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Chiara Agliassa[1], Ravishankar Narayana[2], John M. Christie[3], and Massimo E. Maffei[1]

[1] Plant Physiology Unit, Dept. Life Sciences and Systems Biology, University of Turin, Via Quarello 15/A, 10135 Turin, Italy; [2] Department of Entomology, Penn State University, W249 Millennium Science Complex, University Park, PA 16802, USA; [3] Institute of Molecular, Cell and Systems Biology, Bower Building, University of Glasgow, University Avenue, G12 8QQ Glasgow, UK

Correspondence: M.E. Maffei, Plant Physiology Unit, Dept. Life Sciences and Systems Biology, University of Turin, Via Quarello 15/A, 10135 Turin, Italy E-mail: massimo.maffei@unito.it. Tel. +39 011 6705967; Fax +39 011 2365967

ABSTRACT

The geomagnetic field (GMF) is an environmental element whose instability affects plant growth and development. Despite known plant responses to GMF direction and intensity, the mechanism of magnetoreception in plants is still not known. Magnetic field variations affect many light-dependent plant processes, suggesting that the magnetoreception could require light. The objective of this work was to comprehensively investigate the influence of GMF on *Arabidopsis thaliana* (*Col-0*) photoreceptor signaling. Wild-type Arabidopsis seedlings and photoreceptor-deficient mutants (*cry1cry2*, *phot1*, *phyA* and *phyAphyB*) were exposed to near null magnetic field (NNMF, ≤ 40 nT) and GMF (~43 μT) under darkness and different light wavelengths. The GMF did not alter skotomorphogenic or photomorphogenic seedling development but had a significant impact on gene expression pathways downstream of cryptochrome and phytochrome photoactivation. GMF-induced changes in gene expression observed under blue light were partially associated with an alteration of cryptochrome activation. GMF impacts on phytochrome-regulated gene expression could be attributed to alterations in phytochrome protein abundance that were also dependent on the presence of cry1, cry2 and phot1. Moreover, the GMF was found to impact photomorphogenic-promoting gene expression in etiolated seedlings, indicating the existence of a light-independent magnetoreception mechanism. In conclusion, our data shows that magnetoreception alters photoreceptor signaling in Arabidopsis, but it does not necessarily depend on light.

Keywords: *Arabidopsis thaliana*, cryptochromes, geomagnetic field, light-regulated genes, magnetoreception, photomorphogenesis, phototropins, phytochromes, skotomorphogensis.

1. Introduction

The Earth's magnetic field, or the geomagnetic field (GMF), is an environmental factor characterized by local differences in its magnitude and direction at the Earth's surface as well as polarity changes during the so called GMF reversals, which are always preceded by a reduction in the magnetic field (MF) intensity [1]. Due to its transient instability, the GMF has always been a natural feature able to influence the biological processes of living organisms, including plants. Over the past years, the progress and status of research on the effect of the MF on plants has been reviewed [2]. Interestingly, a correlation has been found between the occurrence of GMF reversals and the speciation of Angiosperms, implying a role for the GMF in plant evolution [1]. Furthermore, artificial reversal of the GMF has confirmed that plants can respond not only to MF intensity but also to MF direction and polarity [3].

One of the most interesting plant responses to GMF variations is the delay in flowering time, especially after exposure of plants to Near Null Magnetic Field (NNMF, $\leq$ 40 nT) conditions [4, 5]. Along with flowering time alteration, many other light-dependent plant processes appear to be influenced by MF variations including germination, leaf movement, stomatal conductance, chlorophyll content and plant vegetative growth [2, 6]. However, despite a plethora of reports on plant MF effects, the molecular basis underlying plant magnetoreception is still not known. A growing body of evidence supports a possible role for plant photoreceptors in magnetoreception. A better evaluation of MF effects on plant photoreceptor action is therefore warranted given their key role in regulating many aspects of plant development.

Photoreceptors perceive different light quality, quantity and intensity, and control multiple aspects of plant development largely through coordinated changes in gene expression. Despite their wavelength-dependent activation, crosstalk is known to occur between different photoreceptor families, especially photoperiodic flowering and photomorphogenesis [7]. The role of photoreceptors in mediating the response to MF changes has been mainly studied for cryptochrome, because the

radical pair mechanism forming the basis of Arabidopsis cryptochrome 1 and 2 (cry1 and cry2) blue light-activation appears to be affected by the external MF [8-10]. Indeed, cryptochrome plays an important role with regards to the NNMF reported delay in flowering [11] and its associated changes in auxin [12] and gibberellin [13] levels. In addition to cryptochrome, phytochrome B (phyB) transcription appears to be enhanced by NNMF [4], thus indicating a possible role for this photoreceptor in mediating NNMF-induced flowering delay.

MF influences on photomorphogenesis that have been observed under blue light appear to be cryptochrome-dependent in Arabidopsis. However, expression of the photomorphogenesis-promoting transcription factor elongation hypocotyl 5 (HY5) is not altered in response to different MF intensities suggesting that the GMF influences other photomorphogenic signaling pathways [14, 15]. Besides cryptochromes and phytochromes, phototropins (phot1 and phot2) are also important for optimizing photosynthetic efficiency and promoting plant growth independent of gene expression regulation [16, 17]. Thus, considering that the coordination of light-mediated plant development involves multiple photoreceptors [18] and that the effects of the GMF on gene expression pathways downstream of photoreceptor activation have been poorly explored, the main objective of this work was to comprehensively investigate the influence of the GMF on photoreceptor signaling in Arabidopsis.

To discriminate whether the GMF affects specific photoreceptor signaling pathways, we exposed wild-type (WT) Arabidopsis seedlings and *cry1cry2, phot1, phyA* and *phyAphyB* mutants to GMF and NNMF conditions. Photoreceptor phosphorylation is a primary event [17] associated with cryptochrome, phototropin and phytochrome signaling. We therefore analyzed the influence of the GMF on photoreceptor activation by monitoring their phosphorylation status and protein abundance. Crosstalk between different photoreceptor pathways was also evaluated. To assess whether GMF effects on cryptochrome and phytochrome activation could impact downstream signaling, we evaluated the GMF influence on the expression of photomorphogenesis-promoting genes in addition to photomorphogenic development by exposing WT Arabidopsis and photoreceptor-deficient

mutants to NNMF and GMF conditions. Taken together, our data provide further evidence for the impact of the GMF on plant photoreceptor activation and signaling both in the presence and absence of light.

2. Materials and Methods

2.1. Plant material and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (*Col-0*) wild type (WT), *cry1cry2*, *phyA*, *phyAphyB* and *phot1* seeds have been described previously [19]. Seeds were surface sterilized with 70 % v/v ethanol for 2 min and then with 5% w/v calcium hypochlorite for 5 min. After 3-4 washes with sterile water, seeds were sown on the surface of sterile agar plates (12x12 cm) containing half-strength Murashige and Skoog (MS) medium [20]. Plates were vernalized for 48 h and then exposed vertically under a homogenous and continuous light source at 120 μmol m^{-2} s^{-1} and 21°C (± 1.5) before being kept in the darkness at room temperature for 72 h. Plates were then transferred, in the same laboratory and at the same time, under either NNMF (see "GMF control system") or GMF (controls) and exposed to different light regimes for a variable time (see "Light Treatment").

2.2. NNMF control system

In order to reduce the GMF to NNMF, we built an octagonal triaxial Helmholtz coils (THC) system which operates as reported earlier [3, 5]. Each pair of coils was connected to a DC power supply (dual range: 0-8V/5A and 0-20V/2.5A, 50W) and to a computer via a GPIB connection. A three-axis magnetometer probe, which was connected to the same computer, was inserted in the middle of the THC. The real-time measurement of $B_{x,y,z,}$ at the probe position was achieved by collecting 10 s interval data which were transformed in total B by a software (VEE, Agilent Technologies) as detailed elsewhere [3].

2.3. Light sources and treatments

Under both GMF and NNMF, white light was provided by a high-pressure sodium lamp source (SILVANIA, Grolux 600W, Belgium), red light by an array of LEDs (SUPERLIGHT, Ultra bright LED, λ 645-665) and blue light by an array of LEDs (SUPERLIGHT, Ultra bright LED, λ 465-475). LED circuitry and spectral analysis is shown in Supporting Figure S1. Plates exposed to continuous darkness were kept in paper boxes internally covered by a black cardboard.

Different exposure times and light fluencies were adopted to selectively induce photoreceptor activation. Specifically, to monitor differences in cry2 degradation, WT, *phyA* and *phyAphyB* seedlings were exposed to 0.5 μmol m^{-2} s^{-1} blue light for 8 h in the morning [21]. To evaluate the phosphorylation level of cry1 and phot1, WT, *phot1*, *cry1cry2* and *phyAphyB* seedlings were exposed to 20 μmol m^{-2} s^{-1} blue light for 15 min at noon [22]. To evaluate the possible influence of the magnetic field intensity on phyA and phyB degradation, WT and *cry1cry2* plants were exposed under 60 μmol m^{-2} s^{-1} red light for 3 h and 9 h, respectively in the morning [23].

For gene expression and morphological experiments, WT, *cry1cry2*, *phyAphyB* and *phot1* seedlings were exposed for 72 h to different light regimes, depending on the set up of the experiment: (i) 16-8 h light/darkness long-day white light (LD), (ii) 150 μmol m^{-2} s^{-1}continuous white light (CW), (iii) continuous darkness (CD), (iv) 20 μmol m^{-2} s^{-1} continuous blue light (BL), and (v) 60 μmol m^{-2} s^{-1} continuous red light (RL).

2.4. Protein extraction and phosphatase treatment

Three-day-old etiolated seedlings were harvested after the light treatment (see above) and then ground directly in 100 μl 2x SDS buffer. After 4 min of incubation at 100°C, samples were centrifuged at 13,000 x *g* for 8 min and the supernatant used for SDS-PAGE. To confirm that reduced electrophoretic mobility shifts observed reflected cry1 and phot1 phosphorylation, we also examined the effect of λ-phosphatase treatment according to Shalitin et al. [24].

137 *2.5. SDS-PAGE and Western Blot analysis*

138 Thirty microliters of each sample were loaded on a 7.5% SDS-polyacrylamide (40% Acrylamide/Bis
139 Solution, 37.5:1, Biorad) gel and separated at 200 V for 40 min. Gel-run proteins were transferred on
140 a nitrocellulose membrane at 100 V for 1 h. After 1h blocking in 8% milk, membranes were probed
141 with the following primary antibodies overnight: anti-phyA (Agrisera); anti-phyB [25]; anti-cry1
142 [26], anti-cry2 [27], anti-phot1 [28] and anti-UGPase (Newmarket Scientific, U.K.) as a loading
143 control. Three TBS-T washings of 10 min each were performed before the incubation with the
144 secondary antibodies (anti-rabbit or anti-mouse horseradish peroxidase (HRP)-conjugated secondary
145 antibody (Promega, Italy) at room temperature for 1 h. All membranes were developed using Pierce®
146 ECL Plus Western blotting chemiluminescence substrate (Thermo Fisher Scientific, Rodano, Italy).
147 Membranes were stripped and re-probed to detect all protein of interest.

148 *2.6. Total RNA isolation and cDNA synthesis*

149 Arabidopsis WT, *cry1cry2*, *phyAphyB* and *phot1* roots and shoots were separately collected 72 h after
150 each light treatment under GMF and NNMF, immediately frozen in liquid N_2 and kept at -80°C for
151 further analysis. Thirty mg of frozen shoots and 10 mg of frozen roots were ground in liquid nitrogen
152 with mortar and pestle. Total shoot RNA was isolated using the Agilent Plant RNA Isolation Mini
153 Kit (Agilent Technologies, Santa Clara, CA, US), while total root RNA was isolated using the
154 RNAeasy Micro Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's protocols.
155 RNA quality and quantity were monitored as reported previously [3]. cDNA was synthesized starting
156 from 1 µg RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystem, Foster
157 City, CA, US), in accordance with the manufacturer's recommendations. Reaction mixtures were
158 prepared and incubated as already detailed [3].

159 *2.7. Quantitative real time-PCR (qPCR)*

160 qPCR assays were processed on a Stratagene Mx3000P Real-Time System (La Jolla, CA, USA) using
161 SYBR green I with ROX as an internal loading standard. The reaction mixture was 10 µl, comprising
162 5 µL 2X Maxima^{TM} SYBR Green qPCR Master Mix (Fermentas International, Inc, Burlington, ON,
163 Canada), 0.6 µl 1:5 diluted cDNA and 300 nM primers (Integrated DNA Technologies, Coralville,
164 IA, US). Non-template controls (water template) were included. Primers were designed using Primer
165 3.0 software. Primers used for qPCR are reported in Supporting Table S1. The following genes were
166 analyzed: *ANS* (anthocyanidin synthase, At4g22880), *CHS* (chalcone synthase, At5g13930); *GST*
167 (glutathione S-transferase, At1g1037); *HY5* (elongated hypocotyl 5, At5g11260); *HYH* (HY5-
168 homolog, At3g17609); *LAF1* (MYB domain protein 18, At4g25560); *NDPK2* (nucleoside
169 diphosphate kinase 2, At5g63310); *PIF3* (phytochrome interacting factor 3, At1g09530); *PIN1* (pin-
170 formed 1, At1g73590); *PIN3* (pin-formed 3, At1g70940); *PKS1* (phytochrome kinase substrate 1,
171 At2g02950).

