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ERRATUM

Erratum to: Daily Changes in the Competence for Photo- and Gravitropic Response by Potato Plantlets

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Abstract Competence for phototropic (PT) and gravitropic (GT) bending by potato plantlets grown in vitro manifests regular daily changes indicating possible involvement of circadian regulation. Unilateral stimulation with the blue light of plantlets at dawn resulted in moderate PT response regarding both attained curvature and long lag phase. The PT response was the strongest between 8:00 and 12:00 h. Throughout the afternoon and in the evening, bending rate and maximal PT curvature declined significantly until 23:00 h. The GT response was fastest and strongest for plantlets stimulated early in the morning and late in the evening. During the rest of the day, GT competence did not change much apart from a minimum at 15:00. In conditions comprising either prolonged day or prolonged night, plantlets appeared to maintain rhythmicity of competence for PT and GT at least in a short-term. Introduction of dark period prior to the tropic stimulation at 11:00 h when both PT and GT responses were strong resulted in the opposite effect: PT was depressed and GT was enhanced. There was a time threshold of 60 min for the duration of dark period so the plants can sense interruption in the daylight. Levels of relative expression of a *PHOT2* gene indicate rhythmic daily changes. *PHOT2* gene was present at high levels during morning hours and late in the evening. As the mid-day and the afternoon hours approached, *PHOT2* expression decreased and reached daily minimum at 17:00 h. We believe that our data offer strong support for the conclusion that there is an involvement of circadian rhythms in control of both PT and GT.

Keywords Potato · Phototropism · Gravitropism · Circadian rhythm

Due to the inadvertent publication of the article in its uncorrected form, the article is now republished. This erratum contains the corrected version and is the version of record.

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Introduction

Although considered as sessile, higher plants are well equipped to respond to environmental changes performing movements known as tropic responses. Phototropism as the response to unidirectional light stimulation and gravitropic response to the gravity stimulation are considered as main factors regulating spatial position of plant body. They enable plants to quickly rearrange their position in order to optimally utilize the incoming light.

Photo- and gravitropism are complex physiological responses occurring both in shoots and roots as a consequence of differential cell elongation. These are synchronised responses of plant organs and not of individual cells or cell groups. Tropisms are traditionally

studied in dark grown, etiolated seedlings with only a limited number of studies of shoot and root responses reported for green, light grown plants.

Recently we described tropic responses of potato using plantlets produced in vitro from single node explants (SNE; Vinterhalter and others 2012). Under conditions of a long day 16/8 LD photoperiod, light-grown plantlets manifested vigorous movements after 2 h of tropic stimulation. During our initial studies we noticed that competence for tropic response of SNE plantlets significantly varied through the day indicating possible involvement of circadian regulation. Circadian rhythms are internally driven plant responses which help plants to synchronize their daily activities. They enable plants to anticipate and correctly respond to the regular daily shifts of night and day compensating seasonal changes. They also prevent plants to respond to unexpected light and temperature stimuli. The complex and sophisticated gene expression machinery underlying circadian rhythms (Mas 2005) presents a significant adaptational advantage (Johnson 2001).

The major breakthrough in the circadian rhythm studies in plants was made in 1990s with development of a method enabling bioluminescence variation measurements in transgenic plants carrying a construct containing CAB2 promoter fused to a functional firefly luciferase *Luc* gene (Millar and others 1992). This method provided a simple and accurate non-invasive technique demonstrating cycling of daily levels of the CAB2::LUC fusion protein. Method was later widely accepted in various approaches including those enabling the isolation of individual components of the circadian clock. Studies done in Arabidopsis pigment mutants showed a major role for cryptochrome, phytochrome (Somers and others 1998), and to less extent zeitlupe genes (Somers and others 2000) in the entrainment of the circadian clock. None of these or other studies showed direct involvement of phototropin in the clock entrainment (Millar 2003; Webb 2003) and as a consequence phototropism was not considered as a process with a possible circadian regulation. However, it should be noted that a co-action between phototropins and cryptochromes in phototropism has been reported by Whippo and Hangarter (2003).

Circadian rhythms have been studied mostly in the model plant Arabidopsis thaliana. Covington and others in (2008) showed that the true number of genes manifesting daily changes in mRNA expression was ~ 36 % of the total genome which is higher than previously reported (Harmer and others 2000). From their results it became obvious that many plant processes and responses are probably affected by circadian rhythms. According to McClung (2006), Arabidopsis exhibits myriad of rhythmic outputs of the clock including (1) rhythmic cotyledon and leaf movement (2) elongation rate of abaxial and adaxial cells of the cotyledonele and leaf petiole, (3) elongation of hypocotyl, and (4) elongation of inflorescence. Circadian regulation was also found to affect or regulate other processes like mineral nutrition and solute transport (Haydon and others 2011) or stomatal conductance and CO_2 assimilation (Dodd and others 2004). Unfortunately, the experimental approaches for many processes affected by circadian regulation like those presented here are still cumbersome and time consuming.

In trying to elucidate possible involvement of circadian regulation in the phototropic bending of potato plantlets we focused our attention to kinetics of the bending process at various times of day and under the free running conditions comprising prolonged day or prolonged night. As supporting evidence, we also studied the relative expression of *PHOT2* gene throughout the day looking for signs of its daily cycling shown to exist for other blue light receptors (Fankhauser and Staiger 2002).

A limitation of our initial study of potato plantlets tropisms (Vinterhalter and others 2012) was that it showed the response in a single time point, recording the data only at the end of 2 h of tropic stimulation. In the present study, we opted to investigate the kinetics of the bending process utilizing time-lapse digital photography, recording the tropic movements of individual plantlets throughout the whole period of tropic stimulation. It enabled us to get a clear insight into different phases of the bending process. Apart from measuring the maximum and bending angle after 120 min we also calculated the lag phase of bending as time required by shoots to reach 10° of bending curvature. Thus, the main goals in this study were to document daily changes in the kinetics of the photo- and gravitropic responses of potato plantlets, and investigate how the changes in light regime and free running conditions affect the observed diurnal changes of tropic competence.

