 h post excision simultaneously with the dark incubation (*black bars*) or high-intensity light (*white bars*), followed by exposure to 4 wk. continuous high light (~ 100 µmol m^{-2} s^{-1}) without inhibitors. In panels (*a*) and (*b*), an *asterisk* denotes that the rooting frequency was significantly different from the wild-type control under the same light treatment ($p < 0.05$). For *panels* (*c*) and (*d*), *percentage declines* in rooting under high light compared to darkness are provided above histograms. Asterisks next to *change in rooting percentages* denote statistically significant changes ($p < 0.05$) compared to the *untreated* control. *Asterisks* directly above histograms denote statistically significant declines compared to the respective dark-treated control ($p < 0.05$). See Table 3 for statistical analysis. *Error bars* represent the standard error of the mean (SEM). Each histogram is the mean of 5–18 replicate Petri dishes ($n = 26$ cotyledons per replicate).

inhibitors previously shown to block photoprotective pigment production or increase ROS, followed by a transfer to basal medium for the remainder of the experiment. Based on the previous results, the inhibitor treatments were executed in the 2-d dark and high-intensity light or continuous high-intensity light treatments. Compared to "mock" (solvent-only) treated L*er*-0 cotyledons, all three chemical treatments caused the explants to become significantly more hypersensitive to the negative effects of early light exposure, and the root masses as well as rooting percentages for these explants were significantly reduced (Fig. 5*c*, *d*). However, the chemical treatments had only minor effects on dark-treated explants (Fig. 5*c*, *d*; Table 3).

Role of phytochromes in the rooting responses of explants exposed to light immediately after excision PhyA and phyB are generally considered to be the dominant receptors for many photomorphogenic R/FR responses (Casal 2000). Assays were conducted for the adventitious root regeneration responses of mutants in these receptors: phyA (*phyA-201*; Nagatani *et al.* 1993) and phyB (*phyB-1*; Koornneef *et al.* 1980; Reed *et al.* 1993). Explants of the *hy1-1* mutant were also included, a mutant with disrupted chromophore biosynthesis for all five phytochromes (Koornneef *et al.* 1980; Muramoto *et al.* 1999; Fig. 6*a*, *b*). Of the mutants analyzed, only *phyA-201* explants showed significant differences in root regeneration capacity, compared to wild-type explants. Explants of this mutant regenerated fewer roots and had lower root mass per explant, and in total, fewer explants initiated adventitious roots in continuous light in comparison to 24-h dark-treated explants (Table 3).

Effect of early exposure to blue/UVA wavelengths on root regeneration Previous experiments evaluated the effects of light intensity on adventitious root induction. Next, the effect of the spectral composition of the light on root regeneration was tested (Fig. 6*c*–*h*). Evaluations were focused on blue/UVA light, because the light spectrum of the fluorescent bulbs used in this study display intense peaks for these wavelengths (F72T12CW/VHO, Osram Sylvania). First, the regeneration response of explants defective in four blue/UVA photoreceptors (*cry1cry2phot1phot2*, referred to as *quadblue*) was tested (Ohgishi *et al.* 2004). The mutant regenerated a similar number of adventitious roots as either parent under darkness, and was similar to one parent (L*er*-0) under light (Fig. 6*c*, *d*; Table 3).

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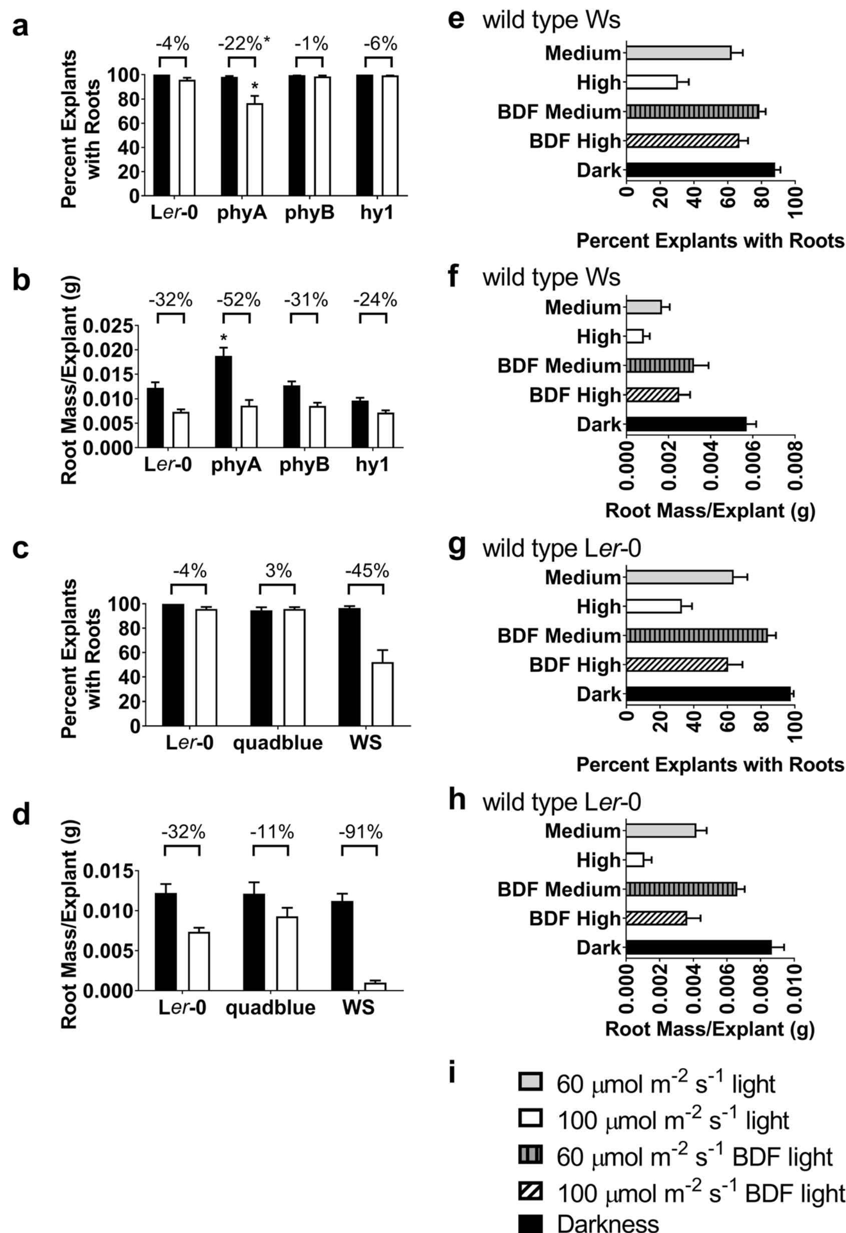

Fig. 6. Effects of early light exposure on adventitious root regeneration of photoreceptor mutants and of blue/UVA deficient light on rooting of *Arabidopsis thaliana* cotyledon explants. (*a*, *b*) Effect of light on red/far red photoreceptor mutants (*phyA*, *phyB*) and a chromophore mutant (*hy1*) on (*a*) the percent of explants with roots, and (*b*) the root fresh weight per explant, 4 wk. after excision. *Ler-0*: control. Cotyledons were either dark treated (*black bars*) or exposed to high-intensity light (~ 100 μmol m^{-2} s^{-1}; *white bars*) for 24 h post excision and then exposed to continuous high-intensity light for 4 wk. (*c*, *d*) The root regeneration percentage and root mass of the hybrid quadruple UVA/blue photoreceptor mutant *cry1cry2phot1phot2* (*quadblue*) compared to the control wild-type parents, ecotypes *Ler-0* and *WS*. (*e–h*) Effect of depleting blue/UVA wavelengths using a blue deficient filter (*BDF*) within the initial 5 d post excision on (*e*, *f*) root regeneration of wild-type ecotype *Ws* and (*g*, *h*) wild-type ecotype *Ler-0*. For (*e–h*), five light treatments, described in panel (*i*), were applied within the initial 5 d post excision, then explants were transferred to continuous high-intensity light (100 μmol m^{-2} s^{-1}). *BDF* blue deficient filter. For *panels* (*a–d*), *asterisks* beside *regeneration change percentages* denote statistically significant changes ($p < 0.05$) compared to the wild-type control (L*er*-0), while *asterisks* directly above *histograms* denote statistically significant declines compared to the respective dark-treated control ($p < 0.05$). *Error bars* represent standard error of the mean (SEM) of 5–18 replicate Petri dishes ($n = 26$ cotyledons per replicate). See Table 3 for statistical analysis.