172 Four different reference genes *ACT1* (actin1, At2g37620), *eEF1Balpha2* (elongation factor
173 1b alpha-subunit 2, At5g19510), *TUB5* (tubulin beta-5 chain, At1g20010), *UBP6* (ubiquitin specific
174 protease 6, At1g51710), were initially used to normalize the results of the qPCR. The best of the four
175 genes was selected using the Normfinder software; the most stable gene was *eEF1Balpha2*. PCR
176 conditions used were as follows: *ACT1, ANS, CHS, LAF1, NDPK2, PIF3, PIN1, PIN3, PKS1, TUB5,*
177 *UBP6*: 10 min at 95°C, 45 cycles of 15 s at 95°C, 20 s at 57°C, and 30 s at 72°C, 1 min at 95°C, 30 s
178 at 55°C, 30 s at 95°C; *eEF1Balpha2*: 10 min at 95°C; 45 cycles of 15 s at 95°C, 30 s at 57°C, and 30
179 s at 72°C; 1 min at 95°C, 30 s at 55°C, 30 s at 95°C; *GST*:10 min at 95°C; 45 cycles of 15 s at 95°C,
180 20 s at 59°C, and 30 s at 72°C; 1 min at 95°C, 30 s at 55°C, 30 s at 95°C; *HYH*:10 min at 95°C; 45
181 cycles of 15 s at 95°C, 20 s at 58°C, and 30 s at 72°C; 1 min at 95°C, 30 s at 55°C, 30 s at 95°C;
182 *HY5*:10 min at 95°C; 45 cycles of 15 s at 95°C, 20 s at 56°C, and 30 s at 72°C; 1 min at 95°C, 30 s at
183 55°C, 30 s at 95°C. Fluorescence was read following each annealing and extension phase. All runs

were followed by a melting curve analysis from 55°C to 95°C. Primer efficiencies for all primer pairs were calculated using the standard curve method.

2.8. Morphological analyses

After 72 h treatments, all plates were photographed just before being sampled. All plate images were used to measure hypocotyl and root lengths. Image analysis was performed using ImageJ software.

2.9. Statistical analyses

All experiments were performed at least three times (three biological replicates) and all data were expressed as mean values with standard deviation. ImageJ software was used to quantify the protein abundance in western blots relative to the loading control UGPase. Significant differences were verified using a Student's t-test. With respect to gene expression experiments, each biological replicate was analyzed using three technical replicates. A Kolmogorov-Smirnov goodness-of-fit test was used to determine the normality of all results. ANOVA followed by a Tukey and Bonferroni *post-hoc* test was used to assess significant differences between treatments and the control. For morphometric measurements, the shoot and root length mean from seedlings on each plate were used in a two-tailed paired t-test analysis to compare the growth of seedlings exposed to the NNMF with those grown simultaneously under GMF conditions. 95% confidence level ($P < 0.05$) was adopted to judge the statistical significance of all our data, using SYSTAT 10.

3. Results

The availability of a triaxial Helmholtz coils (THC) system that could stably reduce the GMF to NNMF was instrumental for investigating the influence of the GMF on photoreceptor signaling cascade in Arabidopsis and to further assess the role of cryptochrome in magnetoreception.

3.1. The GMF enhances cry1 phosphorylation and cry2 degradation in response to BL

To monitor the GMF influence on photoreceptor signaling, we first investigated whether the GMF can modulate photoreceptor activation levels. Therefore, we evaluated the GMF influence on the blue light receptor signaling, by monitoring cry1, cry2 and phot1 activation. In WT, *phot1* and *phyAphyB* seedlings exposed to NNMF, cry1 phosphorylation following exposure to blue light (BL) was practically absent compared to GMF conditions, whereas phosphorylation of the receptor was clearly evident by a detection of a reduced mobility shift (Figure 1, arrow). Under NNMF, a significant ($P < 0.05$) reduction in BL-induced cry2 degradation was also found, thus implying its lower activation level in the absence of the GMF (Figure 2).

Having confirmed the influence of the GMF on cryptochrome activation, we then investigated whether the GMF could affect the photoactivation of phot1, which also promotes the photomorphogenic responses to BL in addition to cryptochrome [29]. To this purpose, we investigated phot1 autophosphorylation under BL (Figure 3). We also included cryptochrome and phytochrome mutants to investigate the involvement of these photoreceptors on phot1 activation in response to changes in the MF. However, our results highlighted the persistence of phot1 autophosphorylation under NNMF (Figure 3, arrow) as was observed under GMF conditions. We therefore conclude that the MF does not affect phot1 autophosphorylation and photoactivation.

3.2. The GMF reduces phyA degradation and increases phyB degradation following RL exposure

We next investigated whether the GMF could affect red light (RL) signaling in Arabidopsis. Activation of phyA and phyB results in their proteasome degradation following translocation to the nucleus. RL-induced changes in phyA and phyB protein abundance was therefore used as a proxy for their activation. After 3 h exposure to RL, phyA degradation was significantly ($P < 0.05$) enhanced in WT seedlings exposed to NNMF with respect to GMF (Figure 4), thus indicating increased activation of phyA in the presence of NNMF. The enhancement in RL-induced phyA degradation under NNMF was less apparent in *cry1cry2* and *phot1* seedlings (Figure 4). These findings therefore

suggest that cryptochromes and phot1 may contribute to accelerating phyA degradation under NNMF conditions..

With regards to phyB, a significantly ($P < 0.05$) lower level of RL-induced degradation was observed in WT plants under NNMF when compared to GMF conditions (Figure 5). Therefore, phyB activation appears to be attenuated by NNMF conditions. Although RL-induced degradation of phyB was clearly apparent in WT seedlings under GMF conditions, this process did not occur in *cry1cry2* or *phot1* seedlings (Figure 5). These findings therefore suggest that efficient phyB activation under GMF conditions depends on the presence of cryptochromes and phot1.

3.3. The GMF impacts Arabidopsis gene expression under different light conditions

Having assessed the influence of the GMF on cryptochrome and phytochrome activation, we investigated the impact of the GMF on gene expression changes under different light conditions and the dependence of any of these changes on photoreceptor signaling. For these experiments, continuous white light (CW) was used to permanently stimulate both cryptochrome and phytochrome photoreception pathways, whereas BL and RL were used to selectively activate BL-responsive receptors (including cryptochromes) and phytochrome, respectively. Continuous darkness (CD) was also used to assess magnetoreception in the absence of light.

To evaluate the impact of the GMF on the expression of photomorphogenic-promoting genes, we analyzed the transcript level of several representative genes that are known to operate downstream of multiple photoreceptors (*HYH, HY5* and *LAF1*), genes encoding for factors mainly regulated by phytochrome signals (*PKS1, PIF3* and *NDPK2*), anthocyanin biosynthesis genes which are transcriptionally regulated by cryptochrome and phytochrome (*ANS* and *CHS*), genes encoding auxin transporters whose transcriptional regulation is under cryptochrome and phytochrome control (*PIN1* and *PIN3*), and finally genes involved in oxidative stress responses (*GST* and *NDPK2*). Considering that roots appear to be one of the primary sites involved in GMF perception [3], we decided to discriminate root and shoot light-dependent gene expression responses to the GMF.

255 Expression of light-related genes were first evaluated in WT seedlings grown under CW. In
256 order to assess the contribution of the GMF, data were expressed as the difference in fold changes
257 between GMF and NNMF conditions (i.e., GMF/NNMF), by considering NNMF as the control
258 condition where MF has a very low contribution. The GMF prompted a significant ($P < 0.05$) down-
259 regulation of *HYH* and *PKS1* and a significant ($P < 0.05$) up-regulation of *GST* and *ANS* in the shoots
260 of light-grown seedlings (Table 1), whereas in roots, the presence of GMF significantly ($P < 0.05$)
261 down-regulated *HYH*, *HY5*, *NDPK2* and *GST*, and up-regulated *PIN3* (Table 1). MF-induced
262 expression changes were also observed for gene targets that are not regulated by light. For instance,
263 a significant ($P < 0.05$) up-regulation of *HYH* in the shoots and roots of WT seedlings and a significant
264 ($P < 0.05$) down-regulation of *NDPK2* and *LAF1* in the roots was observed in the presence of GMF
265 (Table 1). These data clearly show that alteration in MF conditions can impact the expression of light-
266 and non-light-regulated gene targets.

267 We next assessed whether the above gene expression profiles under GMF or NNMF
268 conditions differed when BL or RL was used instead of CW (Supporting Tables S2 and S3).
269 Moreover, a comparison of gene expression profiles between WT seedlings and different
270 photoreceptor mutants was used to discriminate whether MF-induced changes in gene expression
271 could be attributed to a specific light signaling pathway. To simplify our data presentation, we have
272 only focused on those gene whose differential expression showed a significant ($P < 0.05$) difference
273 in the GMF versus NNMF conditions.

274 Overall, we found that under BL conditions (Figure 6), the changes in the MF impacted the
275 expression of 5 gene targets in the shoot of Arabidopsis seedlings (Figure 6a) and 7 gene targets in
276 the roots (Figure 6b). In the shoots of WT seedlings, expression of *HYH*, *PKS1*, *PIN1* and *PIN3* were
277 down-regulated in GMF versus NNMF conditions, whereas *PIF3* was up-regulated (Figure 6a).
278 Shoots obtained from *cry1cry2* mutant seedlings showed an absence of the down-regulation of *PKS1*
279 under GMF conditions. Likewise, both *PKS1* and *PIN3* expression levels were not significantly
280 affected by the GMF in the shoots of *phot1* seedlings. The *phyAphyB* mutant showed no effect of

GMF on the regulation of *PIF3* and *PIN1* in both shoots and roots. In the roots of WT seedlings grown under BL, we found that the expression of *HYH, PIF3, CHS, PIN1* and *PIN3* was upregulated in the presence of GMF versus NNMF, whereas the expression of *PKS1* and *NDPK2* was down-regulated (Figure 6b). In the roots of *cry1cry2* seedlings, *HYH* and *CHS* were not significantly different between GMF and NNMF conditions, whereas, the *phot1* mutant showed no regulation changes for *PKS1, PIN1* and *PIN3* under GMF conditions. Finally, the *phyAphyB* mutant showed no GMF associated changes in the regulation for *PKS1* and *PIN3*. Therefore, these gene expression studies performed under BL (Figure 6 and Supporting Table S2) suggest that the GMF has an impact not only on cryptochrome signaling, but also on phot1 and phytochrome signaling.

Under RL, we found that changes in the MF could affect the expression of 5 gene targets in the shoots (Figure 7a) and 9 gene targets in the roots of Arabidopsis seedlings (Figure 7b). We therefore conclude that the GMF can impact RL signaling by the phytochromes. In the shoots of WT seedlings, expression of *PKS1, PIF3* and *GST* was down-regulated in the presence of GMF versus NNMF, whereas *ANS* and *CHS* were up-regulated. In the shoots of *cry1cry2* mutants, *CHS* and *GST* expression was not significantly affected by changes in the MF under RL conditions. However, the MF changes observed for *PKS1* and *PIF3* expression under RL was lacking in the shoots of the *phyAphyB* mutant, whereas no change in *GST* expression was detected in the shoots of the *phot1* mutant. In the roots of WT seedlings grown under RL, the presence of GMF versus NNMF caused a significant ($p < 0.05$) up-regulation of *LAF1* and a significant down-regulation of the other genes, notably the phytochrome-related factors *PIF3* and *NDPK2* (Figure 7b). When compared to WT seedlings no MF-dependent changes in expression were observed for *CHS* and *PIN3* in the roots of the *cry1cry2* mutant under these light conditions. Likewise, exposure of seedlings to GMF versus NNMF conditions did not alter *PIF3* and *NDPK2* expression in *phot1* mutant plants. *GST* expression was also unaffected by changes in the MF in the roots of the *phyAphyB* mutant. (Figure 7b). Taken together, these gene expression studies performed under RL (Figure 7 and Supporting Table S3) once

again suggest that the presence of the GMF can influence phytochrome, cryptochrome and phot1 signaling.

3.4. Skoto- and Photomorphogenic responses to GMF in Arabidopsis seedlings

Having evaluated that the GMF can impact light signaling by modulating both photoreceptor activation and light-dependent gene expression, we verified whether the GMF could affect the establishment of photomorphogenic responses, by measuring light-regulation of shoot and primary root growth. The skotomorphogenic growth phenotype of Arabidopsis shoots grown under CD, as well as the photomorphogenic growth under CW were not affected by MF variations (Supporting Figure S2). Similar results were also obtained when WT, *cry1cry2*, *phot1* and *phyAphyB* seedlings were exposed to GMF and NNMF and grown under either BL or RL (Supporting Figure S2). Therefore, we conclude that the GMF is unable to influence dark and light-regulated seedling establishment under the conditions used, despite affecting photoreceptor signaling by altering photoreceptor activation and light-related gene expression.

4. Discussion

During early photomorphogenesis, all photoreceptors play a key role in the genome-wide reprogramming of light signaling [30, 31]. Thereby, the evaluation of the GMF effect on different responses related to this process has been useful to investigate the light dependence of GMF influence on light signaling in Arabidopsis and to discriminate photoreceptor involvement in magnetoreception.

