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Photo- and Gravitropic Bending of Potato Plantlets Obtained In Vitro from Single-Node Explants

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Abstract Etiolated and light-grown plantlets obtained from potato shoot cultures were shown to perform vigorous tropic movements. Unilateral blue irradiation actively induced phototropic curvature of the shoots toward the light source, although etiolated plantlets required ten times longer stimulation than the light-grown plantlets to achieve a 90° angle. The fluence requirements for induction of second positive phototropism (PT) of light-grown plantlets spanned almost five orders of magnitude ($\sim$30–1.7 $\times$ 10^5 μmol/m^2). Upon responding to unilateral blue light by curving, plantlets entered the process of straightening after irradiation ended. Straightening also occurred in plantlets placed on a clinostat but it was of lower magnitude. Compared to early-morning and day-time hours, plantlets exhibited a significantly lower PT response in the late afternoon (5 p.m.) and gravitropic (GT) response at the end of the day (11 p.m.), suggesting that these responses may be under the control of circadian rhythms. GT was also recorded for both light-grown and etiolated plantlets. Ninety-degree stimulation, used to induce GT in etiolated plantlets, needed to be 50 % longer than stimulation used for light-grown plantlets to induce a similar response. Straightening was also recorded for the shoots that exhibited GT but was smaller when plantlets were placed on a clinostat compared to straightening exhibited by those plantlets left standing in an upright position for 2 h.

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Introduction

Light is an important factor regulating growth of potato shoot cultures and it has been amply investigated in connection to in vitro propagation (Seabrook 1987; Wilson and others 1993; Aksenova and others 1994) and tuberization (Seabrook and others 1993; Dobranszki and others 1999). The use of shoot cultures in studies of photoperiod control was restricted to initial stages (Macháčkova and others 1998), but in this whole field of study (Sarkar 2010) phytochromes received most of the attention (Heyer and others 1995; Jackson and others 1996; Yanovsky and others 2000). There are also reports on the use of light-emitting diodes (LEDs; Miyashita and others 1997) and lateral irradiation systems (Kitaya and others 1995), but these studies had a common goal of improving potato propagation procedures and none of them reported the occurrence of phototropic bending. Also, in a comprehensive review dedicated to the effect of light in potato in vitro cultures (Seabrook 2005), there were no reports on phototropic movements.

When grown in vitro, potato shoot cultures quickly reorient their spatial growth toward the light source providing the highest irradiance. These observations indicate a strong phototropic potential of potato. Potato shoot cultures are common experimental objects used for different investigations in the field of biotechnology. Features such as fast growth of the apical shoot bud accompanied by high shoot multiplication and subsequent axillary bud elongation make potato shoot cultures ideal for studies of growth and development. The new shoots produced by the apical

bud consist of well-spaced nodes with dormant axillary buds. Upon excision, individual nodes carrying a single axillary bud (single-node explants, SNE) get released from apical dominance, showing remarkable growth potential. The SNE multiplication system of potato based on the use of hormone-free medium (Hussey and Stacey 1981) enables fast and continuous culture cycling. In the absence of a true hypocotyl and cotyledons characteristic for seedlings, potato SNE plantlets have a simplified developmental pattern resembling the growth of the true aerial shoots. Unexpectedly, a feature common to both potato seedlings and SNE plantlets is the formation of hooks. Epicotylar hooks, constitutive in development of true seedlings and absent in light-grown SNE plantlets, appear in etiolated potato SNE plantlets under appropriate growth conditions, a combination of low temperature and low irradiance (Hussey and Stacey 1981, 1984).