Materials and Methods

Plantlet Growth and Tropic Stimulation

Shoot cultures of potato (*Solanum tuberosum* L.) cv. Desiree, confirmed by ELISA tests to be virus-free were obtained from the Agricultural Combine Belgrade (PKB). They were grown on plant growth regulator-free MS medium (Murashige and Skoog 1962) supplemented with 3 % sucrose and 0.7 % agar according to the continuous propagation procedure suggested by Hussey and Stacey (1981). Single node explants were excised from shoots avoiding the 2–3 basal and 1–2 apical nodes. Groups of six SNE explants arranged in a circle were cultured in 270-ml volume glass jars (Φ 60 × 120 mm) with 50 ml of medium and translucent polypropylene closures. Sub-culturing was

done at 3–4 week intervals always prior to the activation of axillary buds. SNEs required 9–14 days in the growth chamber to reach the height suitable for tropic treatments. At this stage, explants had well developed adventitious roots and were therefore referred to as plantlets.

Growth chamber from which flasks with cultures were sampled for treatments was adjusted to maintain temperature at 24.5 ± 0.5 °C and a long day photoperiod (16 h light/8 h darkness). Light was produced by fluorescent lamps (Philips TLD 18w) providing a fluence rate of 74 μmol/m^{2}s as measured by a LiCor 1400 spectrophotometer with a Quantum sensor. The beginning of the day (dawn) in the growth chamber was fixed at 7:00 h and the end of day (dusk) at 23:00 h. For the 13 h light/10 h darkness photoperiod end of day was fixed at 20.00 h. For constant light condition, lights were continuously turned on. Experiments were performed in 60 × 80 × 30 cm (H × L × W) black-walled cabinets (black boxes) situated in a dark room adjusted to the same temperature conditions as the growth chamber. Light isolated growth chamber with cultures was situated in the same dark room. There was no other light (safe light) in the dark room during treatments apart from the light sources providing unilateral blue light for phototropism or brief orange light used in gravitropic studies. Commercial narrow-beam, 1.0 W spot LED lamps produced by Phillips (for the emission spectral characteristics see our previous paper Vinterhalter and others 2012), equipped with a GU10 socket, were used as a sources of unilateral blue light. These blue lamps provided a fluence rate of 24 μmol/m^{2}s at a distance of 40–42 cm. Yellow Orange LED lamps provided less than 2 μmol/m^{2}s, which was sufficient to take photographs during gravitropic stimulation. The peak of the emission spectrum was at about 580 nm for yellow LED lamps as measured by Ocean Optics HR2000-CR UV-NIR spectrometer (data not shown).

During unilateral blue light (BL) stimulation, each culture jar containing six plantlets was continuously illuminated by a single, blue light LED lamp. For gravitropic stimulation, jars with SNE plantlets were turned on the side and placed horizontally (at 90°) in darkness in shallow grooves preventing jars to roll. Jars were briefly illuminated with yellow LED lamps (6–10 s) to enable photographs to be taken.

Data Collection and Analyses

Treatments were applied to four culture flasks each containing six plantlets and were replicated 2–3 times. For both phototropism and gravitropism experiments, flasks were photographed at 3 or 4 min intervals. Close up, 3.5 Mpix large photographs were made with Panasonic Lumix DMC-FZ28 digital camera. Unilateral blue light

illumination used for PT treatments was continuous while in the GT studies yellow light from a mobile light source positioned lateral to cultures was briefly turned on for every photograph. Continuous illumination of cultures with yellow light used in GT studies did not induce visible PT response. Quantitative measurements of curvature angles were done from stored digital images with the UTHSCSA Image tool for Windows 3.0 or Linux Gimp 2.8.

Graphic presentations of tropic curvatures were drawn for each shoot in the treatment. They were all aligned to zero angle at start and their curvatures at 10 min increments, if missing, were extrapolated from graphs. Average curvatures calculated for 10 min increments were used to create the average curvature plots for each treatment. In order to prevent misinterpretation of data due to variability of responses at different times of day, we arbitrarily assigned the angle of 10° to be a threshold for both PT and GT. Therefore, duration of lag phase was determined as the time needed for plantlets to reach 10° of curvature. Under tropic competence or potential we consider the ability of plantlets to perform tropic bending in time. Therefore high competence denotes treatments in which plants exhibit vigorous tropic curvatures in short periods of time. Graphs were drawn and statistics calculated using the Qtiplot for Linux software.

Quantitative Real Time PCR

Total RNA was isolated from samples using GeneJET RNA Purification kit (Thermo Fisher Scientific, Pittsburgh, PA), according to manufacturer's instructions. Samples consisting of upper ∼20 mm of shoots were prepared from 10 plantlets each in order to minimize the individual plant variation in gene expression. The quantity as well as the purity of total RNA was determined by measuring optical density at 260 nm and the A260/A280 absorption ratio using NanoVue spectrophotometer (GE Healthcare, Sweden). Only the RNA samples with A260/A280 ratio between 1.9 and 2.1 and A_{260}/A_{230} greater than 2.0 were used in the analysis. To avoid any genomic DNA (gDNA) contamination, total RNA was treated with DNase I (RNase-free) (AM2222-Ambion, Life Technologies, Carlsbad, CA). First strand cDNA synthesis (starting from 1 μg of RNA) was primed with an oligohexamer primer using RevertAid reverse transcriptase (Thermo Fisher Scientific, Pittsburgh, PA) according to manufacturer's instructions. Primers for *PHOT2* (Table 1) were designed using Primer3 software according to tomato *PHOT2* gene (Acc. No. EU021291.1). As a reference gene *EF1α* (Acc. No. AB061263) was used (Nicot and others 2005). Polymerase chain reactions were performed in a 96-well plate with ABI Prism 7500 (Applied Biosystems, Life Technologies, Carlsbad, CA) thermal cycler, using SYBR Green