As an alternate method to reduce blue/UVA wavelengths from fluorescent light, and to do so transiently only during the initial days after excision, an acetate filter was employed that had previously been shown to block almost all light below 450 nm (Fig. 6*e–h*). Explants of both ecotypes Ws and L*er*-0 showed significantly increased root regeneration when exposed to blue/UVA-deficient light (Fig. 6*e–h*; Table 3). For ecotype L*er*-0, it was calculated that blue/UVA wavelengths

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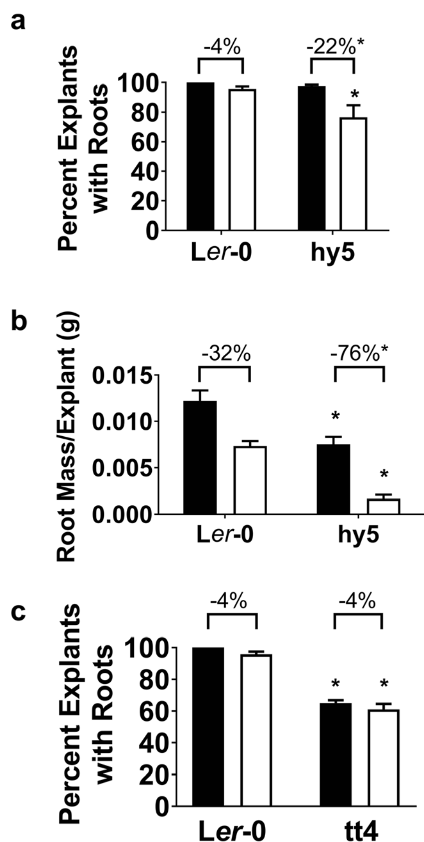

Fig. 7. Hypersensitivity to light and reduced root regeneration in the loss-of-function mutants ELONGATED HYPOCOTYL 5 (HY5) and its downstream target TT4 (chalcone synthase). (*a*, *b*) Effect of the *hy5-1* mutant allele on root regeneration. Cotyledons were exposed to the dark (*black bars*) or to high-intensity light (~ 100 μmol m^{-2} s^{-1}; *white bars*) for 24 h post excision and then treated with continuous high-intensity light for 4 wk. (*c*) Root formation in the *tt4* mutant. *Ler-0* control. *Asterisks* beside *regeneration change percentages* denote statistically significant changes ($p < 0.05$) compared to the wild-type control (L*er*-0), while *asterisks* directly above histograms denote statistically significant declines compared to the respective dark-treated control ($p < 0.05$). *Error bars* represent the standard error of the mean (SEM), and each histogram is the mean of 17 replicate Petri dishes ($n = 26$ cotyledons per replicate). See Table 3 for additional statistical values.

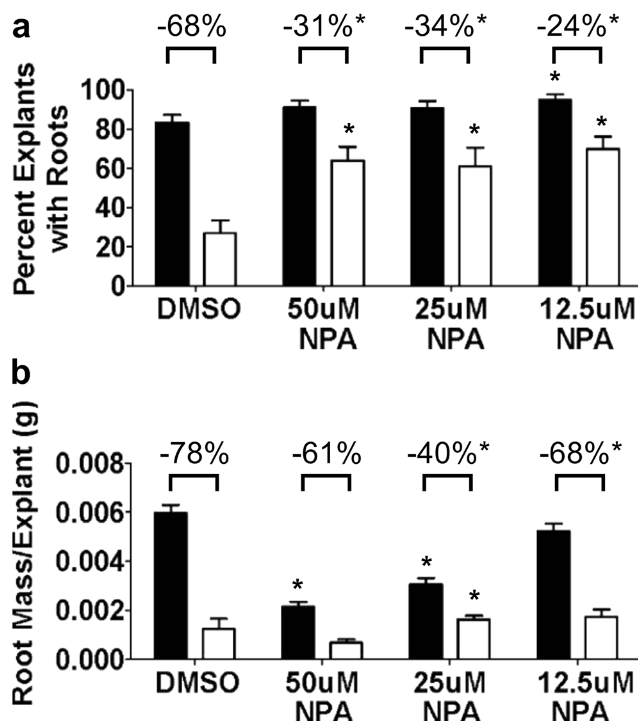

Fig. 8. Decreased high-intensity light inhibition of root regeneration with application of 1-N-naphthylphthalamic acid (NPA) during the initial 24 h after excision. Effect of *NPA* added during the initial 24 h post excision on root regeneration in wild-type L*er*-0 cotyledon explants on (*a*) root regeneration and (*b*) root mass per explant. Dimethyl sulfoxide (*DMSO*) is the non-NPA-treated, solvent control. Cotyledons were either dark treated (*black bars*) or exposed to high-intensity light (~ 100 μmol m^{-2} s^{-1}; white bars) for 24 h post excision, then exposed to continuous high light for 4 wk. *Asterisks* beside *regeneration change percentages* denote statistically significant changes ($p < 0.05$) compared to the *DMSO* control, while *asterisks* directly above histograms denote statistically significant declines compared to the respective dark-treated control ($p < 0.05$). *Error bars* represent the standard error of the mean (SEM) of 10 replicate Petri dishes ($n = 26$ cotyledons per replicate). See Table 3 for additional statistical values.

contributed 28 and 21% of the decline in root regeneration, at these respective light levels (Fig. 6*g*).

Interaction between HY5 and exposure to light immediately after excision The hypothesis that light-inducible transcription factor HY5 is part of the signaling pathway involved in light inhibition of root regeneration was tested. Compared to wild-type explants, cotyledon explants of the loss-of-function *hy5-1* allele (Osterlund *et al.* 2000) were hypersensitive and formed even fewer adventitious roots when exposed to high-intensity light early after excision, in comparison to explants exposed immediately to darkness (Table 3). Whereas 98% of wild-type explants regenerated roots following 24 h of early light exposure, only 77% of *hy5* explants regenerated roots (Fig. 7*a*, *b*).

Table 1. List of *Arabidopsis thaliana* wild-type ecotypes used in this study

Ecotype	Accession	Source
Initial light-dark transfer experiments		
Columbia-0 (Col-0)	WT-2	Lehle
Dijon G (Dij-G)	WT-10	Lehle
Nossen-0 (No-0)	WT-9	Lehle
Estland 1 (Est-1)	WT-6A	Lehle
Landsberg *erecta*-0 (L*er*-0)	WT-4	Lehle
Mutant analysis		
Wassilewskija 2 (Ws2)	CS2360/CS22659	ABRC
Landsberg *erecta*-0 (L*er*-0)	CS20	ABRC
C24 (C24)	CS906	ABRC

WT wild type, *ABRC A. thaliana* Biological Resource Center, *CS* center stock

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Table 2. List of *Arabidopsis thaliana* mutant lines used in this study

Mutant	Accession	Complete name	Ecotype	Description	Reference
hy1-1	CS67	Long hypocotyl mutant 1	L*er*-0 (CS20)	Mutation in heme oxygenase; disrupts chromophore biosynthesis for all phytochromes	Koornneef *et al.* 1980
hy5-1	CS71	Long hypocotyl mutant 5	L*er*-0 (CS20)	Mutation in downstream transcription factor required for photomorphogenesis	Chattopadhyay *et al.* 1998
npq1-2	CS3771	Non-photochemical quenching 1	L*er*-0 (CS20)	Mutation in violaxanthin de-epoxidase; reduced zeaxanthin pigment	Niyogi *et al.* 1998
phyA-201	CS6219	Phytochrome A	L*er*-0 (CS20)	Mutation in phyA photoreceptor (R/FR light)	Nagatani *et al.* 1993
phyB-1	CS6211	Phytochrome B	L*er*-0 (CS20)	Mutation in phyB photoreceptor (R/FR light)	Koornneef *et al.* 1980; Reed *et al.* 1993
quadblue	Line 210 (Sakai Lab)	Cryptochrome 1 Cryptochrome 2 Phototropin 1 Phototropin 2	Mixed L*er*-0 × Ws2 (CS20 × and CS2360/CS22659)	Mutations in all four blue/UVA light photoreceptors	Ohgishi *et al.* 2004
tt4-1	CS85	Transparent testa 4	L*er*-0 (CS20)	Mutation in chalcone synthase; reduced flavonoids and anthocyanins	Ang *et al.* 1998

CS center stock, *Ler* Landsberg *erecta*

Therefore, HY5 could mitigate early light inhibition of root regeneration in *A. thaliana* cotyledon explants.

Interaction between chalcone synthase and early light exposure For the root regeneration responses of a loss-of-function allele of *TRANSPARENT TESTA 4* (*TT4*) (Ang *et al.* 1998; Lee *et al.* 2007), a gene which encodes chalcone synthase, a rate-limiting step in flavonoid and anthocyanin biosynthesis (Shirley *et al.* 1995) was tested. Mutant *tt4* explants were hypersensitive to both early and later light exposure, showing reduced rates of root regeneration compared to the wild type (Fig. 7*c*). These results suggested that flavonoid and/or anthocyanin accumulation might play a positive role during adventitious root regeneration in *A. thaliana*.

Importance of polar auxin transport and early light exposure on inhibition of adventitious root formation The relationship between auxin transport and the negative impact of early light exposure on root regeneration was investigated. Explants were transiently exposed to a non-competitive inhibitor of auxin efflux, NPA (Petrasek *et al.* 2006), which was added into CIM medium. Afterwards, the explants were moved to inhibitor-free CIM medium for the remaining duration of the experiment. Significantly improved long-term root regeneration was observed when NPA was applied for 24 h to explants simultaneously exposed to high light (*white bars* in Fig. 8; Table 3). No significant improvement of the rooting success was observed in the dark-exposed explants. In fact, up to 56% of the inhibition by light on root regeneration could be alleviated by exposure to NPA (Fig. 8*a*). Therefore, the accumulation of a strong auxin, such as 2,4-D, was sufficient to counteract the negative effects of light-induced root formation inhibition in *A. thaliana* cotyledon explants.