Almost all of our knowledge on phototropism in dicotyledonous plants is derived from studies of hypocotyls of etiolated seedlings, mostly *Arabidopsis* (Pedmale and others 2010) but also *Lepidium* (cress; Hart and McDonald 1981b), *Fagopyrum* (buckwheat; Ellis 1987), pea (Britz and Galston 1983), and others. Despite its importance as a crop and undisputed popularity as an experimental material, classical studies in the field of photobiology and plant movements have not been done with etiolated seedlings of potato. A recent survey on the use of in vitro techniques in potato biotechnology (Vinterhalter and others 2008), showed that there are hardly any data on phototropic responses in potatoes.

Our goal in this project was to define the procedure to produce SNE potato plantlets in vitro and to use them to characterize basic features of their of photo- and gravitropic responses.

Materials and Methods

Plant Material and Growing Conditions

Shoot cultures of potato (*Solanum tuberosum* L.) cv. Desiree, Marijke, Kenebeck, and Ostara, obtained from the Agricultural Combine Belgrade (PKB), 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). Stems of plants obtained from shoot cultures were cut into SNEs while trying to avoid the basal 2–3 nodes and 1–2 nodes from the apical leaf whorl. Batches of five SNEs were cultured in 70-mm × 120-mm glass jars (270-ml volume) with 40–50 ml of medium and translucent polypropylene closures. For dark-grown (etiolated) cultures, SNEs were placed individually in 20-mm × 150-mm glass test tubes filled with 8–10 ml of medium and closed with plastic closures. Subculturing was done at 3–4-week intervals prior to the activation of axillary buds. Shoot tips were used to maintain cultures while the SNEs were harvested for experimental treatments and to increase the number of available shoot tips. Three to five SNEs of various heights were aligned in a row across baby-food jars with 20 ml of medium and closed with Magenta B caps. On average, SNEs required 10–14 days in light to reach the height suitable for phototropism (PT) experiments. Etiolated cultures needed only 6–7 days to reach the same height as the light-grown shoots. At this stage, explants had well-developed adventitious roots and were therefore referred to as plantlets.

Cultures were multiplied and maintained in a growth room with a long day photoperiod (16-h light/8-h darkness) and temperature controlled at 24 ± 2 °C. White light in the growth rooms was provided by long fluorescent lamps (Philips TLD 58w/54) at 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 in the growth room was fixed at 7 a.m., with most experiments starting at 11 a.m., that is, 4 h after the beginning of the day. Jars with etiolated plantlets were kept in a tightly closed box placed on the same shelf where plants for other treatments were growing.

The experiments presented here were done with potato cv. Desiree. PT of cvs. Ostara, Marijke, and Kennebeck was also studied initially but in less detail. Ostara exhibited similar PT to that of Desiree. Marijke strongly resembled the growth patterns and responses of Desiree but it was susceptible to early activation of axillary buds and was therefore not suitable for PT studies. Compared to Desiree, Kennebeck plantlets had shorter, thicker, and sturdier shoots. We thought that due to these morphological differences, PT of Kennebeck may differ from PT of Desiree but that was not the case. Therefore we decided to use cv. Desiree.

Light Sources and Irradiation Conditions

Experiments were performed in a black-walled cabinet (black box) situated in a dark room adjusted to the same temperature conditions as the growth rooms. There was no other light (safe light) in the dark room apart from the light sources providing unilateral irradiation. The temperature in the black box used for the etiolated plantlets experiments was maintained at 24 ± 2 °C.

Commercial narrow-beam spot LED lamps produced by Vito and Phillips, equipped with a GU10 socket, were used as a source of unilateral blue irradiation. Lamps provided a fluence rate of 24 μmol/m^2 s at a distance of 40–42 cm.

During unilateral blue light (BL) stimulation, each culture jar was illuminated by a single, separate LED lamp. The peak of the emission spectra was at about 460 nm (Fig. 1). Emission spectra were measured by using an Ocean Optics HR2000-CR UV-NIR spectrometer.