4.1. The GMF affects gene expression in a light-dependent and light-independent manner

Our gene expression analyses surprisingly highlight the occurrence of a light-independent response to the GMF in the roots of WT seedlings. In the absence of light (CD), the most highly regulated gene in response to MF changes is *NDPK2* (Table 1), which is involved in the oxidative stress signaling [32]. This result implies the presence of a light-independent root magnetoreception mechanism that

329 involves an oxidative response. These results are in agreement with our previous studies on GMF
330 reversal [3]. Root light-independent responses to MF variations have been demonstrated in plants
331 under a continuous high gradient MF application, with a magnetophoretic plastid displacement and a
332 consequent induction of root curvature [33]. Therefore, our results indicate the possibility of a light-
333 independent magnetoreception mechanism and further studies are now under way to better understand
334 how roots are involved in magnetoreception.

335 Our gene expression analyses under continuous white light (CW) revealed a light-dependent
336 influence of the GMF on photomorphogenesis-promoting genes (Table 1). GMF was reported not to
337 influence *HY5* expression in the shoot of 7-day-old seedlings grown under LD conditions [4].
338 However, we found that the *HY5* expression level in the roots of WT seedlings is affected by the
339 GMF under CW, thus implying a role of active photoreceptors in promoting this process. The
340 observed down-regulation of *HY5* in the shoot might be related to changes in *CHS* transcription,
341 which is regulated by HY5 during photomorphogenesis [34]. Furthermore, under CW the GMF
342 influence on the expression of auxin signaling (*PIN3*) and anthocyanin biosynthesis (*ANS* and *CHS*)
343 genes could be related not only to changes in the expression of their promoting transcription factors
344 [35, 36] but also to the strong GMF effect on *GST* transcription, whose involvement in the
345 photomorphogenic response is mediated by multiple photoreceptors [37]. Therefore, our results
346 suggest that the light signaling cascade is influenced by the GMF especially under light exposure.

347 *4.2. The GMF influences blue light photoreceptor signaling*

348 In agreement with previous reports [12, 13], we confirmed that the GMF affects gene expression
349 under BL (Figure 6). In contrast to previous studies [15], our analyses showed an influence of the
350 GMF on *CHS* transcripts in roots under BL, thus implying a possible GMF effect on anthocyanin
351 expression levels under this light treatment. In this regard, the influence of BL on anthocyanin
352 production has been already demonstrated at the protein level with MF intensity ten times higher than
353 the GMF (500 μT) [38]. Moreover, the reduction of *PKS1* expression in the shoot under BL suggest

354 a possible influence of the GMF on this gene, because BL normally enhances *PKS1* expression level
355 [39].

356 In WT plants, the opposite trend in *HYH, PIN1* and *PIN3* expression in the shoots compared
357 to the roots underlines a specific organ response to GMF under BL (Figure 6). In particular, the GMF-
358 induced reduction of *PIN1* transcript levels in the shoots is associated with the down-regulation of
359 the bZip transcription factor *HYH* [40] whose expression level is regulated by BL [36]. Conversely,
360 the higher expression level of *PIN1* observed in the roots is associated with the GMF-induced
361 upregulation of *HYH*, whose expression occurs autonomously in the root with respect to the shoot
362 [41].

363 Considering the key role of cryptochrome in promoting photomorphogenesis by modulating
364 auxin signaling and anthocyanin biosynthesis gene expression [42, 43], the GMF-induced regulation
365 of both *PIN1* and *CHS* transcript level (Figure 6) implies a GMF influence on cryptochrome mediated
366 photomorphogenesis. The cryptochrome dependence of GMF regulation of *PIN1* expression is in
367 agreement with previous work on Arabidopsis seedlings grown under BL [12]. *HYH* expression is
368 known to be enhanced by cryptochrome in a BL-dependent manner [40]. The observed cryptochrome-
369 dependent upregulation of *HYH* in the presence of the GMF highlights the possible influence of the
370 GMF on cryptochrome activation. The higher activation levels of cry1 and cry2 in the presence of
371 the GMF could then be directly related to *HYH* and *CHS* upregulation at the root level. We therefore
372 conclude that the gene expression changes detected here in the roots of Arabidopsis under BL could
373 partially depend on the GMF-influence on cryptochrome activation.

374 The finding that cry1 phosphorylation was practically absent in WT, *phot1* and *phyAphyB*
375 mutant lines exposed to BL under NNMF conditions (Figure 1) is in contrast with the recent results
376 that report a lack of difference in cry1 phosphorylation between NNMF and GMF [38]. However, in
377 our experiments, we used a higher fluence rate of BL that allowed us to visualize the GMF influence
378 on cry1 phosphorylation. Our findings also suggest that this impact of the GMF on cry1
379 phosphorylation occurs independently from phot1 and phytochrome. However, cryptochrome

magneto-sensitivity in plants has been hypothesized to play a crucial ecological role by affecting cryptochrome signaling especially under low BL, such as those tested on cry2 activation [44]. In this regard, NNMF conditions almost abolish cry2 degradation, independent of phytochrome signaling (Figure 2). BL is known to reduce cry2 phosphorylation under NNMF [38]. Moreover, cry2 degradation is faster under a MF higher than the GMF [14], probably because of the increase in cry2 phosphorylation rate under high MF intensities [38].

Although there is little evidence to date to suggest that phot1 is involved in regulating gene expression [40], our data highlight that *PKS1* and *PIN3* regulation in the both the roots and shoots of Arabidopsis is partly dependent on phot1 in a GMF-dependent manner (Figure 6). In this regard, *PKS1* expression is known to be regulated by BL via phyA to mediate phototropic bending by phot1 [39], while PIN3 is involved in establishing phototropic curvature both in the shoot [45] and in the root [46]. However, the persistence of phot1 phosphorylation under NNMF conditions (Figure 3) indicates that the GMF appears not to affect phot1 signaling by changing phot1 phosphorylation and therefore its activation level.

Despite the minimal role of phyA in mediating BL regulation of gene expression [40], we observed a phytochrome-mediated regulation of *PIF3* and *PIN1* in the shoots and *PKS1* and *PIN3* in the roots the presence of the GMF (Figure 6). Interestingly, phyA is known to induce *PKS1* transcription under BL [39]. Therefore, the phytochrome-related change in *PSK1* expression level suggests the influence of the GMF on the phytochrome signaling under blue light.

4.3. The GMF influences red light photoreceptor signaling

The present study also shows that gene expression is affected by the GMF not only under BL, but also under RL (Figure 7). The observed GMF regulation of *HY5*, *LAF1*, *PKS1* and *PIF3*, whose gene expression is specifically connected to RL [47], implies that the GMF may affect phytochrome signaling. Moreover, RL treatment induced the regulation of genes related to auxin signaling and anthocyanin biosynthesis, which confirms a GMF effect on genes targeted by *PIF3*, *HY5* and *LAF1*

405 transcription factors during photomorphogenesis [35, 48]. Although *GST* transcript levels are
406 influenced by BL [37], our results shows that the GMF modulates the expression of *GST* in shoots
407 and roots only under RL, thus suggesting the existence of a possible *GST*-specific RL-dependent
408 magnetoreception mechanism.

409 The opposite trend of *CHS* expression changes observed in the roots versus the shoots under
410 GMF conditions (Figure 7) suggests that different response pathways exist in these two organs,
411 particularly under RL. Furthermore, the absence of GMF-induced changes in *HY5* expression levels
412 in the shoot appears to exclude the possible interference of shoot-localized HY5 on the abundance of
413 *HY5* transcripts in the roots, as recently reported [49].

414 The gene expression data obtained for *phyAphyB* seedlings additionally suggest that the GMF
415 impacts on phytochrome signaling (Figure 7). In particular, the observed down-regulation of *PKS1*
416 expression in the shoots might be phyA-dependent, since this gene is known to be specifically
417 regulated by phyA under red light [47]. Moreover, the observed down-regulation of *GST* in the root
418 could also be phyA-dependent, since phyB does not influence *GST* transcription under RL [37]. By
419 contrast, the up-regulation of *CHS* under GMF versus NNMF conditions could to be dependent on
420 phyB. The impact of phytochrome on *CHS* expression is known to be phyB-dependent under RL and
421 is induced by PIF3-promoted degradation [35]. Our western blot analysis suggests that these changes
422 in gene expression could be, in part mediated by the GMF influence on phytochrome activation.
423 Indeed, our data indicates that the GMF appears to positively affect phyB activation and negatively
424 affect phyA activation (Figures 4 and 5).

425 Our results suggest that GMF-mediated alterations in phytochrome signaling may also
426 dependent on cryptochromes and phot1 despite the fact that these photoreceptors are not activated by
427 RL. We found the presence of cryptochromes influenced the GMF-induced expression changes of
428 *PKS1*, *CHS* and *GST* in the shoots of Arabidopsis seedlings, as well as the expression of *NDPK2*,
429 *CHS* and *PIN3* in the root (Figure 7). Moreover, our data suggest that the presence of phot1
430 contributes to GMF-mediated changes in the expression of *PIF3*, *NDPK2* and *GST* both in the roots

and shoots of Arabidopsis seedlings under RL (Figure 7). The GMF regulation of some genes is dependent on phot1 or cryptochromes as is the case for *GST*, whose expression has been already reported to be influenced by the cryptochrome under RL [37]. For other genes such as *PKS1*, *PIN3* and *CHS* the regulation also involves phyA and phyB. Interestingly, the GMF-mediated changes in phytochrome activation levels seem to require the presence of cry1, cry2 and phot1 (Figures 4 and 5). Therefore, the effect of the GMF on phytochrome regulated genes may result from a modulation of phytochrome activation status that is also dependent on cryptochrome and phot1 signaling. Although Arabidopsis seedlings respond to the GMF under both dark and light conditions by altering photoreceptor signaling, we found that the GMF does not affect Arabidopsis skotomorphogenic and photomorphogenic development, at least under the conditions examined in the present study.

5. Conclusions

In conclusion, the results of this work highlight for the first time the influence of the GMF on photoreceptor signaling both under red and blue light. Overall, despite the absence of a GMF-induced changes in Arabidopsis seedling photomorphogenesis, our studies reveal a significant GMF-dependent differential shoot/root regulation of genes expressed following photoreceptor activation after 72 h exposure to GMF with respect to NNMF conditions. Under BL, the GMF regulation of gene expression appears to be partially dependent on cryptochrome activation, which is enhanced in terms of increased cry1 phosphorylation and cry2 degradation. Under RL, the GMF-dependent regulation of light-induced genes is partially mediated by phyA and phyB, whose activation is altered by cry1, cry2 and phot1 in their inactive form (Figure 8). Moreover, considering that the RL response to GMF is not limited to phyA and phyB [50], the contribution of other phytochromes to this response cannot be excluded. Therefore, despite the involvement of cryptochrome, and the possibility of a cryptochrome-based radical pair mechanisms, magnetoreception in Arabidopsis appears to be different from the mechanism thought to be responsible for the ability of migratory songbirds to detect the direction of the geomagnetic field. Our data also support the hypothesis for a possible light-

457 independent root magnetoreception mechanism. Therefore, Arabidopsis magnetoreception alters
458 photoreceptor signaling, but that is does not necessarily depend on light. Other processes besides
459 photoreceptor activation are probably involved in GMF perception and studies are under way to better
460 evaluate this aspect.
461

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469 **CONFLICT OF INTEREST**

470 The authors declare no conflict of interest.

471 **REFERENCES**

472 [1] A. Occhipinti, A. De Santis, M.E. Maffei, Magnetoreception: An Unavoidable Step for Plant
473 Evolution?, Trends Plant Sci, 19 (2014) 1-4.
474 [2] M.E. Maffei, Magnetic Field Effects on Plant Growth, Development, and Evolution, Front Plant
475 Sci, 5 (2014) 445.
476 [3] C.M. Bertea, R. Narayana, C. Agliassa, C.T. Rodgers, M.E. Maffei, Geomagnetic Field (Gmf)
477 and Plant Evolution: Investigating the Effects of Gmf Reversal on *Arabidospis Thaliana*
478 Development and Gene Expression, J Visual Exp, 105 (2015) e53286.

[4] C.X. Xu, X. Yin, Y. Lv, C.Z. Wu, Y.X. Zhang, T. Song, A near-Null Magnetic Field Affects Cryptochrome-Related Hypocotyl Growth and Flowering in Arabidopsis, Adv Space Res, 49 (2012) 834-840.

[5] C. Agliassa, R. Narayana, C.M. Bertea, C.T. Rodgers, M.E. Maffei, Reduction of the Geomagnetic Field Delays *Arabidopsis Thaliana* Flowering Time through Downregulation of Flowering-Related Genes, Bioelectromagnetics, In press (2018).

[6] P. Galland, A. Pazur, Magnetoreception in Plants, J Plant Res, 118 (2005) 371-389.

[7] V.C. Galvao, C. Fankhauser, Sensing the Light Environment in Plants: Photoreceptors and Early Signaling Steps, Current Opinion in Neurobiology, 34 (2015) 46-53.

[8] A. Zeugner, M. Byrdin, J.P. Bouly, N. Bakrim, B. Giovani, K. Brettel, M. Ahmad, Light-Induced Electron Transfer in Arabidopsis Cryptochrome-1 Correlates with in Vivo Function, J. Biol. Chem, 280 (2005) 19437-19440.