Table 1 Primers used in quantitative real time PCR

	F primer sequence 5′–3′	R primer sequence 5′–3′
PHOT2	AGTGGGGATTGACTGTGAGG	CCTCGGATGTCCTTGTTGAT
EF1α	ATTGGAAACGGATATGCTCCA	TCCTTACCTGAACGCCTGTCA

to monitor dsDNA synthesis. Reactions contained 12.5 µl 2X SYBR Green Solution (Thermo Fisher Scientific, Pittsburgh, PA), 10 pmol of each primer and 1 µl of 100 fold diluted cDNA (1.5 ng). The following standard thermal profile was used for all PCR reactions: polymerase activation (95 °C for 10 min), amplification and quantification cycles repeated 40 times (95 °C for 1 min, 60 °C for 1 min). The efficiency of primers was determined using standard curve method (User bulletin #2, Applied Biosystems). The specificity of the amplicons was checked by electrophoresis in 2 % (w/v) agarose gel and a melting-curve analysis performed by the PCR machine after 40 amplification cycles (60–95 °C with one fluorescence read every 0.6 °C). All investigated qPCR products showed only single peaks and no primer-dimer peaks or artifacts. Three biological repetitions were used for the measurement, and three technical replicates were analyzed for each biological repetition. Relative expression of *PHOT2* gene was calculated using ddCt-comparative method (Livak and Schmittgen 2001), using etiolated plants as calibrator.

Results

Kinetics of Phototropic Response

Flasks with potato plantlets growing under 16/8 h light to darkness photoperiod were sampled at different times of day and unilaterally illuminated with blue light in a dark chamber to induce phototropic response. Kinetics for phototropic curvature of obtained at certain time points are presented in Fig. 1a. PT response at different times of the day showed changes in the lag phase duration and slope of the curves representing the rate of PT bending. Response of plantlets at dawn (7:00 h, just before the lights were turned on) took more than 50 min to start, and was moderate but much higher than at the dusk (23:00 h) at the beginning of night. In the first hour of the day (7:00–8:00) lag phase duration rapidly decreased and the magnitude of PT curvature increased. From 8:00 to 12:00 h, PT response was fast and plantlets reached about 90° angle of curvature during 120 min of stimulation. Plantlets stimulated at these times had lag phase between 18 and 22 min long. This significant morning increase was followed by an afternoon decline in rate and magnitude of PT response all the way until the end of day (23:00 h). The drop in PT competence was obvious already at 15:00 h since the plantlets

stimulated at this time had slower rate of curvature and maximal attained angle was 78°. From Fig. 1a it is evident that there was a prominent change in PT competence through the day. Both the highest curvature angles and lag phase durations plotted on a graph against corresponding times of days (Fig. 2a) appeared to change in a distinct daily rhythm.

Kinetics of Gravitropic Response

For the study of GT bending kinetics, plantlets were sampled at different times of day and stimulated in darkness (Fig. 1b). The strongest GT response was recorded in plants at dusk (end of day at 23:00 h). Maximum angle attained was higher than 90° (around 105°) and the lag phase was short (22 min). Plantlets stimulated at dawn responded in similar manner except that the maximal angle they reached was closer to right angle (87°). Turning the light on at dawn induced a small but visible decline in the GT competence. For the plantlets stimulated at 8:00, 10:00, 12:00, and 17:00 h duration of the lag phase was around 30 min and maximal angle of bending between 77° and 85°. The longest lag phase of 40 min was recorded for plantlets stimulated gravitropically at 17:00 h. These plantlets reached maximum angle of curvature of 72° (Fig. 1b). In general, GT response was more consistent throughout the day than the PT response. When plotted on a graph against corresponding times of day, the highest curvature angles and lag phase durations for the GT responses (Fig. 2b) also appeared to follow distinct daily rhythms.