The PT curving response occurred only after illumination with blue and green LED lamps, whereas it was absent following stimulation with light from red and yellow LED lamps. The red-light-producing LED lamps could be used for brief background illumination in dark treatments since they induced no PT responses irrespective of the illumination duration (data not shown). Jars were positioned in such way that the upright axes of the plantlets were perpendicular to the incoming light. The phototropism threshold was determined by using LED lamps of different light intensity, by adjusting the distance between the plants and the light source, and by shielding the light source with transparent foil with mesh drawn onto it.

Experiments with Clinostat

There were four jar holders on the clinostat stand, with each holder rotating a single jar around its longitudinal axis. Thus, the axes of the four jars (and plantlets within) rotated on the clinostat were parallel to the surface of the lab bench. Jars or bundles of test tubes, both wrapped in black plastic foil, were placed on the clinostat and rotated at 35 rpm.

Gravitropic Stimulation

For gravitropic stimulation, jars with SNE plantlets were turned on the side and placed horizontally (at 90°) in darkness on Styrofoam plates. These plates had shallow grooves to prevent the jars from rolling. The tubes with etiolated plantlets were placed into Styrofoam holders and the whole holder was turned at 90° for the duration of the experiment.

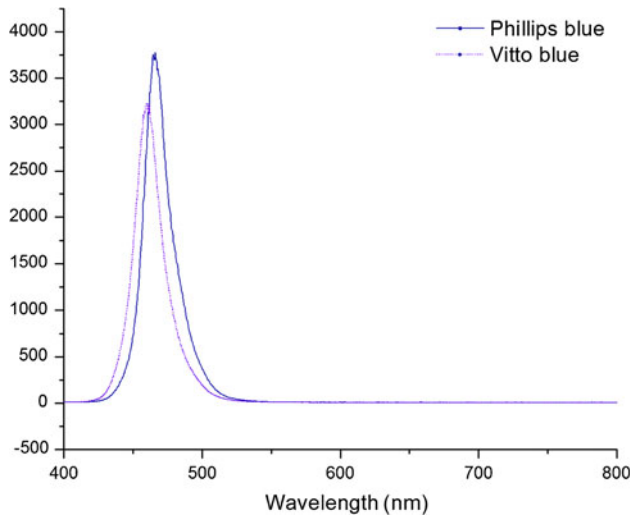


Fig. 1 Emission spectra of Vito and Philips blue LED diode lamps

Measurements of Plantlet Curvature and Length

Following PT and GT stimulation, plantlets were immediately removed from the jars, aligned on plastic Petri dishes placed on graph paper, and digitally photographed for documentation and further measurements. For each photograph, the treatment description, date, time of day, and fluence rate or other data were recorded on a small sticker included in the photograph. Only rooted, 20–35-mm tall plantlets were considered for statistical analysis of PT. Quantitative measurements of stem length and curvature angle were done using the stored digital images with the UTHSCSA Image tool for Windows 3.0.

Statistical Analyses

Light irradiation of two jars each with five SNE plantlets was replicated at least eight times, providing for each treatment no fewer than 54 plantlets suitable for statistical analysis. Dark treatments using ten test tubes each with a single SNE explant were replicated at least six times with no fewer than 52 plantlets per treatment. Means followed by a different letter were significantly different according to Duncan's multiple-range test at $p \leq 0.05$.

Results

Phototropism of light-grown potato SNE plantlets stimulated with unilateral BL for 2 h was 85°–88° when stimulation was started at 7 a.m., 9 a.m., 11 a.m., and 1 p.m. (Table 1). Plantlets irradiated at 1 p.m. needed only 75 min to attain maximum response, whereas plantlets from the other three groups needed a full 2 h. When the stimulation started at 5 p.m., the PT curvature was only about 34° and irradiation that was initiated at 11 p.m. yielded PT curvature of only about 28°. Irrespective of the time of day, stems of plantlets of all groups from this set of experiments were of similar length. The length of the apical, curved portion of the stem was the shortest for the plantlets that were stimulated at 11 p.m., a little longer for those irradiated at 5 p.m., and longest for plantlets irradiated at an earlier time of the day (Table 1).