[9] P.J. Hore, H. Mouritsen, The Radical-Pair Mechanism of Magnetoreception, Annu Rev Biophys., 45 (2016) 299-344.

[10] I.A. Solov'yov, D.E. Chandler, K. Schulten, Magnetic Field Effects in Arabidopsis Thaliana Cryptochrome-1, Biophysical Journal, 92 (2007) 2711-2726.

[11] C. Xu, Y. Li, Y. Yu, Y. Zhang, S. Wei, Suppression of Arabidopsis Flowering by near-Null Magnetic Field Is Affected by Light, Bioelectromagnetics, 36 (2015) 476-479.

[12] C.X. Xu, Y.X. Zhang, Y. Yu, Y. Li, S.F. Wei, Suppression of Arabidopsis Flowering by near-Null Magnetic Field Is Mediated by Auxin, Bioelectromagnetics, 39 (2018) 15-24.

[13] C. Xu, Y. Yu, Y. Zhang, Y. Li, S. Wei, Gibberellins Are Involved in Effect of near-Null Magnetic Field on Arabidopsis Flowering, Bioelectromagnetics, 38 (2017) 1-10.

[14] M. Ahmad, P. Galland, T. Ritz, R. Wiltschko, W. Wiltschko, Magnetic Intensity Affects Cryptochrome-Dependent Responses in *Arabidopsis Thaliana*, Planta, 225 (2007) 615-624.

503 [15] S.R. Harris, K.B. Henbest, K. Maeda, J.R. Pannell, C.R. Timmel, P.J. Hore, H. Okamoto, Effect
504 of Magnetic Fields on Cryptochrome-Dependent Responses in *Arabidopsis Thaliana*, J Royal Soc
505 Interf, 6 (2009) 1193-1205.
506 [16] J.M. Christie, Phototropin Blue-Light Receptors, Annual Review of Plant Biology, 58 (2007)
507 21-45.
508 [17] J.M. Christie, L. Blackwood, J. Petersen, S. Sullivan, Plant Flavoprotein Photoreceptors, Plant
509 and Cell Physiology, 56 (2015) 401-413.
510 [18] J. Su, B.B. Liu, J.K. Liao, Z.H. Yang, C.T. Lin, Y. Oka, Coordination of Cryptochrome and
511 Phytochrome Signals in the Regulation of Plant Light Responses, Agronomy-Basel, 7 (2017).
512 [19] S. Sullivan, J.E. Hart, P. Rasch, C.H. Walker, J.M. Christie, Phytochrome a Mediates Blue-Light
513 Enhancement of Second-Positive Phototropism in Arabidopsis, Front Plant Sci, 7 (2016).
514 [20] T. Murashige, F. Skoog, A Revised Medium for Rapid Growth and Bioassays with Tobacco
515 Tissue Cultures, Physiol. Plant, 15 (1962) 473-497.
516 [21] G. Weidler, S. zur Oven-Krockhaus, M. Heunemann, C. Orth, F. Schleifenbaum, K. Harter, U.
517 Hoecker, A. Batschauer, Degradation of Arabidopsis Cry2 Is Regulated by Spa Proteins and
518 Phytochrome A, Plant Cell, 24 (2012) 2610-2623.
519 [22] S. Sullivan, C.E. Thomson, D.J. Lamont, M.A. Jones, J.M. Christie, In Vivo Phosphorylation
520 Site Mapping and Functional Characterization of Arabidopsis Phototropin 1, Molecular Plant, 1
521 (2008) 178-194.
522 [23] D. Debrieux, M. Trevisan, C. Fankhauser, Conditional Involvement of Constitutive
523 Photomorphogenic1 in the Degradation of Phytochrome A, Plant Physiology, 161 (2013) 2136-2145.
524 [24] D. Shalitin, X.H. Yu, M. Maymon, T. Mockler, C.T. Lin, Blue Light-Dependent in Vivo and in
525 Vitro Phosphorylation of Arabidopsis Cryptochrome 1, Plant Cell, 15 (2003) 2421-2429.
526 [25] R. Yamaguchi, M. Nakamura, N. Mochizuki, S.A. Kay, A. Nagatani, Light-Dependent
527 Translocation of a Phytochrome B-Gfp Fusion Protein to the Nucleus in Transgenic Arabidopsis,
528 Journal of Cell Biology, 145 (1999) 437-445.

529 [26] M. Ahmad, J.A. Jarillo, O. Smirnova, A.R. Cashmore, The Cry1 Blue Light Photoreceptor of
530 Arabidopsis Interacts with Phytochrome a in Vitro, Mol Cell, 1 (1998) 939-948.
531 [27] O. Kleiner, S. Kircher, K. Harter, A. Batschauer, Nuclear Localization of the Arabidopsis Blue
532 Light Receptor Cryptochrome 2, Plant Journal, 19 (1999) 289-296.
533 [28] H.Y. Cho, T.S. Tseng, E. Kaiserli, S. Sullivan, J.M. Christie, W.R. Briggs, Physiological Roles
534 of the Light, Oxygen, or Voltage Domains of Phototropin 1 and Phototropin 2 in Arabidopsis, Plant
535 Physiology, 143 (2007) 517-529.
536 [29] B. Kang, N. Grancher, V. KoyVmann, D. Lardemer, S. Burney, M. Ahmad, Multiple Interactions
537 between Cryptochrome and Phototropin Blue-Light Signalling Pathways in Arabidopsis Thaliana,
538 Planta, 227 (2008) 1091-1099.
539 [30] A. Viczian, C. Klose, E. Adam, F. Nagy, New Insights of Red Light-Induced Development, Plant
540 Cell and Environment, 40 (2017) 2457-2468.
541 [31] H.J. Lee, Y.J. Park, J.H. Ha, I.T. Baldwin, C.M. Park, Multiple Routes of Light Signaling During
542 Root Photomorphogenesis, Trends in Plant Science, 22 (2017) 803-812.
543 [32] Y.H. Kim, M.D. Kim, Y.I. Choi, S.C. Park, D.J. Yun, E.W. Noh, H.S. Lee, S.S. Kwak,
544 Transgenic Poplar Expressing Arabidopsis Ndpk2 Enhances Growth as Well as Oxidative Stress
545 Tolerance, Plant Biotechnology Journal, 9 (2011) 334-347.
546 [33] O.A. Kuznetsov, J. Schwuchow, F.D. Sack, K.H. Hasenstein, Curvature Induced by Amyloplast
547 Magnetophoresis in Protonemata of the Moss *Ceratodon Purpureus*, Plant Physiol, 119 (1999) 645-
548 650.
549 [34] J. Lee, K. He, V. Stolc, H. Lee, P. Figueroa, Y. Gao, W. Tongprasit, H.Y. Zhao, I. Lee, X. Deng,
550 Analysis of Transcription Factor Hy5 Genomic Binding Sites Revealed Its Hierarchical Role in Light
551 Regulation of Development, Plant Cell, 19 (2007) 731-749.
552 [35] J. Shin, E. Park, G. Choi, Pif3 Regulates Anthocyanin Biosynthesis in an Hy5-Dependent in an
553 Hy5-Dependent Manner with Both Factors Directly Binding Anthocyanin Biosynthetic Gene
554 Promoters in Arabidopsis (Vol 49, Pg 961, 2007), Plant Journal, 50 (2007) 933-933.

[36] M. Sassi, Y.F. Lu, Y.H. Zhang, J. Wang, P. Dhonukshe, I. Blilou, M.Q. Dai, J. Li, X.M. Gong, Y. Jaillais, X.H. Yu, J. Traas, I. Ruberti, H.Y. Wang, B. Scheres, T. Vernoux, J. Xu, Cop1 Mediates the Coordination of Root and Shoot Growth by Light through Modulation of Pin1-and Pin2-Dependent Auxin Transport in Arabidopsis, Development, 139 (2012) 3402-3412.

[37] H.W. Jiang, M.J. Liu, I.C. Chen, C.H. Huang, L.Y. Chao, H.L. Hsieh, A Glutathione S-Transferase Regulated by Light and Hormones Participates in the Modulation of Arabidopsis Seedling Development, Plant Physiology, 154 (2010) 1646-1658.

[38] C. Xu, Y. Lv, C. Chen, Y. Zhang, S. Wei, Blue Light-Dependent Phosphorylations of Cryptochromes Are Affected by Magnetic Fields in *Arabidopsis*, Adv. Space Res, 53 (2014) 1118-1124.

[39] P. Lariguet, I. Schepens, D. Hodgson, U.V. Pedmale, M. Trevisan, C. Kami, M. de Carbonnel, J.M. Alonso, J.R. Ecker, E. Liscum, C. Fankhauser, Phytochrome Kinase Substrate 1 Is a Phototropin 1 Binding Protein Required for Phototropism, Proceedings of the National Academy of Sciences of the United States of America, 103 (2006) 10134-10139.

[40] Y. Jiao, H. Yang, L. Ma, N. Sun, H. Yu, T. Liu, Y. Gao, H. Gu, Z. Chen, M. Wada, M. Gerstein, H. Zhao, L.J. Qu, X.W. Deng, A Genome-Wide Analysis of Blue-Light Regulation of Arabidopsis Transcription Factor Gene Expression During Seedling Development, Plant Physiol, 133 (2003) 1480-1493.

[41] Y.H. Zhang, C. Li, J.X. Zhang, J.J. Wang, J.W. Yang, Y.X. Lv, N.A. Yang, J.P. Liu, X.B. Wang, G. Palfalvi, G.D. Wang, L.L. Zheng, Dissection of Hy5/Hyh Expression in Arabidopsis Reveals a Root-Autonomous Hy5-Mediated Photomorphogenic Pathway, Plos One, 12 (2017).

[42] L.G. Ma, J.M. Li, L.J. Qu, J. Hager, Z.L. Chen, H.Y. Zhao, X.W. Deng, Light Control of Arabidopsis Development Entails Coordinated Regulation of Genome Expression and Cellular Pathways, Plant Cell, 13 (2001) 2589-2607.

[43] C.P. Cluis, C.F. Mouchel, C.S. Hardtke, The Arabidopsis Transcription Factor Hy5 Integrates Light and Hormone Signaling Pathways, Plant Journal, 38 (2004) 332-347.

[44] J. Vanderstraeten, P. Gailly, E.P. Malkemper, Low-Light Dependence of the Magnetic Field Effect on Cryptochromes: Possible Relevance to Plant Ecology, Front Plant Sci, 9 (2018).

[45] Z.J. Ding, C.S. Galvan-Ampudia, E. Demarsy, L. Langowski, J. Kleine-Vehn, Y.W. Fan, M.T. Morita, M. Tasaka, C. Fankhauser, R. Offringa, J. Friml, Light-Mediated Polarization of the Pin3 Auxin Transporter for the Phototropic Response in Arabidopsis, Nature Cell Biology, 13 (2011) 447-U222.

[46] K.X. Zhang, H.H. Xu, T.T. Yuan, L. Zhang, Y.T. Lu, Blue-Light-Induced Pin3 Polarization for Root Negative Phototropic Response in Arabidopsis, Plant Journal, 76 (2013) 308-321.

[47] J.M. Tepperman, Y.S. Hwang, P.H. Quail, Phya Dominates in Transduction of Red-Light Signals to Rapidly Responding Genes at the Initiation of Arabidopsis Seedling De-Etiolation, Plant Journal, 48 (2006) 728-742.

[48] B.S. Park, W.G. Sang, J.T. Song, B.H. Lee, J.H. Kim, H.S. Seo, Auxin Is Involved in the Regulation of Leaf and Root Development by Laf1 under Short Day Conditions, Biol Plantarum, 55 (2011) 647-652.

[49] X.B. Chen, Q.F. Yao, X.H. Gao, C.F. Jiang, N.P. Harberd, X.D. Fu, Shoot-to-Root Mobile Transcription Factor Hy5 Coordinates Plant Carbon and Nitrogen Acquisition, Current Biology, 26 (2016) 640-646.

[50] A.R. Jeong, S.S. Lee, Y.J. Han, A.Y. Shin, A. Baek, T. Ahn, M.G. Kim, Y.S. Kim, K.W. Lee, A. Nagatani, J.I. Kim, New Constitutively Active Phytochromes Exhibit Light-Independent Signaling Activity, Plant Physiology, 171 (2016) 2826-2840.

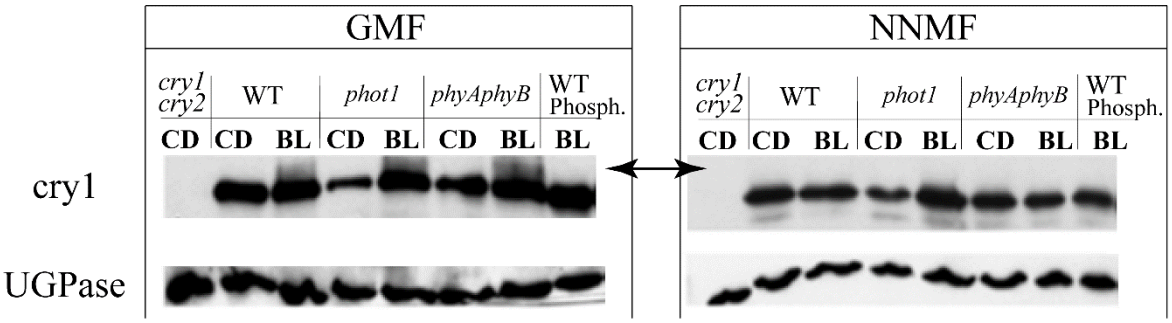
Table 1. GMF-dependent shoot and root gene expressions in 3-day-old etiolated *Arabidopsis* WT seedlings grown for 72 h under either GMF or NNMF conditions using different light conditions. Data are expressed as fold changes (mean ± SD) with respect to NNMF (i.e., GMF/NNMF).