Effect of Interruption of Day with the Short Period of Darkness

Since both the PT and GT response were strongly affected at dawn when the light was turned on, we investigated how a dark pretreatment applied in midst of the morning PT maximum (4–5 h after the beginning of day) would affect the response of potato plantlets. A 60 min long period of darkness applied at the time of day when plants exhibit vigorous PT response induced a drop in competence represented by a loss of sensitivity to subsequently applied light (Fig. 3a). The PT response was delayed for at least 20 min but the magnitude of curvature was unchanged after 140 min of stimulation (Fig. 3a). The introduction of 60 min long period of darkness at 11:00 h prior to GT stimulation produced the effect opposite to one induced in
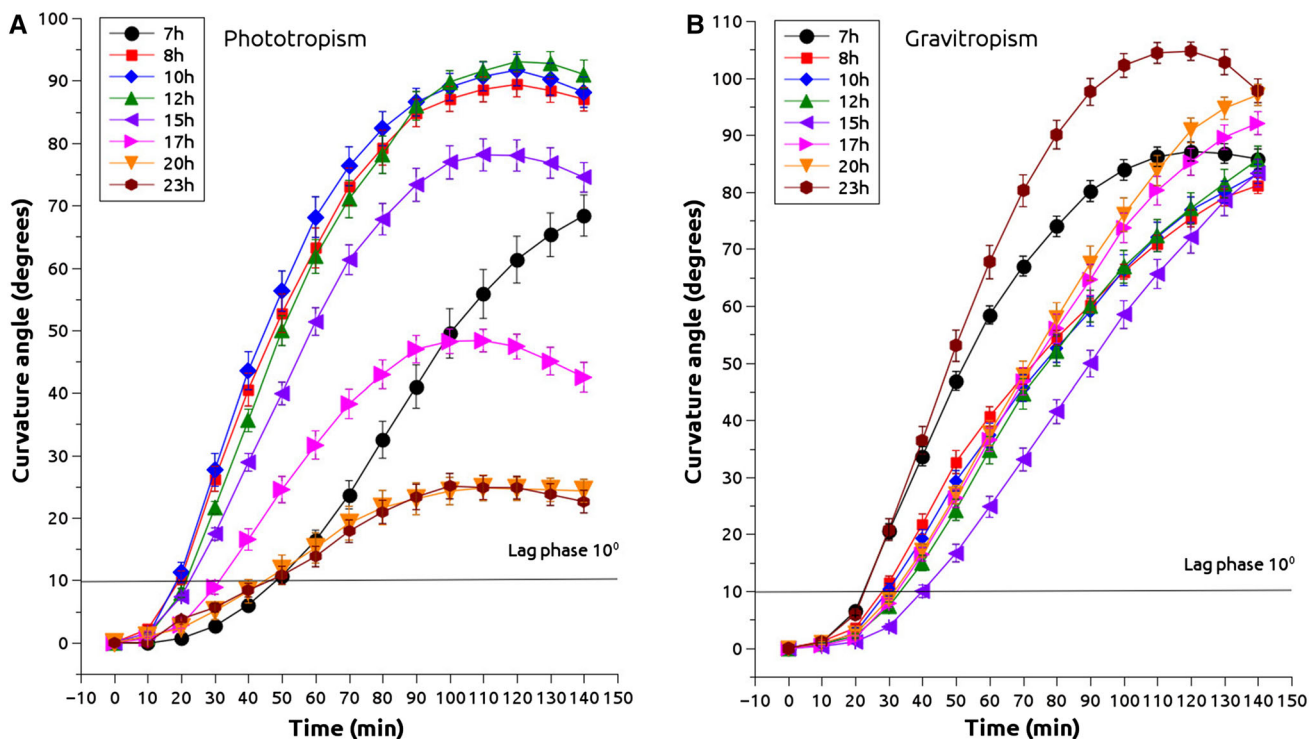
Fig. 1 Tropic curvatures of potato plantlets grown in 16 WL/8 D photoperiod at different times of day. Culture flasks each containing six plantlets were transferred from a growth chamber and: **a** placed in the beam of a single blue light emitting LED lamp for PT; **b** overturned at 90° and placed in darkness for GT. The flasks were briefly (5–6 s) illuminated with yellow LED lamps to take photographs
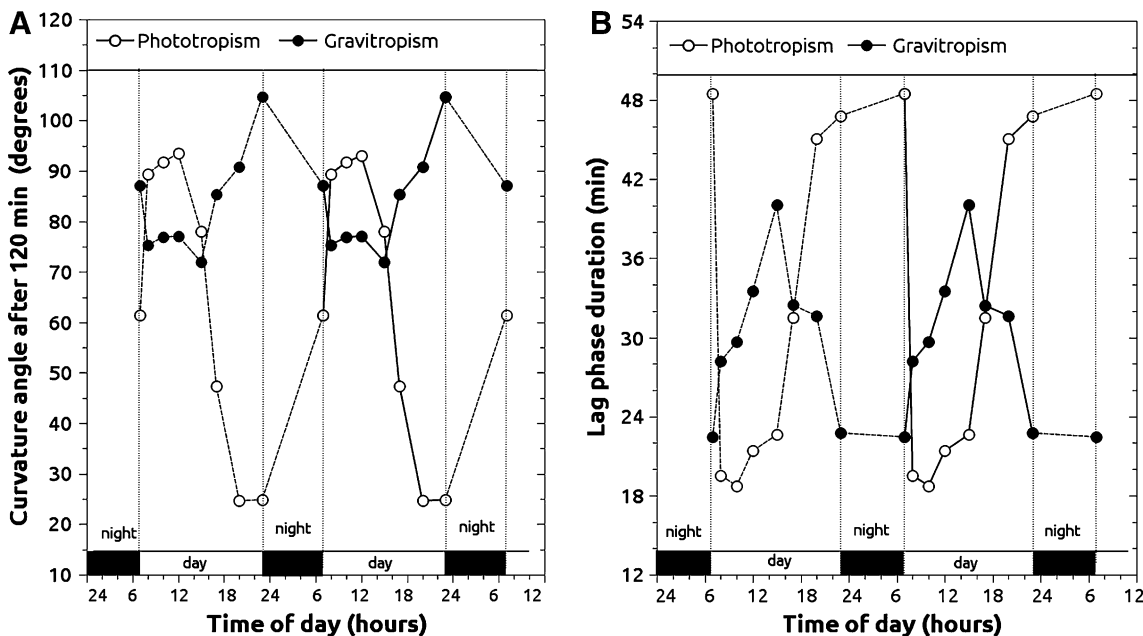

Fig. 2 Daily changes of parameters defining PT and GT response: **a** magnitude of tropic curvature after 120 min stimulation, **b** duration of lag phase calculated as time to reach 10° curvature. Plants grown under 16 WL/8 D photoperiod were used in these experiments

plantlets responding to unilateral BL. The lag phase of GT response was not prolonged, and the maximum angle of curvature was higher when compared to response of