Shoot length had no effect on the efficiency of PT bending (Table 2). Although the length of the curved portion of the shoot was bigger in taller plantlets, proportionally it represented a smaller part of the total length (~54–60 %) in comparison to short plantlets (<25 mm in

Table 1 Phototropic response of light-grown SNE plantlets to unilateral BL irradiation

Time of day and treatment duration	*n*	Plantlet length (mm)	Length of the apical curved portion of the shoot (mm)	Angle of curvature (°)
7 a.m. (120 min)	57	27.92 ± 0.65 a	16.57 ± 0.5 c	86.33 ± 1.89 c
9 a.m. (120 min)	56	27.53 ± 0.77 a	16.97 ± 0.6 c	87.86 ± 1.92 c
11 a.m. (120 min)	64	29.71 ± 0.83 a	17.84 ± 0.4 c	86.71 ± 1.44 c
1 p.m. (75 min)	51	28.87 ± 0.96 a	17.24 ± 0.5 c	84.94 ± 1.52 c
5 p.m. (120 min)	55	27.59 ± 0.78 a	14.32 ± 0.5 b	33.84 ± 2.03 b
11 p.m. (120 min)	53	29.82 ± 1.0 a	12.72 ± 0.4 a	27.78 ± 1.51 a

Means followed by the same letter are not statistically different according to Duncan's multiple-range test

n Number of plantlets

Table 2 Relationship between phototropic response and the length of the curved apical portion of light-grown SNE plantlets to the total length of plantlets

SNE shoot length (mm)	*n*	Curved apical shoot portion (mm)	Percentage of total length of plantlet bent toward BL (approx.)	Angle of curvature (°)
Over 40	13	22.84 ± 1.15 d	53.75	83.09 ± 2.47 a
35–40	20	20.47 ± 0.92 c	54.59	89.98 ± 3.49 a
30–35	41	17.67 ± 0.60 b	54.37	86.19 ± 1.78 a
25–30	85	16.42 ± 0.31 ab	59.71	83.26 ± 1.39 a
20–25	58	15.96 ± 0.44 ab	70.93	90.72 ± 1.55 a
Under 20	8	15.17 ± 1.20 a	86.69	88.25 ± 4.95 a

Tropic stimulation started at 11 a.m.

For calculation of percentage of total length of plantlet bent toward BL, the following lengths were used: 42.5, 37.5, 32.5, 27.5, 22.5, and 17.5 mm

Means followed by the same letter are not statistically different according to Duncan's multiple-range test

n Number of plantlets

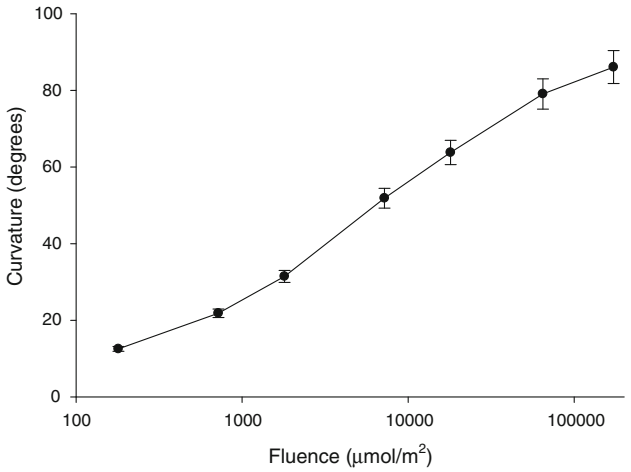


Fig. 2 Fluence–response relationship for induction of the second positive phototropism in light-grown potato plantlets irradiated for 2 h with unidirectional BL

length) where it reached over 70 % of total length. Thus, curvature was localized more in the apical region of taller plantlets.