Function	***Gene***	**CD**		**CW**	
		Shoot	*Root*	*Shoot*	*Root*
Transcription factors regulated by COP1/SPA1 complex	*HYH*	**2.00 (± 0.00)**	**1.45 (± 0.36)**	**-1.58 (± 0.06)**	**-1.41 (± 0.12)**
	HY5	-1.35 (± 0.47)	1.22 (± 0.19)	1.08 (± -0.13)	**-1.61 (± 0.06)**
	LAF1	n.e.	**-1.30 (± 0.09)**	n.e.	1.06 (± -0.16)
Phytochrome-related factors	*PKS1*	-1.28 (± 0.03)	-1.08 (± 0.11)	**-1.91 (± 0.03)**	1.23 (± -0.15)
	PIF3	1.32 (± 0.26)	1.08 (± 0.1)	-1.10 (± 0.12)	-1.07 (± 0.18)
	**NDPK2*	-1.50 (± 0.26)	**-3.42 (± 0.51)**	1.14 (± -0.17)	**-2.09 (± 0.35)**
Anthocyanin biosynthesis	*ANS*	n.e.	1.11 (± 0.19)	**3.85 (± -1.04)**	-1.02 (± 0.12)
	CHS	n.e.	1.16 (± 0.41)	-1.43 (± 0.13)	-1.70 (± 0.13)
Auxin signaling	*PIN1*	-1.03 (± 0.04)	1.22 (± 0.09)	-1.09 (± 0.41)	-1.17 (± 0.07)
	PIN3	1.72 (± 0.48)	1.02 (± 0.04)	1.01 (± -0.2)	**1.25 (± -0.05)**
Oxidative response	*GST*	-1.59 (± 0.44)	-1.59 (± 0.44)	**2.04 (± -0.17)**	**-2.68 (± 1.01)**

Boldfaced numbers indicate a significant ($P < 0.05$) difference between NNMF and GMF treatment. CD, continuous darkness; CW, continuous white light; n.e.= not expressed; *= this gene is also associated to the oxidative response.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.



618

619 **Figure 1.** cry1 phosphorylation level in 3-day-old WT, *phot1* and *phyAphyB* etiolated seedlings
620 exposed to either GMF or NNMF conditions and grown either in continuous darkness (CD) or under
621 20 μmol m^{-2} s^{-1} blue light (BL) for 15 min. Arrows indicate the position of the phosphorylated cry1
622 protein. Phosph., phosphatase treatment. UGPase, loading control.

623

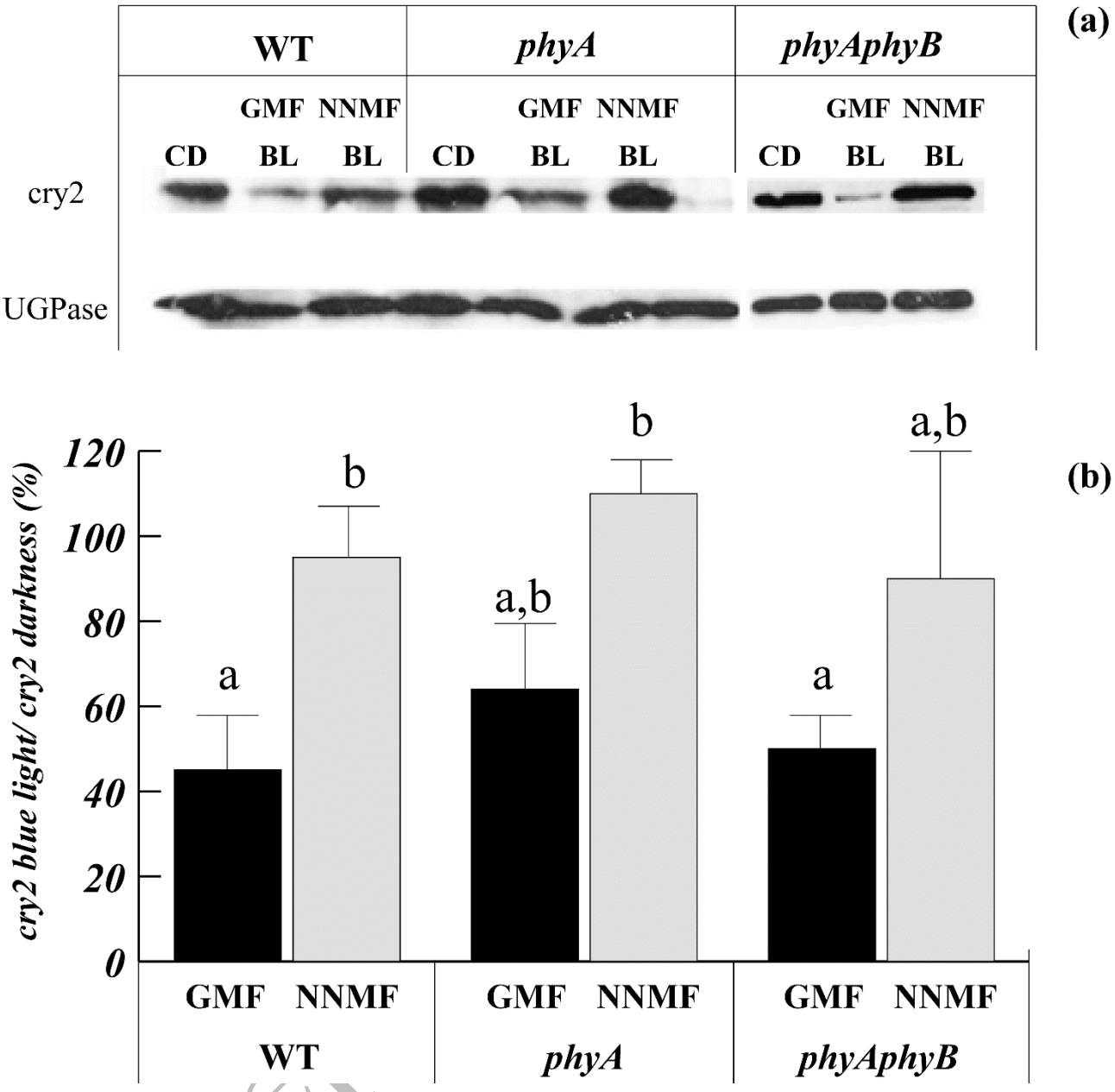


Figure 2. cry2 degradation in 3-day-old WT, *phyA* and *phyAphyB* etiolated seedlings exposed to either GMF or NNMF conditions under either continuous darkness (CD) or 0.5 μmol m^{-2} s^{-1} blue light (BL) for 8 h. (**a**) Western blot analysis with anti-cry2 antibody and anti-UGPase antibody. (**b**) Western blot image analysis expressed as the percentage of cry2 protein quantity after the blue light treatment with respect to dark controls. Bars indicate SD. Different letters in the same group indicate significant ($P < 0.05$). differences.

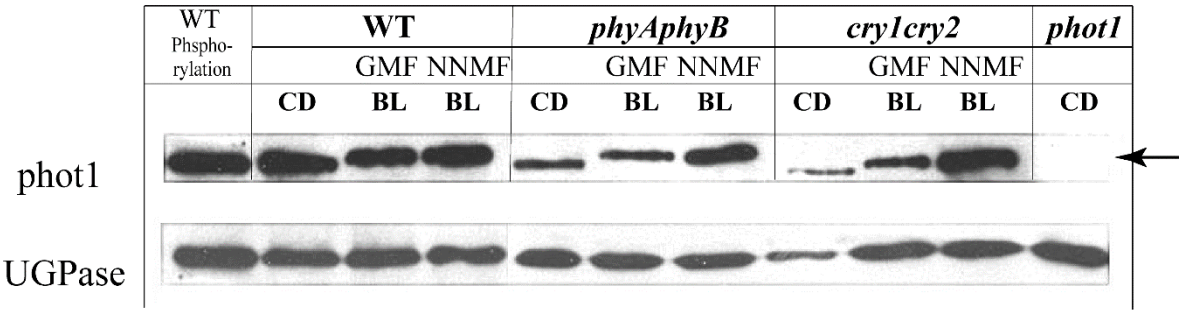
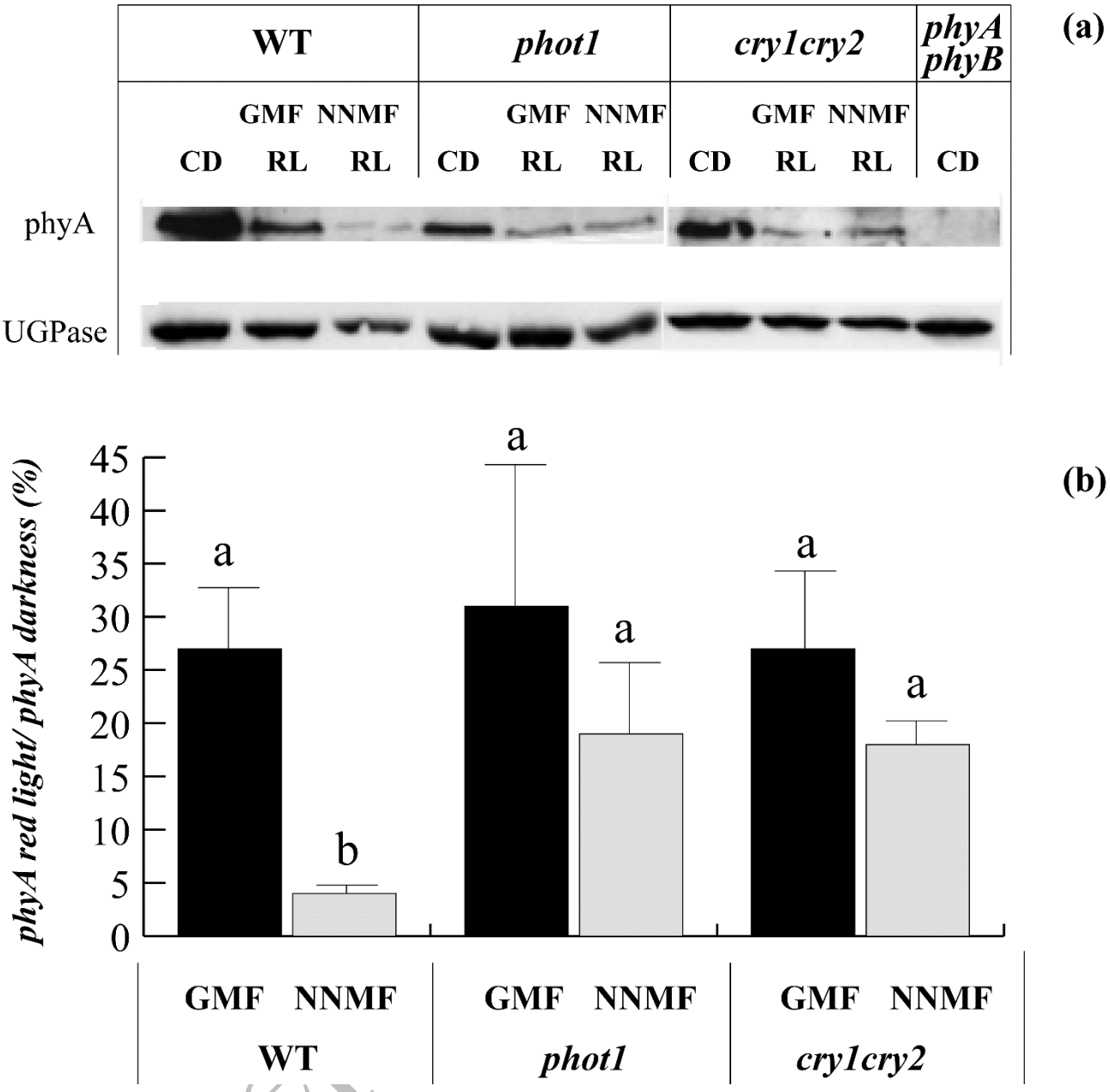


Figure 3. phot1 phosphorylation in 3-day-old WT, *phot1*, *phyAphyB* etiolated seedlings exposed to either GMF or NNMF conditions under either continuous darkness (CD) or 20 μmol m^{-2} s^{-1} blue light (BL) for 15 min. The arrow indicates the position of the phosphorylated protein. UGPase, loading control.



639

640 **Figure 4.** phyA degradation in 3-day-old WT, *phot1*, *cry1cry2* and *phyAphyB* etiolated seedlings
641 exposed to either GMF or NNMF conditions under either continuous darkness (CD) or 60 μmol m^{-2}
642 s^{-1} red light (RL) for 3 h. (**a**) Western blot analysis with anti-phyA antibody and anti-UGPase
643 antibody. (**b**) Western blot image analysis expressed as the percentage of phyA protein quantity after
644 the red-light treatment with respect to dark controls. Bars indicate SD. Different letters in the same
645 group indicate significant ($P < 0.05$). differences.