Discussion

In an earlier study, it was reported that exposure of *A. thaliana* cotyledon explants to light immediately after explant excision inhibited shoot regeneration and that the underlying mechanisms involved ROS, phytochrome A, blue/UVA, HY5, ethylene, and auxin transport (Nameth *et al.* 2013). This present paper reports the parallel concomitant root regeneration data. An important caveat was that the root organogenesis reported here occurred after an initial high dose of auxin (CIM medium), in a cytokinin-containing medium (OM) more optimal for shoot regeneration. Nevertheless, efficient root regeneration was observed under these conditions. Inadvertent or variable light exposure on the first day after explant excision may thus contribute to the reported variability in regeneration responses in otherwise apparently identical tissue cultures. To identify modulators of the early light-sensitivity interval, genetic mutants and pharmacological agents were utilized, which were expected to be involved in differential effects on root regeneration in the light, compared to darkness, immediately after excision. These pathways may comprise part of a complex physiological and genetic network that acts early after excision to modulate how light regulates adventitious root regeneration in *A. thaliana* cotyledon explants; furthermore, wounding is itself well known to induce flavonoid accumulation (Likic and Rusak 2014).

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Table 3. Summary of root regeneration of each *Arabidopsis thaliana* cotyledon explant, *mutant*, and treatment comparison presented in this study

$Name_a$	$Name_b$	Percent explants with roots							Root mass (g)/explant						
		n_a	$Mean_a$	n_b	$Mean_b$	*p* value	*U*-stat	*U'*	n_a	$Mean_a$	n_b	$Mean_b$	*p* value	*U*-stat	*U'*
Mutants															
CS20 D	CS20 H	22	100.0	23	95.8	0.1075	184	322	17	0.0122	18	0.0073	0.0008	51	255
phyA D	*phyA* H	18	98.1	18	76.7	0.0005	51	273	18	0.0188	18	0.0086	< 0.0001	33	291
phyB D	*phyB* H	23	99.6	23	98.5	0.5734	239.5	289.5	18	0.0127	18	0.0085	0.0004	49	275
hy1-1 D	*hy1-1* H	20	100.0	19	99.4	0.7441	178.5	201.5	15	0.0097	14	0.0072	0.0032	39	171
hy5 D	*hy5* H	10	97.6	10	76.5	0.3591	37.5	62.5	5	0.0075	5	0.0017	0.0079	0	25
npq D	*npq* H	10	14.0	9	7.8	0.0788	23	67	10	0.0002	9	0.0002	0.8421	42	48
tt4 D	*tt4* H	5	64.0	5	60.6	0.4015	8	17	N/A	N/A	N/A	N/A	N/A	N/A	N/A
quad D	quad H	20	94.6	20	95.8	0.6489	183	217	18	0.0121	18	0.0077	0.0328	94	230
WS2 D	WS2 H	10	96.6	10	52.2	0.0004	3	97	5	0.0112	5	0.0010	0.0079	0	25
CS20 D	*phyA* D	22	100.0	18	98.1	0.1774	149.5	246.5	17	0.0122	18	0.0188	0.0039	65	241
CS20 D	*phyB* D	22	100.0	23	99.6	0.7911	241.5	264.5	17	0.0122	18	0.0127	0.5199	133	173
CS20 D	*hy1-1* D	22	100.0	20	99.9	0.9893	219	221	17	0.0122	15	0.0097	0.0894	82	173
CS20 D	*hy5* D	22	100.0	10	97.6	0.0325	57.5	162.5	17	0.0122	5	0.0075	0.0114	11	74
CS20 D	*npq* D	22	100.0	10	14.0	< 0.0001	0	220	17	0.0122	10	0.0002	< 0.0001	0	170
CS20 D	*tt4* D	22	100.0	5	64.0	< 0.0001	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CS20 D	quad D	22	100.0	20	94.6	0.2192	172.5	172.5	17	0.0122	18	0.0121	0.6323	138	168
CS20 D	WS2 D	22	100.0	10	96.6	0.0945	69	69	17	0.0122	5	0.0112	0.9396	41	44
quad D	WS2 D	20	94.6	10	96.6	0.7549	92.5	92.5	18	0.0121	5	0.0112	0.9713	44	46
quad H	WS2 H	20	95.8	10	52.2	< 0.0001	8	8	18	0.0077	5	0.0010	0.0243	15	75
CS20 H	WS2 H	23	95.8	10	52.2	< 0.0001	11	11	18	0.0073	5	0.0010	< 0.0001	0	90
CS20 H	quad H	23	95.8	20	95.8	0.6919	213.5	213.5	18	0.0073	18	0.0077	0.8371	155	169
CS20 H	*tt4* H	23	95.8	5	60.6	0.0009	1.5	113.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CS20 H	*npq* H	23	95.8	9	7.8	< 0.0001	0	207	18	0.0073	9	0.0002	< 0.0001	0	162
CS20 H	*hy5* H	23	95.8	10	76.5	0.1189	75	155	18	0.0073	5	0.0017	< 0.0001	0	90
CS20 H	*hy1-1* H	23	95.8	19	99.4	0.2231	171.5	265.5	18	0.0073	14	0.0072	0.9394	123.5	128.5
CS20 H	*phyB* H	23	95.8	23	98.5	0.3754	225	304	18	0.0073	18	0.0085	0.3039	129	195
CS20 H	*phyA* H	23	95.8	18	76.7	0.0024	91	323	18	0.0073	18	0.0086	0.7397	151	173
Blue deficient filter															
WS2 5DD	L*er* 5DD	7	88.1	7	97.7	0.0522	9	40	7	0.0057	7	0.0087	0.0041	3	46
WS Bd100	L*er* Bd100	8	67.0	7	60.5	0.3247	19	37	8	0.0025	7	0.0036	0.281	18	38
WS Bd60	L*er* Bd60	8	78.9	7	84.0	0.224	17	39	8	0.0032	7	0.0066	0.0059	5	51
WS H100	L*er* H100	7	30.5	7	33.0	0.949	23.5	25.5	7	0.0008	7	0.0011	0.8048	22	27
WS H60	L*er* H60	7	62.4	7	63.3	0.8982	23	26	7	0.0017	7	0.0042	0.007	4	45
WS2 5DD	WS Bd100	7	88.1	8	67.0	0.0093	6	50	7	0.0057	8	0.0025	0.0022	3	53
WS2 5DD	WS Bd60	7	88.1	8	78.9	0.1324	14.5	41.5	7	0.0057	8	0.0032	0.0205	8	48
WS2 5DD	WS H100	7	88.1	7	30.5	0.0006	0	49	7	0.0057	7	0.0008	0.0006	0	49
WS2 5DD	WS H60	7	88.1	7	62.4	0.0111	5	44	7	0.0057	7	0.0017	0.0006	0	49
WS Bd100	WS Bd60	8	67.0	8	78.9	0.1148	16.5	47.5	8	0.0025	8	0.0032	0.5737	26	38
WS H100	WS H60	7	30.5	7	62.4	0.0105	4	45	7	0.0008	7	0.0017	0.0973	11	38
WS Bd100	WS H100	8	67.0	7	30.5	0.0018	0.5	55.5	8	0.0025	7	0.0008	0.0289	9	47
WS Bd60	WS H60	8	78.9	7	62.4	0.0558	11	45	8	0.0032	7	0.0017	0.1206	14	42
WS Bd60	WS H100	8	78.9	7	30.5	0.0003	0	56	8	0.0032	7	0.0008	0.0093	6	50
WS Bd100	WS H60	8	67.0	7	62.4	0.5236	22	34	8	0.0025	7	0.0017	0.3357	19	37
L*er* 5DD	L*er* Bd100	7	97.7	7	60.5	0.0125	4.5	44.5	7	0.0087	7	0.0036	0.0012	1	48
L*er* 5DD	L*er* Bd60	7	97.7	7	84.0	0.0111	5	44	7	0.0087	7	0.0066	0.0262	7	42
L*er* 5DD	L*er* H100	7	97.7	7	33.0	0.0006	0	49	7	0.0087	7	0.0011	0.0006	0	49
L*er* 5DD	L*er* H60	7	97.7	7	63.7	0.0012	1	48	7	0.0087	7	0.0042	0.0012	1	48
L*er* Bd100	L*er* Bd60	7	60.5	7	84.0	0.053	9	40	7	0.0036	7	0.0066	0.0111	5	44
L*er* H100	L*er* H60	7	33.0	7	63.7	0.035	7.5	41.5	7	0.0011	7	0.0042	0.0041	3	46
L*er* Bd100	L*er* H100	7	60.5	7	33.0	0.0175	6	43	7	0.0036	7	0.0011	0.0379	8	41
L*er* Bd60	L*er* H60	7	84.0	7	63.7	0.053	9	40	7	0.0066	7	0.0042	0.0175	6	43
L*er* Bd60	L*er* H100	7	84.0	7	33.0	0.0027	0.5	48.5	7	0.0066	7	0.0011	0.0006	0	49
L*er* Bd100	L*er* H60	7	60.5	7	63.7	0.7104	21	28	7	0.0036	7	0.0042	0.8048	22	27
Chemicals															
L*er* D	L*er* H	17	98.9	16	73.2	0.0002	31.5	240.5	17	0.0100	16	0.0053	< 0.0001	29	243
DTT D	DTT H	10	94.7	9	52.7	< 0.0001	2	88	10	0.0124	9	0.0028	< 0.0001	0	90

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Table 3. (continued)

