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Geomagnetic field impacts on cryptochrome and phytochrome signaling

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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.

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, ≤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

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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 (12 × 12 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: 0–8 V/5A and 0–20 V/2.5A, 50 W) 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 600 W, 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 Fig. 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 2× 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].

2.5. SDS-PAGE and Western Blot Analysis

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

2.6. Total RNA Isolation and cDNA Synthesis

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