Two-hour unilateral stimulation of light-grown SNE plantlets with BL of different intensities was used to determine fluence requirements for the phototropic response. At a fluence above 1.7×10^5 µmol/m^2, curvature reached a maximum of almost 90° (Fig. 2). The threshold for the PT is at a fluence of 20–30 µmol/m^2 judging from the slope of the initial portion of the curve. The middle part of the curve follows a logarithmic scale with PT dependent on the increasing fluence (Fig. 2).

Light-grown SNE plantlets exhibited a strong GT response during the day. When the 2-h stimulation began at 7 a.m., the GT response was 75.78° (Table 3). GT of plantlets turned at 90° for 2 h at 9 a.m., 11 a.m., and 1 p.m. was between 85° and 88°, and for those stimulated at 5 p.m., GT was consistently above 90° and statistically higher than the response of plantlets stimulated at earlier time points (Table 3). Gravity stimulation late in the evening (11 p.m.) produced the lowest GT response of the day at 59.76°. The length of plantlets was similar in all tested populations, whereas the length of the curved portion of the stem varied slightly between the populations.

Table 3 Gravitropic response of light-grown SNE plantlets

Time of day (duration)	*n*	Plantlet length (mm)	Length of the apical curved portion of the shoot (mm)	Angle of curvature (°)
7 a.m. (120 min)	56	28.11 ± 0.73 a	16.46 ± 0.50 c	75.78 ± 2.16 b
9 a.m. (120 min)	55	27.28 ± 0.72 a	15.93 ± 0.54 bc	87.64 ± 2.14 c
11 a.m. (120 min)	54	29.52 ± 0.96 a	13.07 ± 0.50 a	88.45 ± 2.13 c
1 p.m. (120 min)	52	27.97 ± 0.98 a	14.76 ± 0.56 b	85.26 ± 2.14 c
5 p.m. (120 min)	53	28.85 ± 0.82 a	15.31 ± 0.55 bc	95.41 ± 2.88 d
11 p.m. (120 min)	52	29.86 ± 1.08 a	15.18 ± 0.50 bc	59.76 ± 3.08 a

Means followed by the same letter are not statistically different according to Duncan's multiple-range test

n Number of plantlets

A kinetic study of PT and GT responses at 1 p.m., presented in Fig. 4, shows that PT was significantly faster than GT. The lag phase for PT was about two times shorter than that for GT (13 vs. 25 min). The time needed to reach maximum response for PT was about 75 min, whereas it took about 140 min for the plantlets to reach 90° after being flipped on the side for 2 h (Fig. 3).

Stems of light-grown plantlets that were irradiated (at 11 a.m.) with unilateral BL for 2 h and left in the darkness for an additional 2 h attained a curvature of 19.75° (Table 4). If 2 h of phototropic stimulation by BL were followed by 2 h of rotation on a clinostat, stems assumed an angle of 47.67°. Stems of plantlets turned on the side at a 90° angle for 2 h of gravity stimulation and then repositioned into the upright position for an additional 2 h started straightening and ended up with a curvature of 22.06°. When 2 h of gravitropic stimulation was followed by 2 h of rotation on a clinostat, stems maintained a curvature of 32.19° (Table 4).

Unilateral irradiation with BL for 2 h was not sufficient to induce PT in stems of etiolated plantlets (2.26°; Table 5). Longer (24-h) BL stimulation produced a curvature of 90.49°. When etiolated plantlets were returned to darkness for 2 h following 21 h of unilateral BL irradiation, their stems straightened back to a curvature of 30.05° (Table 5). If etiolated plantlets were rotated for 2 h on a clinostat in darkness following phototropic stimulation, their stems maintained a curvature of 47.75° (Table 5).