Name$_a$	Name$_b$	Percent explants with roots							Root mass (g)/explant						
		n_a	Mean$_a$	n_b	Mean$_b$	*p* value	*U*-stat	*U'*	n_a	Mean$_a$	n_b	Mean$_b$	*p* value	*U*-stat	*U'*
NF D	NF H	10	96.6	10	43.7	< 0.0001	0	100	10	0.0120	10	0.0018	< 0.0001	1	99
PQ D	PQ H	8	91.4	8	28.4	0.0002	0	64	8	0.0104	8	0.0013	0.0002	0	64
NPA50 μM D	NPA50 μM	10	91.1	10	63.9	0.0015	10	90	10	0.0021	10	0.0007	0.0001	4	96
NPA25 μM D	NPA25 μM	10	90.8	10	60.8	0.019	18.5	81.5	10	0.0030	10	0.0016	0.0003	6	94
NPA12.5μM D	NPA12.5 μM	10	94.8	10	69.8	0.0051	12.5	87.5	10	0.0052	10	0.0017	< 0.0001	0	100
DMSO D	DMSO H	5	83.3	5	26.8	0.0079	0	25	5	0.0060	5	0.0012	0.0079	0	25
L*er* D	DTT D	17	98.9	10	94.7	0.2196	60.5	109.5	17	0.0100	10	0.0124	0.1035	52	118
L*er* D	NF D	17	98.9	10	96.9	0.4251	69	101	17	0.0100	10	0.0120	0.2231	60	110
L*er* D	PQ D	17	98.9	8	91.4	0.0373	32	104	17	0.0100	8	0.0104	0.8867	65	71
L*er* D	DMSO D	17	98.9	5	83.3	0.0015	1.5	83.5	17	0.0100	5	0.0060	0.0009	4	81
L*er* H	DTT H	16	73.2	9	52.7	0.0741	40	104	16	0.0053	9	0.0028	0.0568	38	106
L*er* H	NF H	16	73.2	10	43.7	0.0032	23.5	136.5	16	0.0053	10	0.0018	0.0028	25	135
L*er* H	PQ H	16	73.2	8	28.4	0.0008	8.5	119.5	16	0.0053	8	0.0013	0.0007	12	116
L*er* H	DMSO H	16	73.2	5	26.8	0.0012	4	76	16	0.0053	5	0.0012	0.005	5.5	74.5
DMSO D	NPA50 μM	5	83.3	10	91.1	0.1292	12	38	5	0.0060	10	0.0021	0.0007	0	50
DMSO D	NPA25 μM	5	83.3	10	90.8	0.2544	15	35	5	0.0060	10	0.0030	0.0007	0	50
DMSO D	NPA12.5 μM	5	83.3	10	94.8	0.008	4	46	5	0.0060	10	0.0052	0.2065	14	36
DMSO H	NPA50 μM	5	26.8	10	63.9	0.0027	2	48	5	0.0012	10	0.0007	0.371	17	33
DMSO H	NPA25 μM	5	26.8	10	60.8	0.0753	10	40	5	0.0012	10	0.0016	0.0992	11	39
DMSO H	NPA12.5 μM	5	26.8	10	69.8	0.0007	0	50	5	0.0012	10	0.0017	0.2544	15	35
Percent decline															
CS20	*phyA*	22	4.0	18	22.0	0.0192	111.5	284.5	17	31.8	18	51.8	0.1332	107	199
CS20	*phyB*	22	4.0	23	1.0	0.5589	227.5	278.5	17	31.8	18	30.7	0.4	127	179
CS20	*hy1*	22	4.0	20	5.6	0.6131	200	240	17	31.8	14	24.1	0.2145	87	151
CS20	*hy5*	22	4.0	10	22.1	0.0856	67.5	152.5	17	31.8	5	76.1	0.0032	7	78
CS20	quadblue	22	4.0	20	− 2.8	0.1977	169	271	17	31.8	15	11.3	0.0695	79	176
CS20	WS	22	4.0	10	45.3	0.0005	29	191	17	31.8	5	90.7	< 0.0001	0	85
Ws	quadblue	10	45.3	20	− 2.8	0.0002	20	180	5	90.7	15	11.3	0.0001	0	75
CS20	*npq*	22	4.0	9	26.7	0.2306	71	127	17	31.8	3	− 440.0	0.0018	0	51
L*er*	DTT	16	26.2	10	51.4	0.0356	40	120	16	49.2	9	76.0	0.0053	24	120
L*er*	NF	16	26.2	10	54.9	0.0022	24	136	16	49.2	10	84.8	0.0001	12	148
L*er*	PQ	16	26.2	8	70.1	0.0006	7.5	120.5	16	49.2	8	88.1	0.0002	8	120
DMSO	NPA50 μM	5	68.1	10	30.9	0.008	4	46	5	78.2	10	61.1	0.5135	19	31
DMSO	NPA25 μM	5	68.1	10	33.7	0.0553	9	41	5	78.2	10	40.4	0.0127	5	45
DMSO	NPA12.5 μM	5	68.1	10	26.6	0.0047	3	47	5	78.2	10	67.9	0.0553	9	41
WS 100	WS BDF10	7	64.7	7	25.5	0.0088	3.5	45.5	7	85.2	7	58.8	0.0379	8	41
WS 60	WS BDF60	7	28.9	7	10.2	0.0844	10.5	38.5	7	69.9	7	51.4	0.2086	14	35
L*er* 100	L*er* BDF100	7	66.6	7	38.6	0.0175	6	43	7	87.8	7	57.0	0.0262	7	42
L*er* 60	L*er* BDF60	7	34.9	7	13.6	0.0379	8	41	7	51.1	7	20.6	0.0111	5	44
L*er*	*tt4*	22	4.0	5	4.3	0.2845	37	73	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Each *row* lists the two *treatments and genotypes* that are compared (*Name$_A$* vs. *Name$_B$*), the sample size (n_a or n_b = number of replicates consisting of 26 cotyledons per replicate), *mean percentage* of explants successfully forming adventitious roots, and whether the comparison between *columns A* and *B* is statistically significant based on Mann-Whitney tests, for which the corresponding *p value*, *U-statistic*, and *U′* value are indicated

CS center stock, *D* dark, *H* high light intensity, *phy phytochrome*, *hy elongated hypocotyl*, *npq non-photochemical quenching*, *tt* transparent testa, *quad*, *quadblue* quadruple UVA/blue photoreceptor mutant *cry1cry2phot1phot2*, *WS* Landsberg *erecta*, *Bd* blue/ultraviolet-A deficient, *DTT* dithiothreitol, *NF* Norflurazon, *PQ* paraquat, *DMSO* dimethyl sulfoxide, *NPA* 1-N-naphthylphthalamic acid, *L* low light intensity

The importance of the initial hours after excision Earlier studies, using transfers between different hormone treatments, showed that the first d after excision are important, because this is when tissues become competent to regenerate into organs (Christianson and Warnick 1983; Valvekens *et al.* 1988; Sugiyama 1999). Adventitious roots were shown to initiate as early as 12–48 h following auxin-mediated induction from intact hypocotyls (Byrne *et al.* 1975). A more recent transcriptome study (Sena *et al.* 2009) showed that dividing cells adjacent to a root-tip excision zone can regain stem cell identity starting within hours after excision. The results presented here supported the importance of the initial h post excision for adventitious root regeneration, and demonstrated that light modulates this early-acting organogenetic pathway.

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Cotyledons possess pericycle-like, stem cell-like cells with organogenic potential, which can give rise to callus and regenerate organs (Atta *et al.* 2009; Sugimoto *et al.* 2010; Sugimoto *et al.* 2011). It is useful to understand how environmental factors such as light impact pericycle cell identity and developmental progression in cotyledon explants used for regeneration experiments.

Though a similar trend was observed across ecotypes, there was also considerable variation between ecotypes regarding their sensitivity to early light exposure, with respect to root regeneration success (Figs. 2 and 3). The reason for ecotype variation is not known and would be interesting to investigate in future studies, for example, by making genetic crosses between the ecotypes and discovering natural variation alleles.

Another observation from this study was that light sometimes differentially affected the percentage of explants initiating roots, compared to total regenerated root mass (*e.g.*, Fig. 5). Understanding such differences would require considerable future investigation, as a greater root biomass could be the result of more roots initiated per explant, more branching, or longer roots. Distinguishing between these causes would be a huge undertaking, and without this information, it would be speculative to suggest an explanation.

The role of photooxidative stress and/or signaling in root regeneration In agreement with the photooxidative stress data reported here, antioxidants have previously been shown to improve *in vitro* plant regeneration (Dan 2008). Seedling cuttings of tomato showed higher root regeneration when treated with ascorbate or similar antioxidants (Tyburski *et al.* 2006). Moreover, an endogenous increase in antioxidant activity has also been observed during *in vitro Pinus strobus* L. (pine) rooting (Fei *et al.* 2016). In many species, hydrogen peroxide (H_2O_2) has been shown to mediate auxin-induced adventitious root formation (Li *et al.* 2009; Libik-Konieczny *et al.* 2015; Takac *et al.* 2016). Subsequently, H_2O_2 was shown to be part of a complex signaling network regulating adventitious rooting in mung bean, involving nitric oxide (NO), calcium, cyclic guanosine monophosphate (cGMP), and mitogen-activated protein kinase (MAPK) (Li and Xue 2010). The data presented here add to the previously demonstrated importance of photooxidative stress or signaling to root regeneration in the initial hours after explant excision. This report also found indications that the early inhibitory effect of light on root regeneration was modulated by photooxidative signaling components in *A. thaliana.*

In studies using intact mung bean seedlings, a relationship between photooxidative stress, auxin transport, and root regeneration was demonstrated. There, the auxin transport inhibitor, TIBA (2,3,5-triiodobenzoic acid), was shown to inhibit adventitious root regeneration, but its effects could be reversed by the addition of exogenous H_2O_2 (Li *et al.* 2009). An independent study using intact *A. thaliana* seedlings, as well as roots and root segments, showed that H_2O_2 acts as a signal to promote adventitious roots, perhaps by enhancing auxin-dependent reactivation of formative cell divisions (Pasternak *et al.* 2005). Several studies have indeed confirmed that ROS can act as signaling molecules, potentially by creating chemical gradients that interact with phytohormones (Tognetti *et al.* 2012; Choudhury *et al.* 2013; del Rio 2015). Reactive oxygen species were shown to modulate auxin gradients, to relocate auxin export proteins (*i.e.*, PIN-FORMED (PIN) proteins), and to modify auxin conjugation (Tognetti *et al.* 2012). Results presented here show that dark regulation of root regeneration can be modulated by addition of chemicals known to induce ROS accumulation or block auxin efflux (Figs. 5 and 8). A hypothesis may be that ROS and auxin pathways interact directly with one another to modulate how light regulates root regeneration.