plantlets that stayed in WL all the time (Fig. 3b). Placing plantlets into darkness for 20 min did not induce any change in either PT or GT response.
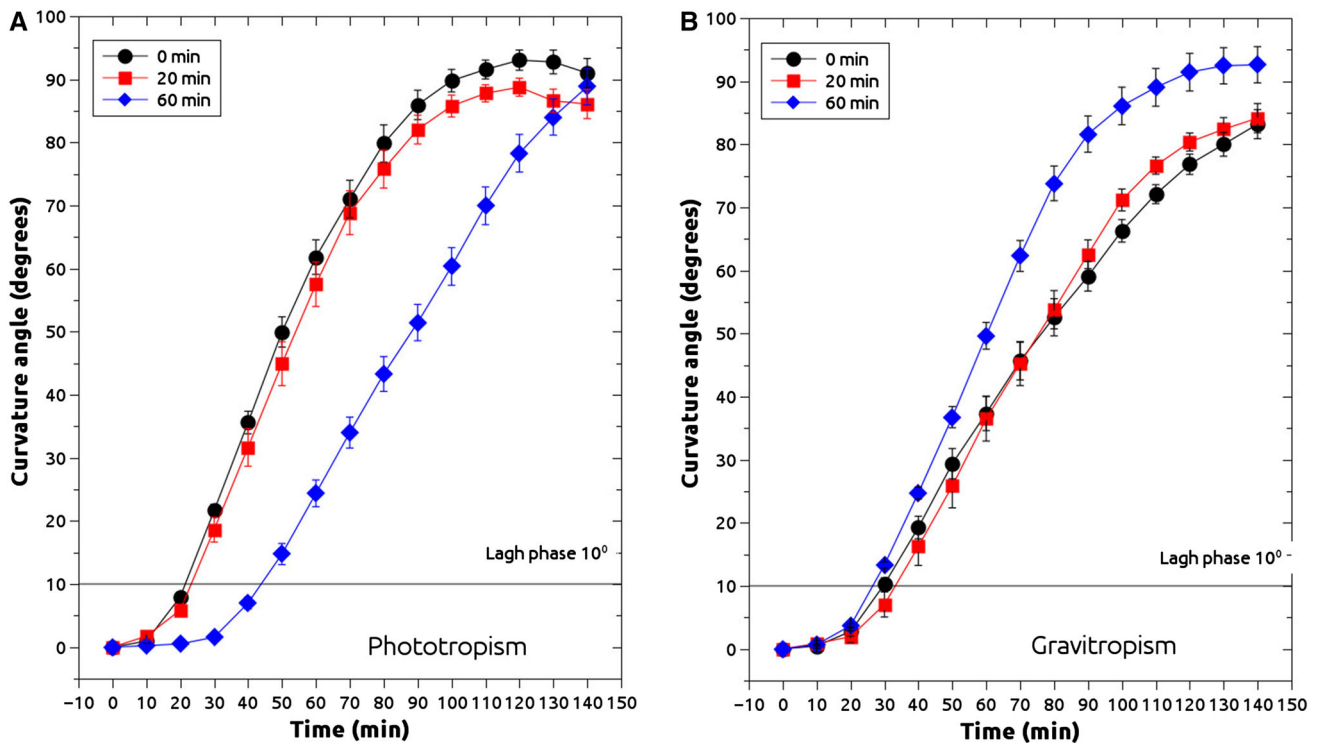
Fig. 3 Effect of 20 and 60 min long dark pretreatments on tropic responses of plants grown under 16 WL/8 D photoperiod: **a** phototropic response, **b** gravitropic response. Plants were placed in the dark for different period of time at 11:00 h

Therefore a 60 min long dark pretreatment delayed the PT response by prolonging the lag phase duration but at the same time it improved the GT response increasing the maximum curvature angle while the lag phase duration remained the same.

Prolonged Day and Night Experiments

To evaluate the possibility that daily changes in the potato tropic capacities are under circadian regulation it would be necessary to place cultures under free-running conditions meaning continuous night and/or continuous day. Although such conditions can be easily established for material grown in vitro, they seriously affect PT response of potato plantlets making this approach unsuitable. After a single day in continuous darkness, lag phase duration of PT bending was extended and lasted nearly 3 h. In continuous light, PT response maintained constantly a magnitude similar to one recorded in the afternoon (between 15:00 and 17:00 h) for the plants grown in 16/8 light to darkness photoperiod (Figs. 1a, 4a).

Our approach for getting a better insight into how potato plantlets grown under "free running conditions" respond to PT and GT stimulation was to prolong the length of the last day or night of the plantlets that were grown in the 16/8 light to darkness photoperiod prior to tropic stimulation. Like this we were able to record response trends which clearly indicated that potato plantlets anticipate the next night-to-day or day-to-night change of photoperiod.

When the duration of the last day (before treatment) was extended from 16 to 24 h, the maximum curvature of PT response has not changed much (26° vs. 30°; Fig. 4a) while the lag phase duration somewhat decreased. When the "day" was prolonged to 30 h, bending rate improved and maximum was achieved already after 70 min while the lag phase did not change significantly (Fig. 4a). Under conditions of 34 h long day, PT bending response dropped to a very low magnitude and the lag phase duration was extended to 55 min (Fig. 3a).

SNE potato explants kept continuously in light from the time of subculturing onward developed into plantlets manifesting no detectable rhythms in either PT or GT response. Their PT response (Fig. 4a) was constant regardless of the time of day reaching a maximum curvature angle of 56° after 70 min of unilateral BL stimulation following a 22 min long lag phase. GT response of these plantlets was constantly at maximum (data not shown).