646

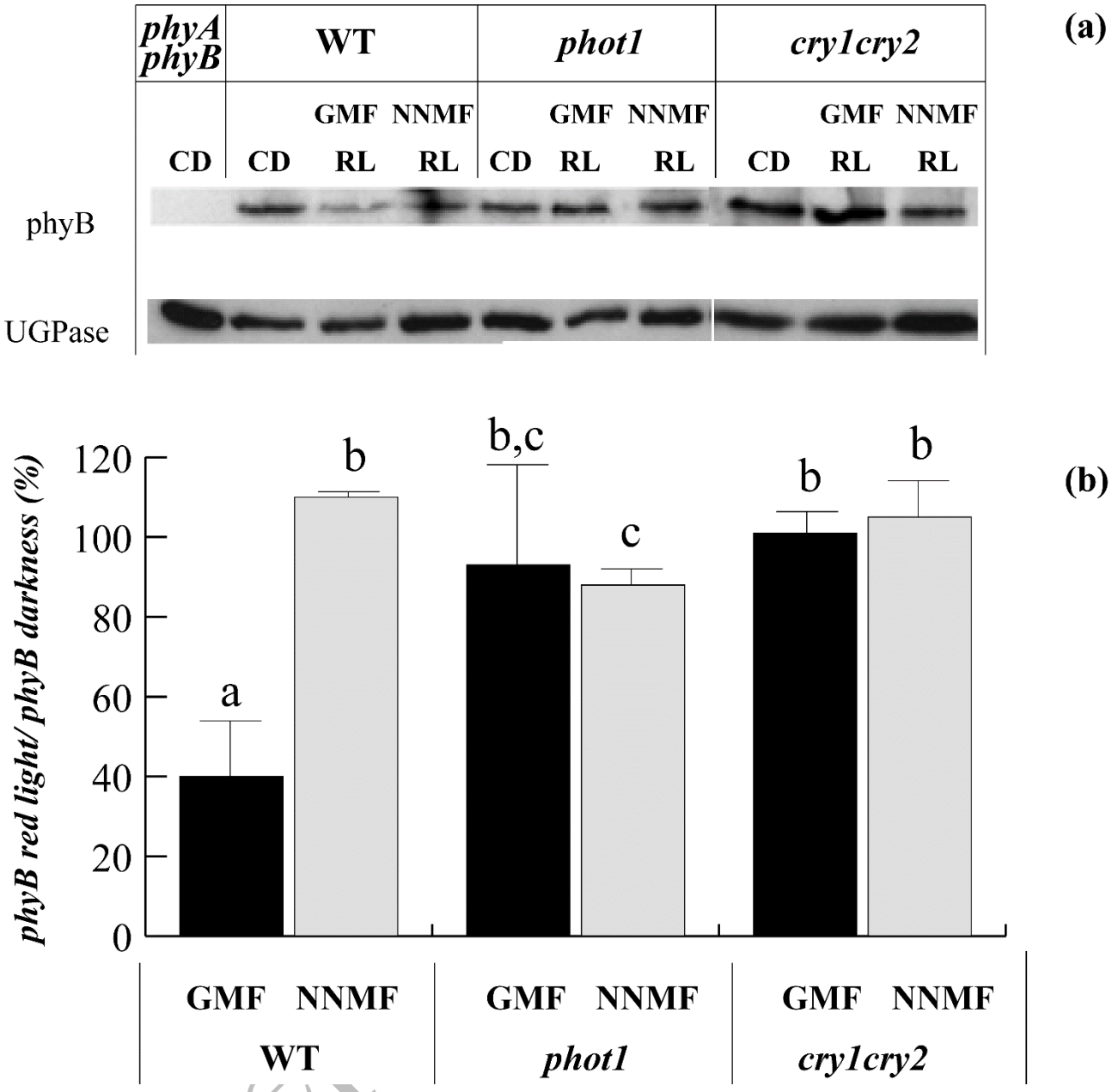


Figure 5. phyB degradation in 3-day-old WT, *phot1*, *cry1cry2* and *phyAphyB* etiolated seedlings exposed to either GMF or NNMF conditions under either continuous darkness (CD) or 60 μmol m^{-2} s^{-1} red light (RL) for 3 h. (**a**) Western blot analysis with anti-phyB antibody and anti-UGPase antibody. (**b**) Western blot image analysis expressed as the percentage of phyB protein quantity after the red-light treatment with respect to dark controls. Bars indicate SD. Different letters in the same group indicate significant ($P < 0.05$). differences.

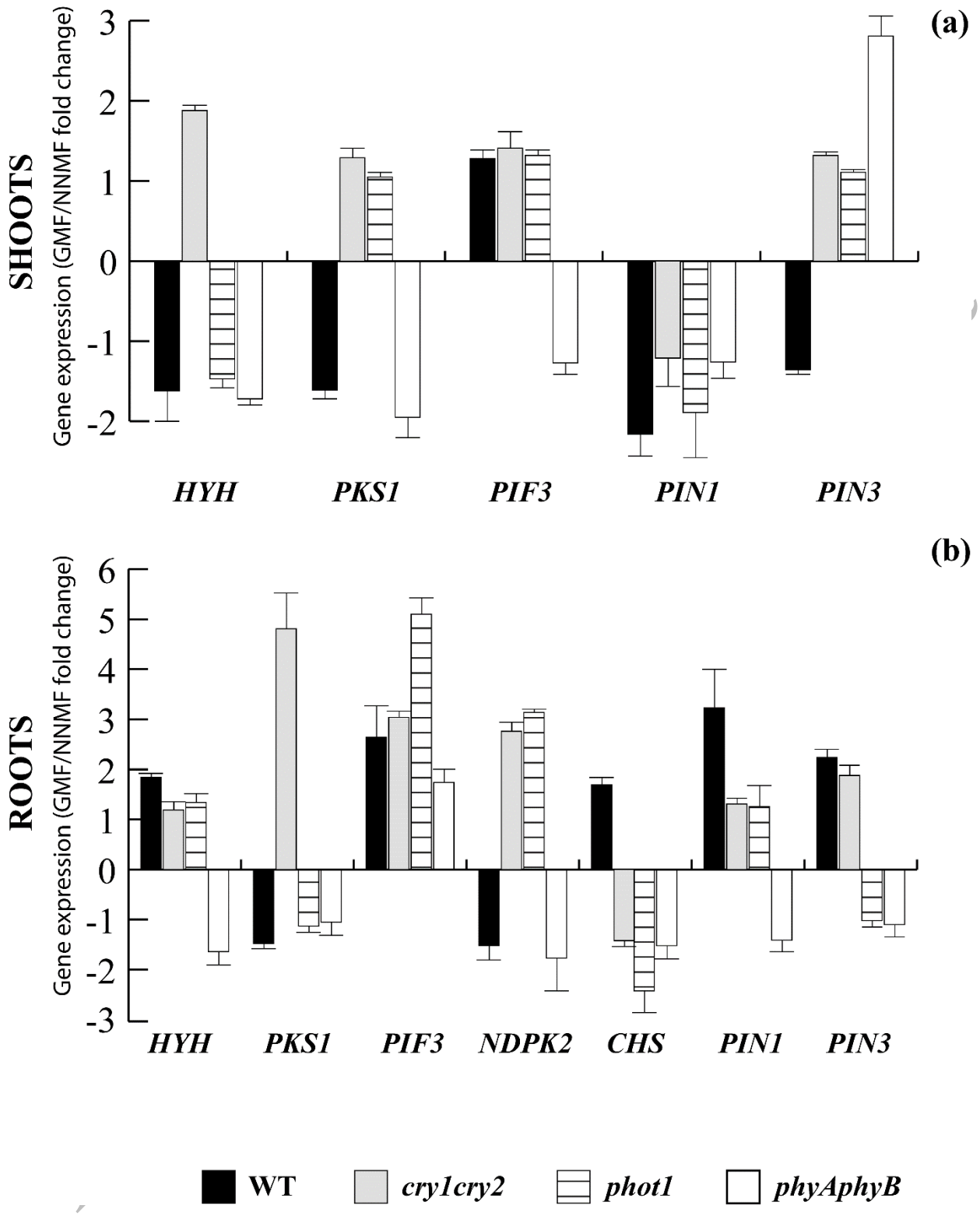
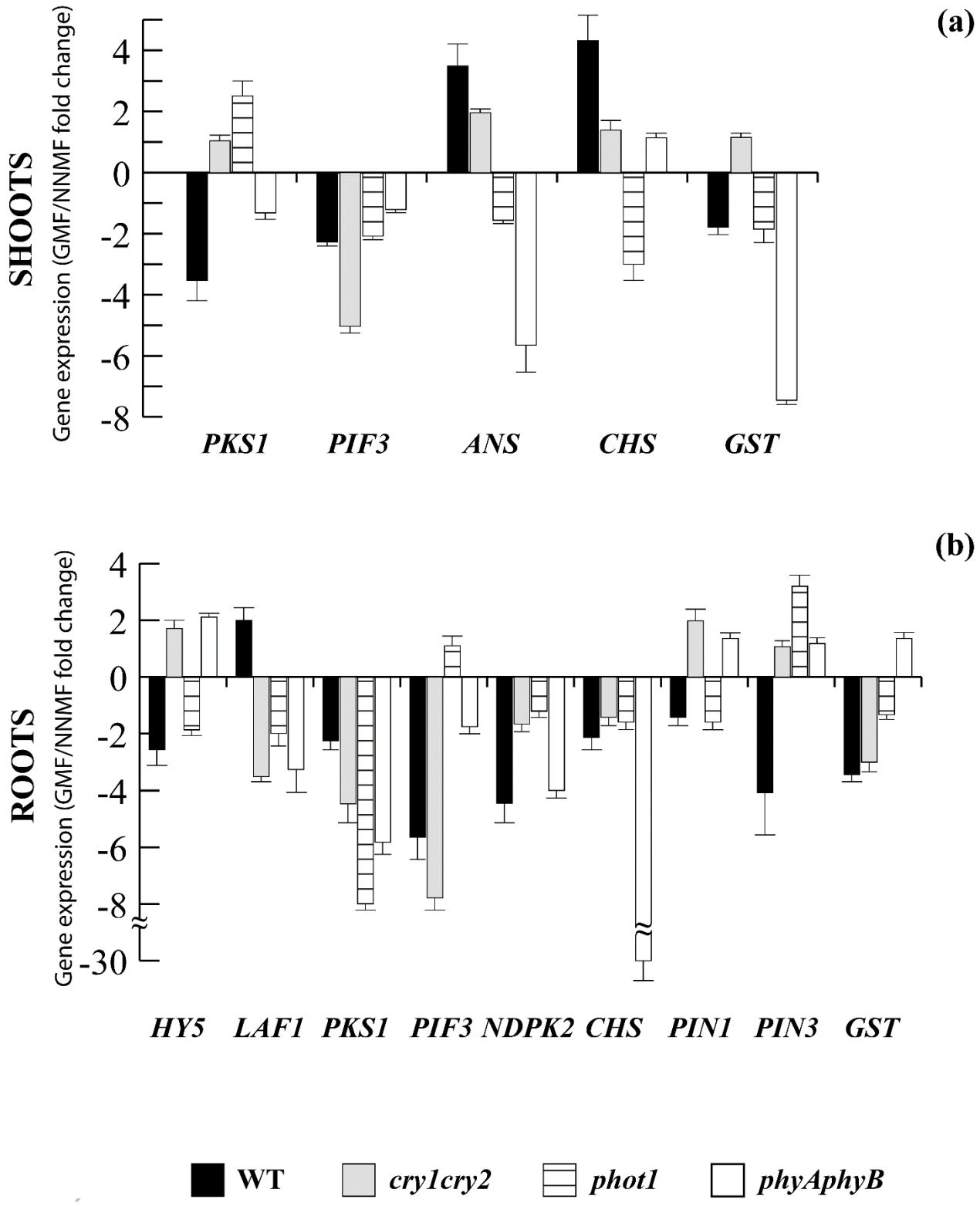
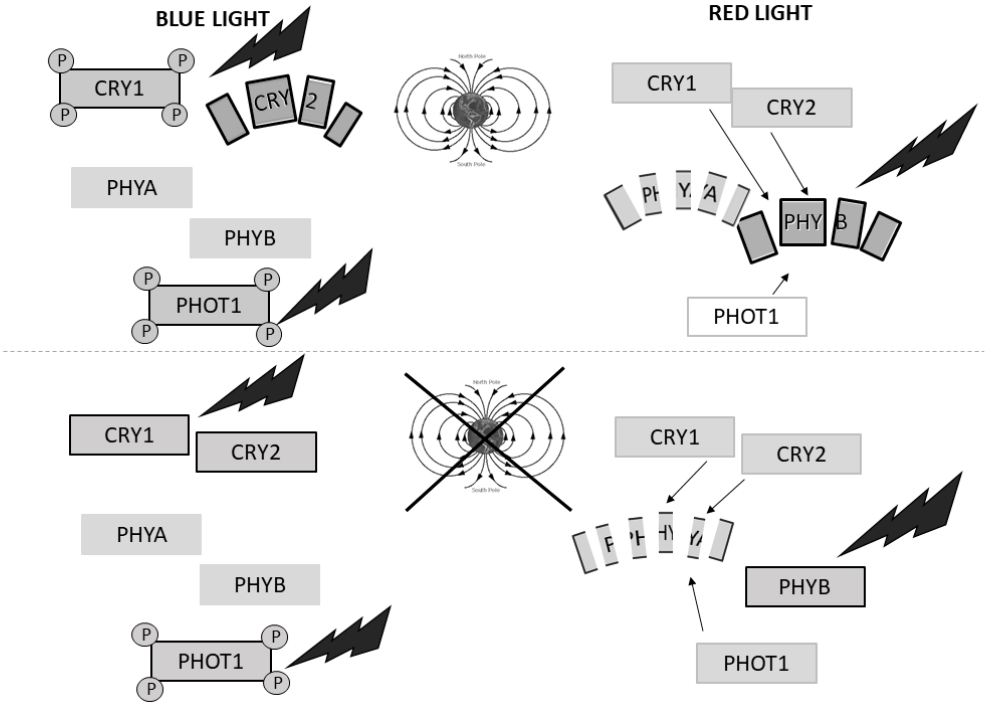


Figure 6. GMF effects on the expression of gene targets in either the shoots (**a**) or roots (**b**) of 3-day-old etiolated *Arabidopsis WT, cry1cry2, phot1* and *phyAphyB* seedlings grown for 72 h in the presence of GMF or NNMF conditions under continuous blue light. Data are expressed as fold changes (mean ± SD) with respect to NNMF conditions (i.e., GMF/NNMF). Bars indicate SD.