The role of blue/UVA wavelengths The observation that blue light inhibited root regeneration (Fig. 6) might be surprising, given that blue/UVA light has previously been shown to promote photoprotective responses in intact seedlings (Kubasek *et al.* 1992; Ahmad *et al.* 1995). Indeed, blue light was shown to promote root regeneration from *Vitis* spp. nodal explants (Chee 1986) and to increase root growth in *Fragaria* spp. explants, compared to white fluorescent lamps (Hung *et al.* 2015). Numerous other instances indicate an either stimulatory or inhibitory effect of blue light on root regeneration from stem cuttings. For example, increased fluorescent light and blue light strongly inhibited root formation in *Prunus* cuttings (Fuernkranz *et al.* 1990; Rossi *et al.* 1993) and from birch (*Betula* sp.) shoot tip cultures (Pinker *et al.* 1989). Although the root induction percentage remained high, blue light greatly reduced the root number and length, compared to white light in explants of *Scrophularia takesimensis* Nakai (Jeong and Sivanesan 2015). Since blue/UVA wavelengths are high in energy, they may cause damage to cells critical to root regeneration in the initial hours after explant excision. Moreover, blue light has been shown to regulate polar auxin transport in intact plants (Jensen *et al.* 1998; Canamero *et al.* 2006; Christie and Murphy 2013) and in roots (Zhang *et al.* 2013; Mo *et al.* 2015).

The role of transcription factor HY5 The results from this present study, showing that the *hy5* mutant was hypersensitive to light inhibition of root regeneration (Fig. 7*a*, *b*), was consistent with earlier reports, which demonstrated that transcription factor HY5 is necessary for normal root development (Oyama *et al.* 1997; Cluis *et al.* 2004). ELONGATED HYPOCOTYL 5 mediates downstream signaling from R/FR and blue/UVA photoreceptors (Gyula *et al.* 2003; Zheng *et al.* 2013) and has been implicated in coordinating light and hormone signaling

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(Cluis *et al.* 2004; Lee *et al.* 2007). In darkness, the E3-ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) accumulates and targets HY5 for degradation (Osterlund *et al.* 2000; Xu *et al.* 2016b). Light onset destabilizes COP1 protein to permit HY5 protein accumulation. ELONGATED HYPOCOTYL 5 then mediates a multitude of photomorphogenic responses by binding to G-box motifs in light-activated promoters. The regulator HY5 alters the expression of at least 1100 genes, and binding sites have been identified in over 9000 regulatory regions (representing 30% of the *A. thaliana* genome), including the promoters of key mediators of auxin signal transduction and biosynthesis (Lee *et al.* 2007; Zhang *et al.* 2011), as well as the promoter of chalcone synthase (*TT4*) (Ang *et al.* 1998; Lee *et al.* 2007). In *A. thaliana*, HY5 also regulates anthocyanin and flavanol biosynthesis (Stracke *et al.* 2009; Shin *et al.* 2013), and the tomato ortholog of *HY5*, *LeHY5*, has been shown to upregulate carotenoid biosynthesis in tomato fruits (Liu *et al.* 2004). Thus, an intriguing possibility exists that HY5 signals during the initial post-excision period to regulate root regeneration by coordinating the various modifiers: photoprotective carotenoids, flavonoids, anthocyanins, phyA, blue/UVA light, and polar auxin transport.

A minor role for far red light and influence of phyA-related components The modest effect of a phyA mutant on root regeneration (Fig. 6*a*) appeared to suggest that FR light, which is primarily sensed by phyA (Casal 2000), played only a minor role in regulating root regeneration from *A. thaliana* cotyledon explants. Caution must be used in interpreting this result, however, as the fluorescent light source used in this study was low in FR wavelengths (F72T12CW/VHO, Osram Sylvania), which may have artificially reduced the impact of the *phyA* mutant allele. Regardless, this report did suggest that normal phyA signaling immediately after excision partially mitigated early light inhibition of root regeneration. Since phyA is upstream of HY5 and TT4 is a downstream target of HY5 (Ang *et al.* 1998; Lee *et al.* 2007), one possibility was that phyA suppressed light inhibition of root regeneration by stimulating synthesis of anthocyanin, a pigment that shields plant cells against high-energy blue-green wavelengths (Gould *et al.* 2002). Phytochrome A may also be acting through HY5, which regulates PIN auxin efflux carriers and auxin metabolism (Lee *et al.* 2007; Laxmi *et al.* 2008; Zhang *et al.* 2011). Indeed, phyA signaling has been shown to regulate polar auxin transport in intact and segmented seedlings (Jensen *et al.* 1998; Canamero *et al.* 2006; Liu *et al.* 2011).

Interactions between polar auxin transport, flavonoid pigments, and light The most striking result of this study was that treatment with the auxin efflux inhibitor NPA during the initial 24 h post excision could revert ~ 50% of the negative effect of light exposure on adventitious root initiation (Fig. 8). Unlike genetic mutants of auxin, the application of the chemical NPA provided the opportunity to transiently modify auxin transport. Previous studies have demonstrated an interaction between auxin and light in plant tissue culture. For example, in mung bean stem cuttings, addition of auxin was found to be very beneficial to root regeneration in the light and less beneficial in darkness (Jarvis and Ali 1985). In callus-derived plantlets of *Prunus* GF 655/2, rooting required NAA in darkness, but not in light (Rossi *et al.* 1993). A study using tomato hypocotyl explants showed that addition of the polar transport inhibitor TIBA suppressed adventitious root regeneration, if applied for 24 h during the first 48 h after excision, but the quantity of TIBA required for this repression was ~ 10-fold higher in continuous white light compared to darkness (Tyburski and Tretyn 2004). Both TIBA and NPA are auxin efflux transport inhibitors, and hence, this result may contradict the finding that NPA increased the percentage of root regeneration in *Arabidopsis* under high light reported here, suggesting that species or tissue explant sources may be critical for this interaction.

It was also observed that *tt4* mutant explants, deficient in flavonoid accumulation, showed overall lower rates of root regeneration (Fig. 7*c*). Studies involving *tt4* mutants and exogenous application of flavonoid (quercetin) have previously been shown to inhibit adventitious root regeneration in *A. thaliana* excised leaves (Brown *et al.* 2001; Correa *et al.* 2012). Flavonoids inhibit auxin transport (Besseau *et al.* 2007), and auxin transport is enhanced in *tt4* mutant plants (Brown *et al.* 2001; Li and Zhachgo 2013). Therefore, the *tt4* data presented here may demonstrate an interaction between flavonoids, auxin efflux transport, and light, in the regulation of root regeneration. It is noteworthy to mention that in previous *Arabidopsis* reports (Brown *et al.* 2001; Correa *et al.* 2012), flavonoids showed contradictory effects on adventitious root regeneration, which has recently been speculated to involve light (Correa *et al.* 2012). In *Sorghum bicolor* (L.) Moench (sorghum), exogenous flavonoids were shown to inhibit root growth (Franco *et al.* 2015). Similarly, in *Medicago truncatula* Gaertn., flavonoid deficiency was shown not to inhibit lateral root formation (Wasson *et al.* 2009). Furthermore, it is well known that flavonoids induce nodule formation in legumes (Taylor and Grotewold 2005). In *A. thaliana*, light induces flavonoid accumulation in roots (Hemm *et al.* 2004). In the present study, there were no differences identified in root regeneration from *tt4* explants exposed to early light, compared to early darkness (Fig. 7*c*). More research will be required to understand these results, including an examination of the cross-talk between flavonoids, auxin transport, and light at specific time points in early root regeneration.

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ELONGATED HYPOCOTYL 5 may be critical in a mechanism by which a reduction in auxin transport might suppress the inhibitory effects of light on root regeneration. Chromatin immunoprecipitation (ChiP) experiments have revealed HY5 promoter-binding sites upstream of genes encoding auxin efflux carriers, PIN1, a predicted PIN3, and PINOID (PID) (Lee *et al.* 2007), which has been further implicated in the polar localization of PIN1 proteins (Friml *et al.* 2004). Localization of PIN2 to the plasma membrane was also shown to require HY5, which was degraded during darkness in the vacuole (Laxmi *et al.* 2008). If the collective effect of darkness on auxin efflux transporters is a reduced polar auxin transport, the observed beneficial NPA treatment in the light (Fig. 7*b*, *c*) may be phenocopying dark-mediated degradation of HY5. The above observations may indicate a potential mechanism by which light-induced auxin transport could inhibit root regeneration. Specifically, since auxin maxima promote root meristem initiation, it is possible that increased light increases auxin efflux, thereby reducing the auxin maxima and hence reducing root regeneration. Detailed understanding of how light and polar auxin transport interact to regulate root regeneration will require further experiments.