Gravitropic stimulation for 2 h at 90° produced a curvature of 64.58° in stems of etiolated plantlets (Table 5). Increasing the duration of stimulation to 3 h resulted in a curvature of 72.71°. Straightening of etiolated plantlets left in an upright position after 3 h of gravistimulation decreased the curvature angle to 14.14°. However, if the 2-h static period for straightening was replaced by rotation on a clinostat for 2 h, then the stems maintained a curvature of 22.18°. The average length of stems from different groups of plantlets used in this series of experiments was similar (Tables 4, 5).

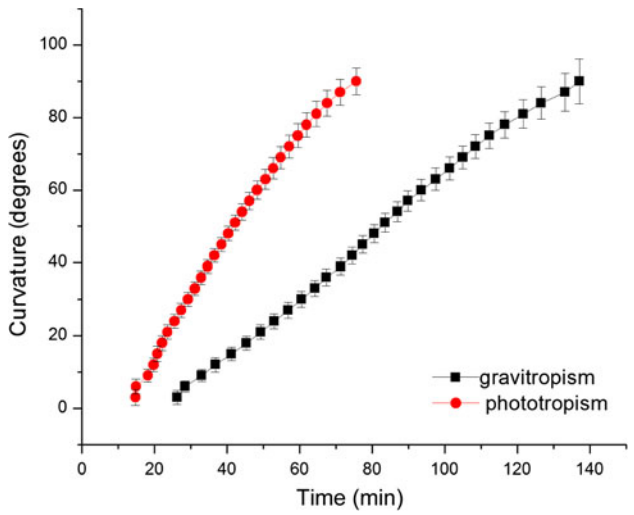


Fig. 3 Kinetics of the PT and GT responses of light-grown plantlets. Stimulation lasted for 2 h and started at 11 a.m.

Discussion

Potato plantlets obtained from in vitro manipulated SNE explants performed fairly uniform vigorous photo- and gravitropic movements. We have therefore succeeded in producing both light-grown and etiolated experimental objects from potato shoot cultures that can be employed as a useful model system for tropic studies (Fig. 4a).

Plant tropisms were studied mostly in hypocotyls of etiolated or briefly de-etiolated seedlings. Studies of PT in light-grown seedlings and plantlets are rare and were mostly centered on the first positive response that is induced by brief pulses of unidirectional light (Baskin 1986; Ellis 1987; Iino 1990); hence, it is difficult to compare and evaluate results that we obtained with potato. Studies by Everett (1974) on radish and Hart and MacDonald (1981a) on lettuce, cress, mustard, and radish demonstrated that light-grown plants strongly manifest the second positive phototropic response. The phototropic response that we recorded for stems of potato SNE

Table 4 Curvature of light-grown SNE plantlets that experienced photo- and gravitropic stimulations for 2 h followed either by 2 h in the upright position or 2 h of rotation on a clinostat in darkness

Treatments (duration)	*n*	Plantlet length (mm)	Angle of curvature (°)
BL (2 h) + static (2 h)	60	26.49 ± 0.75 a	19.75 ± 1.80 a
BL (2 h) + clinostat (2 h)	55	26.9 ± 0.97 ab	47.67 ± 2.73 c
90° (2 h) + Static (2 h)	50	29.0 ± 0.096 b	22.06 ± 1.75 a
90° (2 h) + Clinostat (2 h)	51	28.05 ± 1.01 ab	32.19 ± 2.48 b

Tropic stimulation started at 11 a.m.

Means followed by the same letter are not statistically different according to Duncan's multiple-range test