660

661 **Figure 7.** GMF effect on the expression of gene targets in either the shoots (**a**) or roots (**b**) of 3-day-
662 old etiolated *Arabidopsis WT, cry1cry2, phot1* and *phyAphyB* seedlings grown for 72 h in the presence
663 of GMF or NNMF conditions under continuous red light. Data are expressed as fold changes (mean
664 ± SD) with respect to NNMF conditions (i.e., GMF/NNMF). Bars indicate SD.



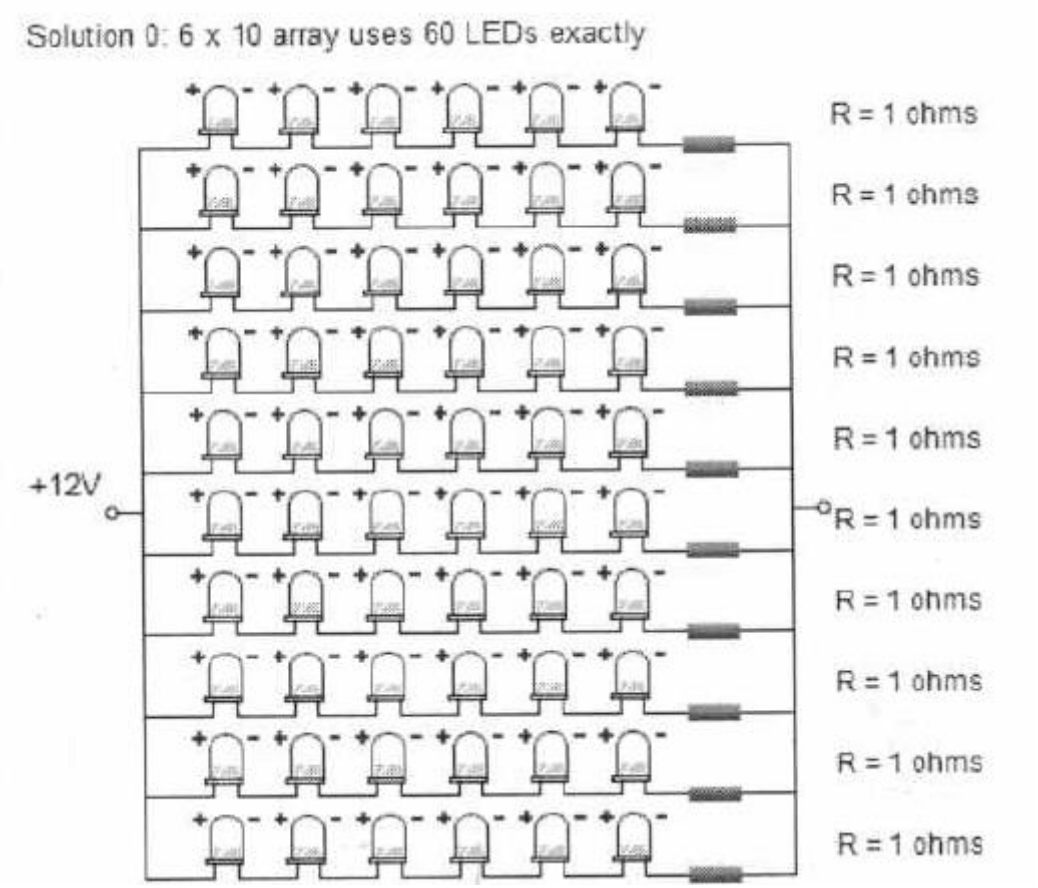
665

666 **Figure 8.** Geomagnetic field influence on photoreceptor activation and signaling. Under blue light,
667 the GMF regulation of gene expression is mainly dependent on cryptochromes, whose activation is
668 enhanced in terms of increased cry1 phosphorylation and cry2 degradation. By contrast, phot1
669 phosphorylation is not affected by the GMF. Under red light, cry1 and phot1 in their inactive form
670 contribute to the GMF-dependent increase in phyB activation and the GMF-dependent decrease in
671 phyA: phyB degradation is indeed enhanced by the GMF, whereas that of phyA is enhanced under
672 NNMF conditions.

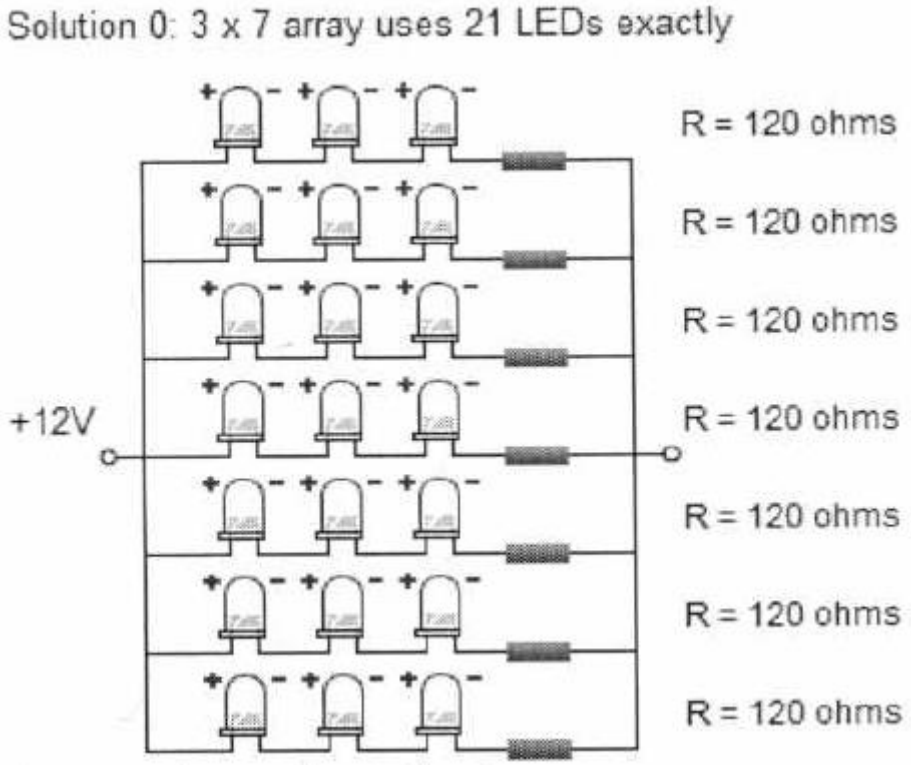
673

Supplementary Figure S1. Circuitry and spectral analysis of LEDs

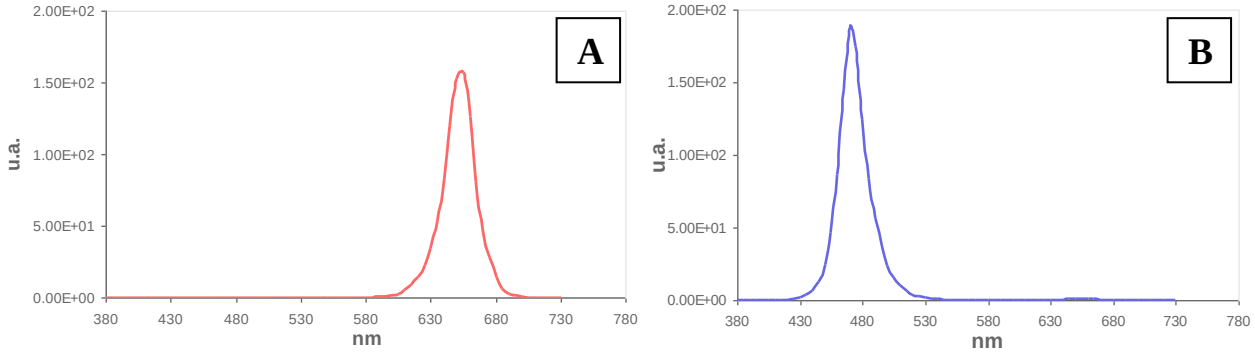
LEDs were arranged in arrays as depicted below, according to the manufacturer's instructions. Red LEDs were assembles using the following scheme:



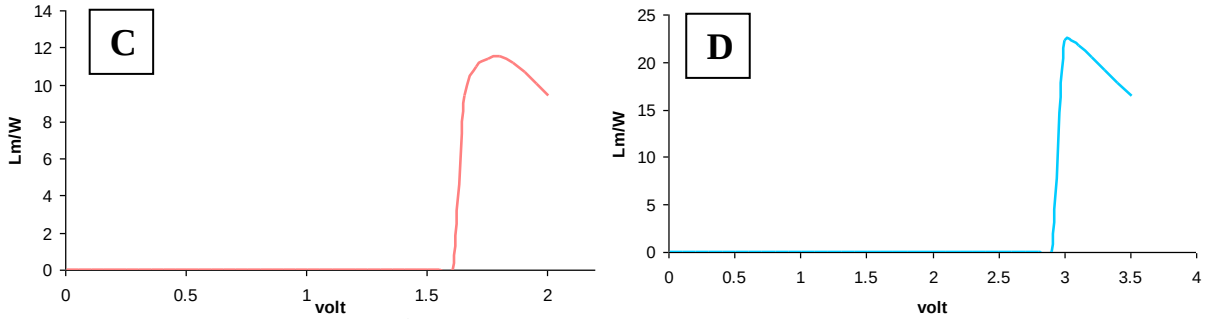
Blue LEDs were assembles using the following scheme:



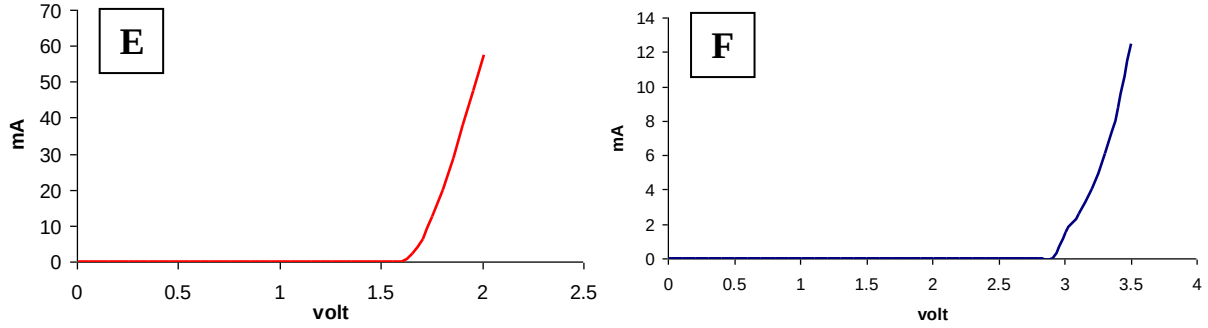
The determination of the emission wavelength was accomplished by means of spectroradiometry by measuring the radiation emitted on a whithe plane and directly from the LEDs.
The red LEDs showed a peak emission at 655 nm (Figure A), whereas blue LEDs had a peak emission at 470 nm (Figure B) (u.a., arbitrary units).