Conclusions

A. thaliana explants were hypersensitive to light during the initial hours after explant excision, with respect to root regeneration. This hypersensitivity was based on a multifaceted network, involving different wavelengths of light, photoreceptor signaling, ROS, photoprotective pigments, and auxin. The complexity and timing of this signaling network may help explain some of the variation observed in root regeneration responses between replicate experiments, treatments, varieties, and species. Specifically, allelic variation at genes associated with this complex signaling network, such as at genetic regulatory regions (*e.g.*, promoters and enhancers), may underlie the variation observed between genotypes with respect to the impact of light on rooting. Similarly, differences in gene expression between tissues within a genotype may explain the variation observed between explants.

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References

Ahmad M, Lin CT, Cashmore AR (1995) Mutations throughout an Arabidopsis blue light photoreceptor impair blue light responsive anthocyanin accumulation and inhibition of hypocotyl elongation. Plant J 8(5):653–658. https://doi.org/10.1046/j.1365-313X.1995.08050653.x

Ang LH, Chattopadhyay S, Wei N, Oyama T, Okada K, Batschauer A, Deng XW (1998) Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development. Mol Cell 1(2):213–222. https://doi.org/10.1016/S1097-2765(00)80022-2

Atta R, Laurens L, Boucheron-Dubuisson E, Guivarc'h A, Carnero E, Giraudat-Pautot V, Rech P, Chriqui D (2009) Pluripotency of Arabidopsis xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown *in vitro*. Plant J 57(4):626–644. https://doi.org/10.1111/j.1365-313X.2008.03715.x

Baldwin IT, Kessler A, Halitschke R (2002) Volatile signaling in plant-plant-herbivore interactions: what is real? Curr Opin Plant Biol 13: 351–354

Besseau S, Hoffmann L, Geoffroy P, Lapierre C, Pollet B, Legrand M (2007) Flavonoid accumulation in Arabidopsis repressed in lignin synthesis affects auxin transport and plant growth. Plant Cell 19(1): 148–162. https://doi.org/10.1105/tpc.106.044495

Bramley PM, Britton G (1993) Inhibition of carotenoid biosynthesis. In: Young AJ, Britton G (eds) Carotenoids in photosynthesis. Chapman and Hall, London, pp 127–159. https://doi.org/10.1007/978-94-011-2124-8_5

Brown DE, Rashotte AM, Murphy AS, Normanly J, Tague BW, Peer WA, Taiz L, Muday GK (2001) Flavonoids act as negative regulators of auxin transport *in vivo* in Arabidopsis. Plant Physiol 126(2): 524–535. https://doi.org/10.1104/pp.126.2.524

Byrne JM, Collins KA, Cashau PF, Aung LH (1975) Adventitious root development from seedling hypocotyl of *Lycopersicon esculentum*. Am J Bot 62(7):731–737. https://doi.org/10.2307/2442062

Canamero RC, Bakrim N, Bouly JP, Garay A, Dudkin EE, Habricot Y, Ahmad M (2006) Cryptochrome photoreceptors CRY1 and CRY2 antagonistically regulate primary root elongation in *Arabidopsis thaliana*. Planta 224(5):995–1003. https://doi.org/10.1007/s00425-006-0280-6

Carmody M, Crisp PA, d'Alesandro S, Ganguly D, Gordon M, Havaux M, Albrecht-Borth V, Pogson BJ (2016) Uncoupling high light responses from singlet oxygen retrograde signaling and spatial-temporal systemic acquired acclimation. Plant Physiol 171(3): 1734–1749. https://doi.org/10.1104/pp.16.00404

Casal JJ (2000) Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. Photochem Photobiol 71(1):1–11. https://doi.org/10.1562/0031-8655(2000)071<0001:PCPPII>2.0.CO;2

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inze D, Sandberg G, Casero PJ, Bennett M (2001) Auxin transport promotes Arabidopsis lateral root initiation. Plant Cell 13(4):843–852. https://doi.org/10.1105/tpc.13.4.843

Chattopadhyay S, Ang LH, Puente P, Deng XW, Wei N (1998) Arabidopsis bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. Plant Cell 10(5):673–683. https://doi.org/10.1105/tpc.10.5.673

Chee R (1986) *In vitro* culture of *Vitis*—the effects of light spectrum, manganese sulfate and potassium iodide on morphogenesis. Plant Cell Tissue Organ Cult 7(2):121–134. https://doi.org/10.1007/BF00043036

Chen X, Yao Q, Gao X, Jiang C, Harberd NP, Fu X (2016) Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. Curr Biol 26(5):640–646. https://doi.org/10.1016/j.cub.2015.12.066

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Cho M, Cho HT (2013) The function of ABCB transporters in auxin transport. Plant Signal Behav 8(2):e22990. https://doi.org/10.4161/psb.22990

Choudhury S, Panda P, Sahoo L, Panda SK (2013) Reactive oxygen species signaling in plants under abiotic stress. Plant Signal Behav 8(4):e23681. https://doi.org/10.4161/psb.23681

Christianson ML, Warnick DA (1983) Competence and determination in the process of *in vitro* shoot organogenesis. Dev Biol 95(2):288–293. https://doi.org/10.1016/0012-1606(83)90029-5

Christie JM, Murphy AS (2013) Shoot phototropism in higher plants: new light through old concepts. Am J Bot 100(1):35–46. https://doi.org/10.3732/ajb.1200340

Chung JP, Huang CY, Dai TE (2010) Spectral effects on embryogenesis and plantlet growth of Oncidium 'Gower Ramsey'. Sci Hort 124(4): 511–516. https://doi.org/10.1016/j.scienta.2010.01.028

Cluis CP, Mouchel CF, Hardtke CS (2004) The Arabidopsis transcription factor HY5 integrates light and hormone signaling pathways. Plant J 38(2):332–347. https://doi.org/10.1111/j.1365-313X.2004.02052.x

Correa LD, Troleis J, Mastroberti AA, Mariath JEA, Fett-Neto AG (2012) Distinct modes of adventitious rooting in *Arabidopsis thaliana*. Plant Biol 14:100–109

Dan YH (2008) Biological functions of antioxidants in plant transformation. In Vitro Cell Dev Biol-Plant 44(3):149–161. https://doi.org/10.1007/s11627-008-9110-9

De Klerk GJ, Van der Krieken W, De Jong JC (1999) Review—the formation of adventitious roots: new concepts, new possibilities. In Vitro Cell Dev Biol-Plant 35(3):189–199. https://doi.org/10.1007/s11627-999-0076-z

del Rio LA (2015) ROS and RNS in plant physiology: an overview. J Exp Bot 66(10):2827–2837. https://doi.org/10.1093/jxb/erv099

Demmig-Adams B, Adams WW III (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 43(1):599–626. https://doi.org/10.1146/annurev.pp.43.060192.003123

Dietz KJ, Mittler R, Noctor G (2016) Recent progress in understanding the role of reactive oxygen species in plant cell signaling. Plant Physiol 171(3):1535–1539. https://doi.org/10.1104/pp.16.00938

Dodge AD, Harris N, Baldwin BC (1970) The mode of action of Paraquat and Diquat. Biochem J 118:43–44

Efroni I, Mello A, Nawy T, Ip PL, Rahni R, DelRose N, Powers A, Satija R, Birnbaum KD (2016) Root regeneration triggers an embryo-like sequence guided by hormonal interactions. Cell 165(7):1721–1733. https://doi.org/10.1016/j.cell.2016.04.046

Fankhauser C, Christie JM (2015) Plant phototropic growth. Curr Biol 25(9):R384–R389. https://doi.org/10.1016/j.cub.2015.03.020

Fei Y, Xiao B, Yang M, Ding Q, Tang W (2016) MicroRNAs, polyamines, and the activities antioxidant enzymes are associated with *in vitro* rooting in white pine (*Pinus strobus* L.) SpringerPlus 5(1): 416. https://doi.org/10.1186/s40064-016-2080-1

Fett-Neto AG, Fett JP, Goulart LWV, Pasquali G, Termignon RR, Ferreira AG (2001) Distinct effects of auxin and light on adventitious root development in *Eucalyptus saligna* and *Eucalyptus globulus*. Tree Physiol 21(7):457–464. https://doi.org/10.1093/treephys/21.7.457

Franco DM, Silva EM, Saldanha LL, Adachi SA, Schley TR, Rodrigues TM, Dokkedal AL, Nogueira FT, Rolim de Almeida LF (2015) Flavonoids modify root growth and modulate expression of *SHORT-ROOT* and *HD-ZIP III*. J Plant Physiology 188:89–95. https://doi.org/10.1016/j.jplph.2015.09.009

Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PBF, Ljung K, Sandberg G, Hooykaas PJJ, Palme K, Offringa R (2004) A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science 306(5697):862–865. https://doi.org/10.1126/science.1100618