Plotting highest curvature angles and lag phase durations in relation to the time of day reveals that under prolonged day plantlets anticipated the change of light regime
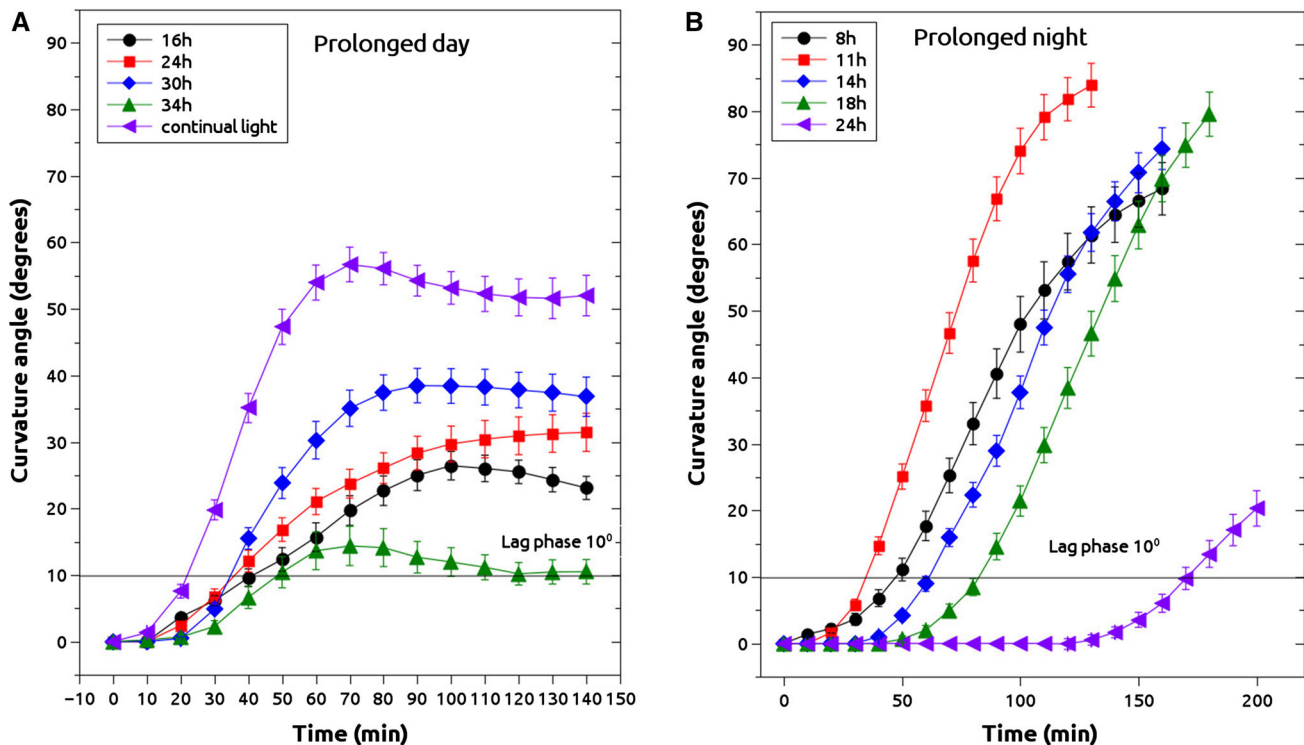
Fig. 4 Phototropic response of plants in conditions of prolonged (extended) last day or night prior to tropic stimulation. Plants grown under 16 WL/8 D photoperiod were used and to create prolonged day treatments (**a**), light was not turned off at 23:00 h at the end of the last day which was extended to last 24, 30, and 34 h. The end of these light periods corresponded to 7:00, 13:00, and 17:00 h of next day's local time, respectively. The exceptions were plants from continuous light treatment that were grown in continuous light through the whole duration of subculture. Plants grown under 16 WL/8 D photoperiod were also used to create prolonged night treatments (**b**), by not turning the light on at 7:00 at the end of the last night which was therefore extended to last 11, 14, 18, or 24 h. The end of these dark periods corresponded to 10:00, 13.00, 17:00 and 23:00 h of next day's local time, respectively

although it actually did not occur. Thus after 30 h of continuous day at the time that corresponded to subjective morning (after the missing night) maximum curvature angle started to increase as an expected "start of new day" response while the lag phase duration started to decrease (Fig. 5a, c).

Prolonging the duration of night from 8 to 11 h resulted in promotion of PT response from both aspects providing shorter lag phase and higher bending rate (Fig. 4b).

Maximum PT response was still high for plantlets that experienced a night lasting 14 and 18 h but then it rapidly deteriorated. After a night lasting 24 h, plantlets reached the average maximum curvature angle of only 180 and the lag phase duration increased to nearly 3 h.

Under prolonged night, plantlets also anticipated the change of light regime. After 11–18 h of continuous night at the time that corresponded to subjective morning, maximum curvature angles remained high (Fig. 5b). The plantlets that were incubated in 14 h of darkness, exhibited prominent but transient decrease in PT lag phase duration (Fig. 5d).