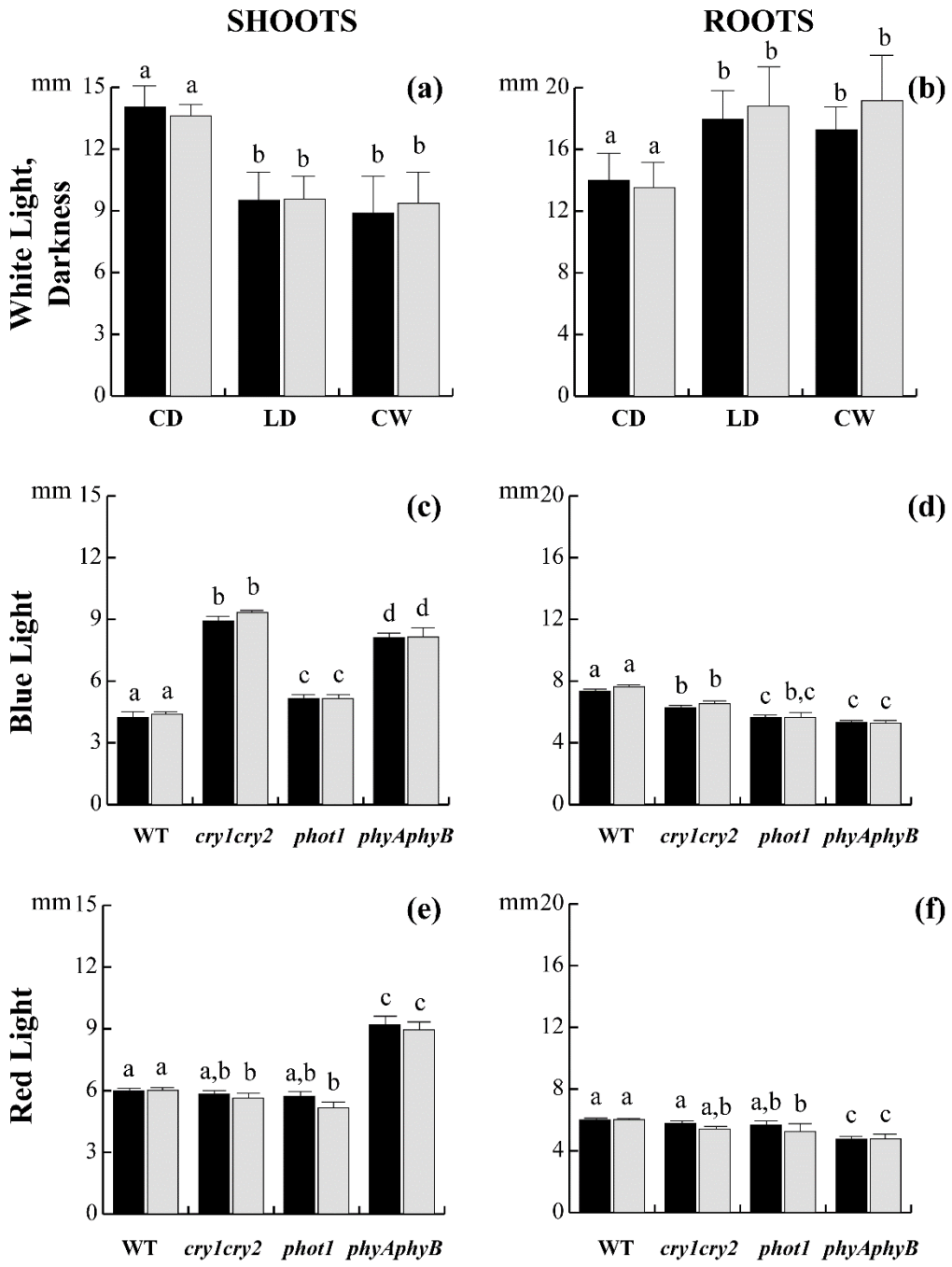
The light efficiencywas measured on individual LEDs by using an integrating sphere.
The luminance, expressed as Lm W^{-1} as a function of the applied tension, is shown for red LEDs (Figure C) and for blue LEDs (Figure D).



Figures E and F, show the I V^{-1} ratio values as a function of applied tension in red and blue LEDs, respectively.



Supporting Figure S2



Morphometric measurements of *Arabidopsis thaliana* WT, *cry1cry2*, *phot1* and *phyAphyB* mutant line seedlings grown under different light conditions for 72 h either in the GMF (black columns) or NNMF (grey columns) conditions. (**a**) WT shoots, (**b**) WT roots, (**c**) blue light exposed shoots, (**d**) blue light exposed roots, (**e**) red light exposed shoots, (**f**) red light exposed roots.

Lengths are reported as mean values (bars indicate SD). *CD* (continuous darkness); *LD* (Long -day white light); *CW* (continuous white light). Different letters in the same group indicate significant ($P < 0.05$) differences.

Supplementary Table S1. Primers used in quantitative real time PCR experiments

Gene code	Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
At4g22880	*ANS*	CTAACAACGCGAGTGGACAA	ACCGACAGAGAGAGCCTTGA
At5g13930	*CHS*	GGCTCAGAGAGCTGATGGAC	CATGTGACGTTTCCGAATTG
At5g15840	*CO*	ATTCTGCAAACCCACTTGCT	CCTCCTTGGCATCCTTATCA
At1g68050	*FKF1*	CTAAGGTCAGGGGAGGCATAC	ACAGTTGCGAAGGAGAGTGAA
At1g10370	*GST*	AACCGGTGAGTGAGTCCAAC	AGCGACAAACCACTTTTCGT
At3g17609	*HYH*	TGATGAGGAGTTGTTGATGG	TGTTGCGCTGATACTCTGTT
At5g11260	*HY5*	ATCAAGCAGCGAGAGGTCAT	CGACAGCTTCTCCTCCAAAC
At4g25560	*LAF1*	ATGGCGAAGACGAAATATGG	GCTTTGATGGGAACAGTGGT
At2g18915	*LKP2*	CGATGCTCTTGAACCTGACA	CCT TGAAACTCGATGCCATT
At5g63310	*NDPK2*	TCCGTCTTTTCTCTCGCAAT	TGCTCCTCAGCCAATTCTTT
At1g09530	*PIF3*	GACTATGGTGGACGAGATCCCTAT	GACAGTAACAGGAGACGACACATC
At1g73590	*PIN1*	AACCACCACGCCGAATTACTC	CACCGTCCGTTGCCAATACT
At1g70940	*PIN3*	GCCGAAGCAAGTCAACGAAA	AGCGACGAGAGCCCAAATAA
At2g02950	*PKS1*	TTGGTGTGTTTGGAGCTGAG	GAGTCGACGACGGTTCTCTC
	Housekeeping genes		
At2g37620	*ACT1*	TGCACTTCCACATGCTATCC	GAGCTGGTTTTGGCTGTCTC
At5g19510	*eEF1Balpha2*	ACTTGTACCAGTTGGTTATGGG	CTGGATGTACTCGTTGTTAGGC
At1g20010	*TUB5*	TGAATGCATGGTCCTCGACA	GCAAGTCACACCGCTCATTGT
At1g51710	*UBP6*	GAAAGTGGATTACCCGCTG	CTCTAAGTTTCTGGCGAGGAG

Supporting table 2. GMF contribution to **hypocotyl** and **root** gene expressions of 3-day-old etiolated *Arabidopsis* **WT, *cry1cry2, phot1*** and ***phyaphyb*** seedlings grown for 72 h under either GMF or NNMF conditions using **blue** light exposition. Data are expressed as fold changes (mean ± SD) with respect to NNMF conditions (i.e., GMF/NNMF).

Function	***Gene***	**WT**		***cry1cry2***		***phot1***		***phyAphyB***	
		Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
Transcription factors regulated by COP1/SPA1 complex	*HYH*	**-1.62 (± 0.37)**	**1.84(±0.08)**	**1.88 (±0.06)**	1.19(±0.18)	**-1.47(±0.11)**	**1.34(±0.09)**	**-1.71(±0.10)**	**-1.64(±0.21)**
	HY5	1.07(±0.20)	1.14(±0.13)	-1.22(±0.33)	-1.16(±0.07)	1.05(±0.07)	**-1.56(±0.04)**	**1.37(±0.20)**	**1.66(±0.06)**
	LAF1	n.e.	1.07(±0.32)	n.e.	-1.13(±0.22)	n.e.	1.44(±0.20)	n.e.	1.18(±0.32)
Phytochrome-related factors	*PKS1*	**-1.61 (± 0.10)**	**-1.48(±0.07)**	**1.29 (±0.11)**	**4.81(±0.76)**	1.05(±0.08)	-1.13(±0.06)	**-1.95(±0.39)**	-1.05(±0.19)
	PIF3	**1.28 (± 0.07)**	**2.64(±0.51)**	**1.41 (±0.26)**	**3.04(±0.06)**	**1.32(±0.10)**	**5.10(±0.31)**	-1.27(±0.17)	**1.74(±0.18)**
	**NDPK2*	-1.12(±039)	**-1.52(±0.29)**	-1.16(±0.17)	**2.16(±0.19)**	-1.14(±0.11)	**3.14(±0.03)**	**-2.17(±0.31)**	**-1.77(±0.60)**
Anthocyanin biosynthesis	*ANS*	1.11(±0.33)	1.17(±0.10)	-1.01(±0.48)	1.77(±0.81)	1.12(±0.09)	1.65(±0.72)	1.34(±0.13)	1.15(±0.21)
	CHS	1.67(±0.81)	**1.69(±0.14)**	5.44(±4.53)	-1.42(±0.12)	1.23(±0.21)	**-3.83(±0.32)**	1.11(±0.30)	**-2.16(±0.25)**
Auxin signaling	*PIN1*	**-2.16 (± 0.36)**	**3.23(±0.87)**	-1.21 (±0.43)	**1.31(±0.04)**	**-1.89(±0.10)**	1.26(±0.24)	-1.26(±0.25)	**-1.41(±0.22)**
	PIN3	**-1.36 (± 0.03)**	**2.24(±0.06)**	**1.32 (±0.01)**	**1.88(±0.17)**	1.11(±0.01)	-1.02(±0.08)	**2.81(±0.30)**	-1.10(±0.18)
Oxidative response	*GST*	1.24(±0.27)	1.16(±0.51)	-1.23(±0.39)	-1.20(±0.09)	**-1.76(±0.29)**	1.05(±0.07)	**1.23(±0.10)**	1.03 (±0.04)

Boldfaced numbers indicate a significant ($p<0.05$) difference between NNMF and GMF treatment; *= this gene is associated to the oxidative response either.

Supporting Table S3. GMF-dependent shoot and root gene expressions in 3-day-old etiolated *Arabidopsis* WT, *cry1cry2*, *phot1* and *phyaphyb* seedlings grown for 72 h under either GMF or NNMF conditions using red light. Data are expressed as fold changes (mean ± SD) with respect to NNMF conditions (i.e., GMF/NNMF).

Function	***Gene***	**WT**		***cry1cry2***		***phot1***		***phyAphyB***	
		Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
Transcription factors regulated by COP1/SPA1 complex	*HYH*	**-2.03(±0.89)**	**-2.05(±0.73)**	**-2.21(±0.48)**	**7.65(±1.65)**	**-2.53(±0.55)**	**1.92(±0.22)**	**-1.51(±0.15)**	**-5.33(±0.78)**
	HY5	-1.02(±0.47)	**-2.56(±0.74)**	-1.17(±0.14)	**1.71(±0.18)**	**-2.53(±0.54)**	**-1.86(±0.13)**	**2.55(±0.41)**	**2.11(±0.09)**
	LAF1	n.e.	**2.00(±0.38)**	n.e.	**-3.51(±0.15)**	n.e.	**-1.99(±0.34)**	n.e.	**-3.26(±0.90)**
Phytochrome-related factors	*PKS1*	**-3.53(±0.67)**	**-2.25(±0.58)**	1.04 (±0.17)	**-4.47(±0.45)**	**2.51(±0.35)**	**-8.00(±0.29)**	-1.32(±0.10)	**-5.82(±0.41)**
	PIF3	**-2.27(±0.14)**	**-5.64(±1.05)**	**-5.03 (±0.21)**	**-7.79(±0.62)**	**-2.08(±0.18)**	1.10(±0.18)	-1.21(±0.16)	**-1.75(±0.12)**
	**NDPK2*	0.96(±0.05)	**-4.45(±0.70)**	-1.41(±0.10)	**-1.66(±0.19)**	**-3.63(±0.72)**	-1.21(±0.09)	**-12.56(±1.07)**	**-4.00(±0.26)**
Anthocyanin biosynthesis	*ANS*	**3.49(±0.72)**	n.e.	**1.96 (±0.06)**	n.e.	**-1.56(±0.04)**	n.e.	**-5.65(±0.84)**	n.e.
	CHS	**4.31(±0.65)**	**-2.13(±0.43)**	1.39 (±0.31)	**-1.42(±0.24)**	**-3.00(±0.46)**	-1.29(±0.13)	1.14(±0.14)	**-30.97(±3.09)**
Auxin signaling	*PIN1*	1.56(±0.57)	**-1.42(±0.24)**	-1.16(±0.16)	**1.98(±0.38)**	**-2.14(±0.31)**	**-1.59(±0.18)**	**-1.38(±0.05)**	**1.36(±0.09)**
	PIN3	1.30(±0.26)	**-4.08(±1.57)**	-1.21(±0.08)	1.07(±0.08)	**-4.09(±0.81)**	**3.20(±0.16)**	1.16(±0.06)	1.18(±0.09)
Oxidative response	*GST*	**-1.78(±0.40)**	**-3.44(±0.21)**	1.15 (±0.11)	**-3.00(±0.34)**	**-1.85(±0.44)**	-1.33(0.10)	**-7.45(±0.16)**	**1.36(±0.07)**

Boldfaced numbers indicate a significant ($p<0.05$) difference between NNMF and GMF treatment.; n.e.= not expressed; *= this gene is also associated to the oxidative response.