Fuernkranz HA, Nowak CA, Maynard CA (1990) Light effects on *in vitro* adventitious root formation in axillary shoots of mature *Prunus serotina*. Physiol Plant 80(3):337–341. https://doi.org/10.1111/j.1399-3054.1990.tb00050.x

Gamborg OL, Miler RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:148–151

Gould KS (2004) Nature's Swiss Army Knife: the diverse protective roles of anthocyanins in leaves. J Biomed Biotechnol 4:314–320

Gould KS, Neill SO, Vogelmann TC (2002) A unified explanation for anthocyanins in leaves? Adv Bot Res 37:167–192. https://doi.org/10.1016/S0065-2296(02)37049-6

Gu AS, Liu WF, Ma C, Cui J, Henny RJ, Chen JJ (2012) Regeneration of *Anthurium andraeanum* from leaf explants and evaluation of microcutting rooting and growth under different light qualities. HortSci 47:88–92

Gutierrez L, Mongelard G, Flokova K, Pacurar DI, Novak O, Staswick P, Kowalczyk M, Pacurar M, Demailly H, Geiss G, Bellini C (2012) Auxin controls *Arabidopsis* adventitious root initiation by regulating jasmonic acid homeostasis. Plant Cell 24(6):2515–2527. https://doi.org/10.1105/tpc.112.099119

Gyula N, Schafer E, Nagy F (2003) Light perception and signalling in higher plants. Curr Opin Plant Biol 6(5):446–452. https://doi.org/10.1016/S1369-5266(03)00082-7

Hemm MR, Rider SD, Ogas J, Murry DJ, Chapple C (2004) Light induces phenylpropanoid metabolism in *Arabidopsis* roots. Plant J 38(5):765–778. https://doi.org/10.1111/j.1365-313X.2004.02089.x

Hung CD, Hong C-H, Jung H-B, Kim S-K, Ket NV, Nam M-W, Choi D-H, Lee H-I (2015) Growth and morphogenesis of encapsulated strawberry shoot tips under mixed LEDs. Sci Hort 194:194–200. https://doi.org/10.1016/j.scienta.2015.08.016

Ikeuchi M, Ogawa Y, Iwase A, Sugimoto K (2016) Plant regeneration: cellular origins and molecular mechanisms. Development 143(9): 1442–1451. https://doi.org/10.1242/dev.134668

Jarvis BC, Ali AHN (1985) The influence of irradiance on root regeneration in stem cuttings of mung bean. New Phytol 101(2):233–239. https://doi.org/10.1111/j.1469-8137.1985.tb02830.x

Jenik PD, Barton MK (2005) Surge and destroy: the role of auxin in plant embryogenesis. Development 132(16):3577–3585. https://doi.org/10.1242/dev.01952

Jensen PJ, Hangarter RP, Estelle M (1998) Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown Arabidopsis. Plant Physiol 116(2):455–462. https://doi.org/10.1104/pp.116.2.455

Jeong BR, Sivansesan I (2015) Direct adventitious shoot regeneration, *in vitro* flowering, fruiting, secondary metabolite content and antioxidant activity of *Scrophularia takesimensis* Nakai. Plant Cell Tissue Organ Cult 123(3):607–618. https://doi.org/10.1007/s11240-015-0864-6

Jung S (2004) Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or Norflurazon on antioxidant systems. Plant Physiol Biochem 42(3):225–231. https://doi.org/10.1016/j.plaphy.2004.01.001

Kagawa T, Wada M (2002) Blue light-induced chloroplast relocation. Plant Cell Physiol 43(4):367–371. https://doi.org/10.1093/pcp/pcf049

Koornneef M, Rolff E, Spruit CJP (1980) Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L) Heynh. Z Pflanzenphysiol 100(2):147–160. https://doi.org/10.1016/S0044-328X(80)80208-X

Kubasek WL, Shirley BW, McKillop A, Goodman HM, Briggs W, Ausubel FM (1992) Regulation of flavonoid biosynthetic genes in germinating Arabidopsis seedlings. Plant Cell 4(10):1229–1236. https://doi.org/10.1105/tpc.4.10.1229

Landi M, Tattini M, Gould KS (2015) Multiple functional roles of anthocyanins in plant-environment interactions. Environ Exp Bot 119:4–17. https://doi.org/10.1016/j.envexpbot.2015.05.012

Laxmi A, Pan J, Morsy M, Chen R (2008) Light plays an essential role in intracellular distribution of auxin efflux carrier PIN2 in *Arabidopsis*

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thaliana. PLoS One 3(1):e1510. https://doi.org/10.1371/journal.pone.0001510

Lee J, He K, Stolc V, Lee H, Figueroa P, Gao Y, Tongprasit W, Zhao HY, Lee I, Deng X (2007) Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell 19(3):731–749. https://doi.org/10.1105/tpc.106.047688

Li SW, Xue LG (2010) The interaction between H_2O_2 and Ca^{2+}, cGMP, and MAPKS during adventitious rooting in mung bean seedlings. In Vitro Cell Dev Biol-Plant 46(2):142–148. https://doi.org/10.1007/s11627-009-9275-x

Li SW, Xue LG, Xu SJ, Feng HY, An LZ (2009) Hydrogen peroxide acts as a signal molecule in the adventitious root formation of mung bean seedlings. Environ Exp Bot 65(1):63–71. https://doi.org/10.1016/j.envexpbot.2008.06.004

Li S, Zhachgo S (2013) TCP3 interacts with R2R3-MYB proteins, promotes flavonoid biosynthesis and negatively regulates the auxin response in *Arabidopsis thaliana*. Plant J 76(6):901–913. https://doi.org/10.1111/tpj.12348

Libik-Konieczny M, Kozieradzka-Kiszkurno M, Desel C, Michalec-Warzecha Z, Miszalski Z, Konieczny R (2015) The localization of NADPH oxidase and reactive oxygen species in *in vitro*-cultured *Mesembryanthemum crystallinum* L. hypocotyls discloses their differing roles in rhizogenesis. Protoplasma 252(2):477–487. https://doi.org/10.1007/s00709-014-0692-2

Likic S, Rusak G (2014) Changes in phenolic compounds in *Nicotiana* species as a response to wounding and viral infection. J Plant Pathol 96:569–575

Liu X, Cohen JD, Gardner G (2011) Low-fluence red light increases the transport and biosynthesis of auxin. Plant Physiol 157(2):891–904. https://doi.org/10.1104/pp.111.181388

Liu YS, Roof S, Ye ZB, Barry C, van Tuinen A, Vrebalov J, Bowler C, Giovannoni J (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. Proc Natl Acad Sci U S A 101(26):9897–9902. https://doi.org/10.1073/pnas.0400935101

Ludwig-Muller J, Vertocnik A, Town CD (2005) Analysis of indole-3-butyric acid-induced adventitious root formation on *Arabidopsis* stem segments. J Exp Bot 56(418):2095–2105. https://doi.org/10.1093/jxb/eri208

Mironova VV, Omelyanchuck NA, Novoselova ES, Doroshokov AV, Kazantsev FV, Kochetov AV, Kolchanov NA, Mjolsness E, Likhoshvai VA (2012) Combined *in silico*/*in vivo* analysis of mechanisms providing for root apical meristem self-organization and maintenance. Ann Bot 110(2):349–360. https://doi.org/10.1093/aob/mcs069

Mo M, Yokawa K, Wan Y, Baluska F (2015) How and why do root apices sense light under the soil surface? Front Plant Sci 6:775

Morini S, D'Onofrio C, Bellocchi G, Fisichella M (2000) Effect of 2,4-D and light quality on callus production and differentiation from *in vitro* cultured quince leaves. Plant Cell Tissue Org Cult 63(1):47–55. https://doi.org/10.1023/A:1006456919590

Muramoto T, Kohchi T, Yokota A, Hwang I, Goodman HM (1999) The Arabidopsis photomorphogenic mutant *hy1* is deficient in phytochrome chromophore biosynthesis as a result of a mutation in a plastid heme oxygenase. Plant Cell 11(3):335–348. https://doi.org/10.1105/tpc.11.3.335

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15(3):473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nagatani A, Reed JW, Chory J (1993) Isolation and initial characterization of Arabidopsis mutants that are deficient in phytochrome A. Plant Physiol 102(1):269–277. https://doi.org/10.1104/pp.102.1.269

Nameth B, Dinka SJ, Chatfield SP, Morris A, English J, Lewis D, Oro R, Raizada MN (2013) The shoot regeneration capacity of excised Arabidopsis cotyledons is established during the initial hours after injury and is modulated by a complex genetic network of light signalling. Plant Cell Environ 36(1):68–86. https://doi.org/10.1111/j.1365-3040.2012.02554.x

Niyogi KK, Grossman AR, Bjorkman O (1998) Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. Plant Cell 10(7):1121–1134. https://doi.org/10.1105/tpc.10.7.1121

Niyogi KK, Truong TB (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosyntheis. Curr Opin Plant Biol 16(3):307–314. https://doi.org/10.1016/j.pbi.2013.03.011

Ohgishi M, Saji K, Okada K, Sakai T (2004) Functional analysis of each blue light receptor, CRY1, CRY2, PHOT1, and PHOT2, by using combinatorial multiple mutants in Arabidopsis. Proc Natl Acad Sci U S A 101(8):2223–2228. https://doi.org/10.1073/pnas.0305984101

Osterlund MT, Hardtke CS, Wei N, Deng XW (2000) Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature 405(6785):462–466. https://doi.org/10.1038/35013076