Daily Changes in PHOT2 Relative Expression Levels

Since both, significant daily changes in the PT bending capacity and the absence of this rhythm in etiolated plantlets may results from the changes in levels of pigments involved in the blue light perception, we investigated abundance of *PHOT2* mRNA throughout 1 day. Using the levels present in etiolated plantlets as standard we showed that the relative abundance of *PHOT2* mRNA changed significantly throughout the day (Fig. 6). At dawn and in the early morning hours, level of *PHOT2* expression levels were four times higher than those recorded in the etiolated plantlets. Later in the morning, abundance of *PHOT2* mRNA decreased and reached minimal values in the afternoon hours between 13:00 and 17:00 h. Relative expression of *PHOT2* increased again later in the afternoon and continued to rise until the end of the day at 23:00 h. *PHOT2* mRNA level in etiolated plantlets was low and it was used as a reference value equaling 1.0. Even after 16–18 h of unilateral irradiation that induced a 90° curvature angle (Vinterhalter and others 2012) estimated relative abundance of *PHOT2* mRNA
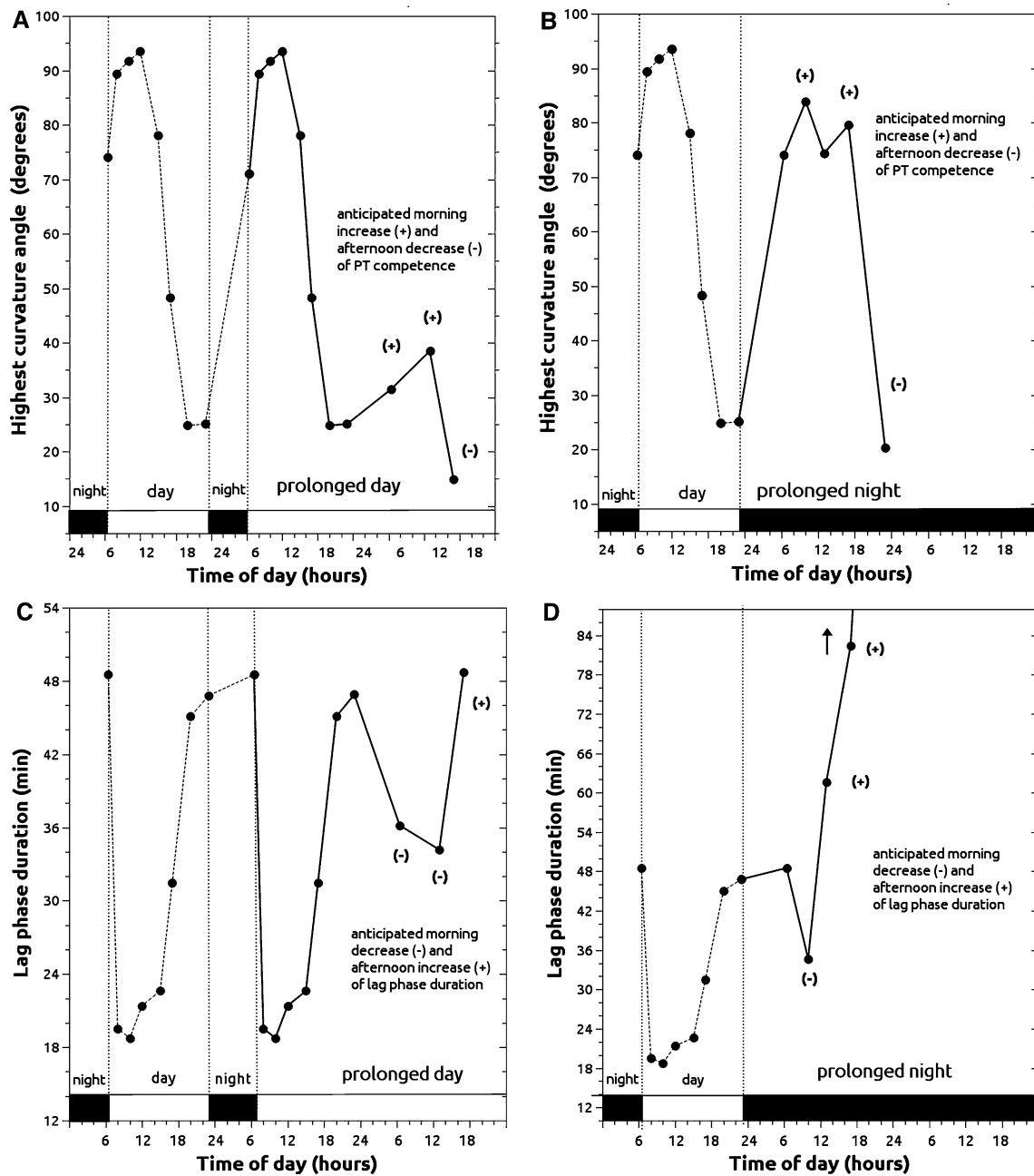
Fig. 5 Changes in maximum curvature angles of phototropic bending (**a**, **b**) and changes in lag phase duration (**c**, **d**) induced by extended duration of last day or night. These data were derived from the Fig. 4.

In **d** the value for duration of lag phase in the night extended to last 24 h was far out of the range (over 180 min) and could not be plotted

was only ×1.1. Highest *PHOT2* mRNA expression levels (up to ×32 times higher than in etiolated material) were detected in plantlets under prolonged night conditions.

Discussion

Our results strongly support the idea that the competence of potato plantlets to perform phototropism is under control of circadian rhythms. Major parameters that define PT

response of potato plantlets exhibited regular daily variations which persisted through conditions of prolonged day and night consistent with other plant responses that are under circadian regulation. As a result of changes in duration of lag phase (Figs. 1a, 2b) and the rate of bending, maximum PT response was recorded in the period that spans over morning and early afternoon hours and minimum was recorded late in the evening (Figs. 1a, 2a).

On the other side, daily variability in gravitropism was far less apparent. Lag phase duration changed throughout
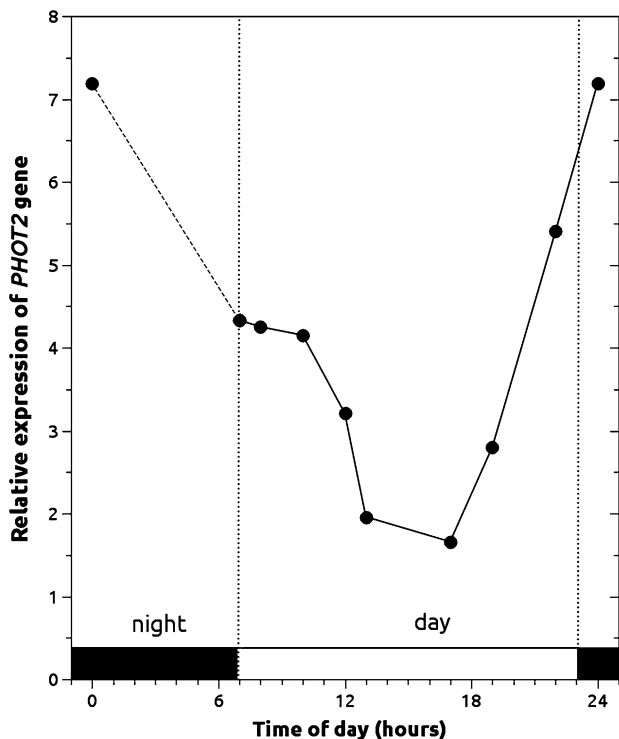
Fig. 6 Diurnal changes of *PHOT2* mRNA relative expression levels in plants grown under 16 WL/8 D photoperiod

the day to a lesser degree for plants exhibiting GT than for those responding to BL (Figs. 1b, 2b). Magnitude of GT response also varied although not as much as it did for the PT response (Figs. 1b, 2a). Plots describing duration of the lag phase and maximum curvature for PT are almost mirror image of those recorded for GT but with smaller amplitudes (Fig. 2). Absence of large magnitude does not negate rhythmic nature of GT which suggests that GT response may also be under control of circadian rhythm.