Oyama T, Shimura Y, Okada K (1997) The Arabidopsis *Hy5* gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes Dev 11(22):2983–2995. https://doi.org/10.1101/gad.11.22.2983

Palme K, Teale W, Dovzhenko A (2016) Plant signaling: HY5 synchronizes resource supply. Curr Biol 26:R319–R337

Pasternak T, Potters G, Caubergs R, Jansen MAK (2005) Complementary interactions between oxidative stress and auxins control plant growth responses at plant, organ, and cellular level. J Exp Bot 56(418):1991–2001. https://doi.org/10.1093/jxb/eri196

Patton DA, Meinke DW (1988) High-frequency plant regeneration from cultured cotyledons of *Arabidopsis thaliana*. Plant Cell Rep 7(4):233–237. https://doi.org/10.1007/BF00272531

Petrasek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertova D, Wisniewska J, Tadele Z, Kubes M, Covanova M, Dhonukshe P, Skupa P, Benkova E, Perry L, Krecek P, Lee OR, Fink GR, Geisler M, Murphy AS, Luschnig C, Zazimalova E, Friml J (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. Science 312(5775):914–918. https://doi.org/10.1126/science.1123542

Pinker I, Zoglauer K, Goring H (1989) Influence of light on adventitious root formation in birch shoot cultures *in vitro*. Biologia Planta 31(4):254–260. https://doi.org/10.1007/BF02907285

Preece JE (2003) A century of progress with vegetative plant propagation. HortSci 38:1015–1025

Reed JW, Nagatani A, Elich TD, Fagan M, Chory J (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol 104(4):1139–1149. https://doi.org/10.1104/pp.104.4.1139

Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell 5(2):147–157. https://doi.org/10.1105/tpc.5.2.147

Robert HS, Grunewald W, Sauer M, Cannoot B, Soriano M, Swarup R, Weijers D, Bennett M, Boutilier K, Friml J (2015) Plant embryogenesis requires AUX/LAX-mediated auxin influx. Development 142:1–10

Rossi F, Baraldi R, Facini O, Lercari B (1993) Photomorphogenic effects on *in vitro* rooting of *Prunus* rootstock GF 655-2. Plant Cell Tissue Organ Cult 32(2):145–151. https://doi.org/10.1007/BF00029836

Sena G (2014) Stem cells and regeneration in plants. Nephron Exp Nephrol 126(2):35–39. https://doi.org/10.1159/000360658

Sena G, Wang XN, Liu HY, Hofhuis H, Birnbaum KD (2009) Organ regeneration does not require a functional stem cell niche in plants. Nature 457(7233):1150–1154. https://doi.org/10.1038/nature07597

Shin DH, Choi M, Kim K, Bang G, Cho M, Choi S-B, Choi G, Park Y-I (2013) HY5 regulates anthocyanin biosynthesis by inducing the

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transcriptional activation of the MYB75/PAP1 transcription factor in Arabidopsis. FEBS Lett 587(10):1543–1547. https://doi.org/10.1016/j.febslet.2013.03.037

Shirley BW, Kubasek WL, Storz G, Bruggemann E, Koornneef M, Ausubel FM, Goodman HM (1995) Analysis of Arabidopsis mutants deficient in flavonoid biosynthesis. Plant J 8(5):659–671. https://doi.org/10.1046/j.1365-313X.1995.08050659.x

Statish L, Rency AS, Rathinapriya P, Ceasar SA, Pandian S, Rameshkumar R, Rao TB, Balachandran SM, Ramesh M (2016) Influence of plant growth regulators and spermidine on somatic embryogenesis and plant regeneration in four Indian geotypes of finger millet (*Eleusine coracana* (L.) Gaertn). Plant Cell Tissue Organ Cult 124(1):15–31. https://doi.org/10.1007/s11240-015-0870-8

Stracke R, Favory J-J, Gruber H, Bartelniewoehner L, Bartels S, Binkert M, Funk M, Weisshaar B, Ulm R (2009) The Arabidopsis bZIP transcription factor HY5 regulates expression of the PFG1/MYB12 gene in response to light and ultraviolet-B radiation. Plant Cell Environ 33:88–103

Sugimoto K, Gordon SP, Meyerowitz EM (2011) Regeneration in plants and animals: dedifferentiation, transdifferentiation, or just differentiation? Trends Cell Biol 21(4):212–218. https://doi.org/10.1016/j.tcb.2010.12.004

Sugimoto K, Jiao YL, Meyerowitz EM (2010) Arabidopsis regeneration from multiple tissues occurs via a root development pathway. Dev Cell 18(3):463–471. https://doi.org/10.1016/j.devcel.2010.02.004

Sugiyama M (1999) Organogenesis *in vitro*. Curr Opin Plant Biol 2(1):61–64. https://doi.org/10.1016/S1369-5266(99)80012-0

Takac T, Obert B, Rolcik J, Samaj J (2016) Improvement of adventitious root formation in flax using hydrogen peroxide. New Biotechnol 33(5):728–734. https://doi.org/10.1016/j.nbt.2016.02.008

Taylor LP, Grotewold E (2005) Flavonoids as developmental regulators. Curr Opin Plant Biol 8(3):317–323. https://doi.org/10.1016/j.pbi.2005.03.005

Tognetti VB, Muhlenbock P, Van Breusegem F (2012) Stress homeostasis—the redox and auxin perspective. Plant Cell Environ 35(2):321–333. https://doi.org/10.1111/j.1365-3040.2011.02324.x

Tyburski J, Jasionowicz P, Tretyn A (2006) The effects of ascorbate on root regeneration in seedling cuttings of tomato. Plant Growth Reg 48(2):157–173. https://doi.org/10.1007/s10725-005-5991-3

Tyburski J, Tretyn A (2004) The role of light and polar auxin transport in root regeneration from hypocotyls of tomato seedling cuttings. Plant Growth Reg 42(1):39–48. https://doi.org/10.1023/B:GROW.0000014896.18601.38

Valvekens D, Van Montagu M, Van Lijsebettens M (1988) *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. Proc Natl Acad Sci U S A 85(15):5536–5540. https://doi.org/10.1073/pnas.85.15.5536

Van Tieghem P, Douliot H (1888) Recherches comparative sur l'origine endogenes dan les plantes vasculaires. Ann Sci Nat Bot 7:1–660

Vandenbussche F, Habricot Y, Condiff AS, Maldiney R, Van der Straeten D, Ahmad M (2007) HY5 is a point of convergence between cryptochrome and cytokinin signalling pathways in *Arabidopsis thaliana*. Plant J 49(3):428–441. https://doi.org/10.1111/j.1365-313X.2006.02973.x

Vanstraelen M, Benkova E (2012) Hormonal interactions in the regulation of plant development. Annu Rev Cell Dev Biol 28(1):463–487. https://doi.org/10.1146/annurev-cellbio-101011-155741

Wasson AP, Ramsay K, Jones MG, Mathesius U (2009) Differeing requirements for flavonoids during the formation of lateral roots, nodules and root knot nematode galls in *Medicago truncatula*. New Phytol 183(1):167–179. https://doi.org/10.1111/j.1469-8137.2009.02850.x

Welander M, Geier T, Smolka A, Ahlman A, Fan J, Zhu LH (2014) Origin, timing, and gene expression profile of adventitious rooting in *Arabidopsis* hypocotyls and stems. Am J Bot 101(2):255–266. https://doi.org/10.3732/ajb.1300258

Xu K, Huang B, Liu K, Qi F, Tan G, Li C, Zhang X (2016a) Peanut regeneration by somatic embryogenesis (SE), involving bulbil-like body (BLB), a new type of SE structure. Plant Cell Tissue Organ Cult 125(2):321–328. https://doi.org/10.1007/s11240-016-0952-2

Xu X, Chi W, Sun X, Feng P, Guo H, Li J, Lin R, Lu C, Wang H, Leister D, Zhang L (2016b) Convergence of light and chloroplast signals for de-etiolation through ABI4–HY5 and COP1. Nat Plants 2(6):16066. https://doi.org/10.1038/nplants.2016.66

Yamamoto HY, Kamite L (1972) Effects of dithiothreitol on violaxanthin deepoxidation and absorbance changes in 500 nm region. Biochim Biophys Acta 267(3):538–543. https://doi.org/10.1016/0005-2728(72)90182-X

Zhang H, He H, Wang X, Wang X, Yang X, Li L, Deng XW (2011) Genome-wide mapping of the HY5-mediated gene networks in Arabidopsis that involve both transcriptional and post-trascriptional regulation. Plant J 65(3):346–358. https://doi.org/10.1111/j.1365-313X.2010.04426.x

Zhang K, Xu H, Yuan T, Zhang L, Lu Y (2013) Blue-light-induced PIN3 polarization for root negative phototrophic response in Arabidopsis. Plant J 76(2):308–321. https://doi.org/10.1111/tpj.12298

Zhao QH, Fisher R, Auer C (2002) Developmental phases and STM expression during Arabidopsis shoot organogenesis. Plant Growth Reg 37(3):223–231. https://doi.org/10.1023/A:1020838712634

Zheng X, Wu S, Zhai H, Zhou P, Song M, Su L, Xi Y, Li Z, Cai Y, Meng F, Yang L, Wang H, Yang J (2013) Arabidopsis phytochrome B promotes SPA1 nuclear accumulation to repress photomorphogenesis under far-red light. Plant Cell 25(1):115–133. https://doi.org/10.1105/tpc.112.107086