Dark pretreatments applied in the midst of the daily PT competence maximum revealed the true importance of light for tropic bending of potato (Fig. 3a, b). We know that continuous blue light irradiation is indispensable for PT bending. If the unilateral BL is turned of in the midst of a PT treatment then the PT bending gets completely arrested after just 25 min of darkness (Vinterhalter and Vinterhalter 2012). Thus, it was somewhat surprising to find that a 60 min long dark pretreatment only postponed the beginning of PT response while it's magnitude was not affected (Fig. 3a). Light is therefore needed through the day to maintain fast PT response (short lag phase) but id doesn't affect the magnitude of bending response. With GT response situation was opposite, absence of light imposed by dark pretreatments apparently favored GT response increasing only its magnitude while the lag phase duration was not affected. This finding actually explains the

observations from Fig. 1b showing that light appearing at dawn decreased somewhat the GT bending competence.

One of the most characteristic features of circadian rhythms distinguishing them from diurnal changes in general is that they continue under free running conditions, comprising continuous day or continuous night (Harmer and others 2000; Nozue and others 2007). Placing plantlets under continuous light or darkness was however not a suitable approach for potato plantlets. Continuous darkness induced rapid increase of lag phase duration within the first 24 h of darkness (Fig. 4b) while in continuous light PT bending was arrested at rather low values (Fig. 4a). We therefore investigated only the initial periods of free running conditions extending the duration of last day or night (Fig. 4a, b) prior to tropic stimulation. With this approach, we established the trends of tropic responses under free running conditions which were sufficient to evaluate the nature of regulatory system essential for the observed diurnal rhythms Both in extended nigh and day plantlets anticipated the "missing" change of light regime (Fig. 5a–d) providing a damped response in expected subjective day or night. Thus, although potato PT response is under circadian regulation, it can quickly respond and adjust to photoperiod changes. We believe that fast damping of tropic response observed under free running conditions (Fig. 5a–d) reflects the ability of plantlets to quickly adapt to new conditions. It should be noted that damping of circadian rhythms in the dark is a common characteristic of clock regulated genes (Hall and others 2001). It has been demonstrated for *CAB2*, phytochromes and some cryptochromes (Kozma Bognar and others 1999; Tóth and others 2001).

The ability of potato plantlets to start PT bending (lag phase duration) decreased from dawn till the end of day (Fig. 4b) but it recovered through the night and significantly increased in the first hour of the new day. This night recovery mechanism deteriorated if the night duration was extended to last 18 h or more. On the other side, although indispensable for fast PT bending light could not prevent drastic fall of the PT bending competence in the second part of the day. Only regular daily changes in the photoperiod (as in case of 16/8 h LD photoperiod could support high, regular and properly timed periods of PT bending.

In potato plantlets grown only under light (continuous light conditions) there were no rhythmic changes in either PT or GT bending competence (Fig. 4a). The lag phase duration, bending rate, and the magnitude of the response were all constant regardless of the time of day when the plantlets were exposed to BL or GT stimulation. PT response of this plants was time limited with a maximum positioned some 70 min after the beginning of BL stimulation. Thus the absence of daily PT and GT bending

rhythms in potato plantlets under conditions of continuous light resembles the arrhythmic response of Arabidopsis (Nozue and others 2007).

When the last night duration approached 24 h, plantlets exhibited weak PT response and extremely extended lag phase duration (Fig. 4b) indicating disruption of circadian rhythm regulation. Thus in prolonged night plantlets quickly approached situation described for etiolated plantlets with 15–16 h long lag period (Vinterhalter and others 2012). It is doubtful that etiolated potato plantlets can exhibit some responses that are rhythmic in nature. Still etiolated hypocotyls of Arabidopsis seedlings entrained with as little as two (Dowson-Day and Millar 1999) or seven (Covington and Harmer 2007) L/D cycles exhibited circadian rhythmicity in elongation growth and expression of many genes, respectively.

Tropisms are complex processes dependent upon proper perception of external signal and redistribution of growth (Poff and others 1994). Auxins that have been implicated as a major contributors to regulation of uneven growth on two sides (Estelle 1996; Liscum and Stowe-Evans 2000) of plant organs exhibiting tropic responses are also heavily controlled by circadian rhythms (Covington and Harmer 2007). We however did not encounter studies showing that PT and GT responses of some plants are under circadian regulation. Among phototropins daily cycling in mRNA expression was confirmed for PHOT1 (Harmer and others 2000).

Our data on daily lag phase duration changes and decrease of PT competence at the end of day suggest that some factors mediating PT are being synthesized (activated) and depleted (de-activated) in accordance with circadian clock. Considering that phot2 is generally accepted as the photoreceptor of second positive phototropism (Jarillo and others 1998) and functions in the range of fluence rates we used herein (Sakai and others 2001), we examined if its levels changed throughout the day. We are well aware that *PHOT2* gene transcription levels do not necessarily offer much information about the levels of phot2 protein. Still, this type of data can indicate the role that phot2 plays in control of PT response. Cycling of *PHOT2* relative abundance through the day was significant (up to sevenfold difference) indicating possible involvement of phot2 in the circadian regulation of PT response.

Since the GT stimulation is constant and unavoidable it is difficult to imagine that there would be some need for plants to have GT response under circadian regulation. However, there is a clear advantage for plants to have their capacity for bending towards incoming light very high during the day and low during the night. By being able to direct their growth towards incoming light, plants can maximize their photosynthesis and further promote overall growth (Dodd and others 2005). For that reason, PT and GT responses of potato plantlets seem to be well coordinated in time complementing each other throughout the day (Fig. 2).

As a conclusion, tropic responses of potato plantlets in vitro appear to be under control of circadian rhythms with phototropism favored by the plantlets during the day and gravitropism during the night.

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