n Number of plantlets

Table 5 Photo- and gravitropic responses of etiolated SNE plantlets

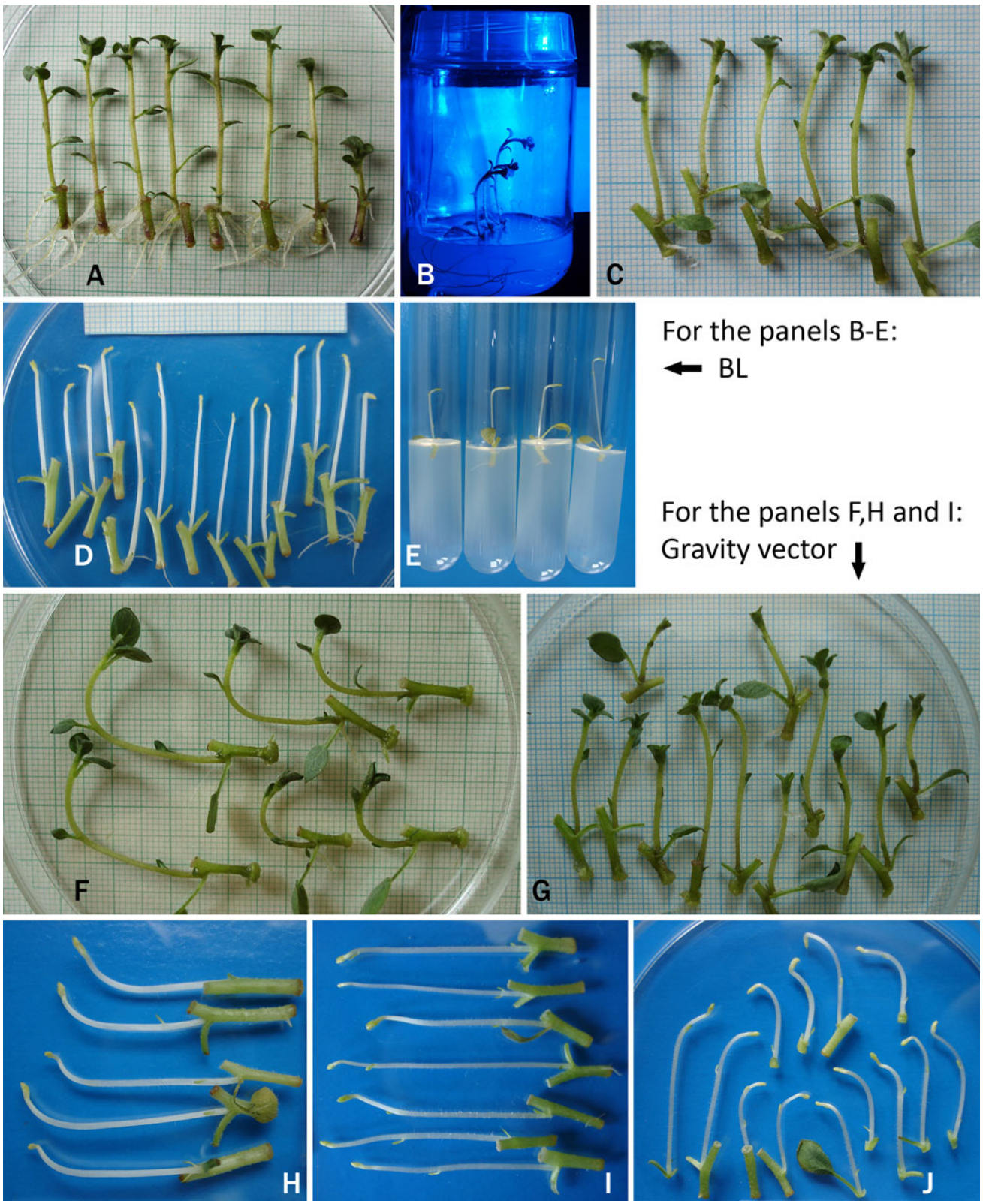
Treatments (duration)	*n*	Plantlet length (mm)	Angle of curvature (°)
Phototropism			
BL (2 h)	36	25.62 ± 0.57 a	2.26 ± 0.36 a
BL (21 h)	60	29.47 ± 0.79 b	90.49 ± 2.00 d
BL (21 h) + static (2 h)	57	26.11 ± 0.81 a	30.05 ± 1.76 b
BL (21 h) + clinostat (2 h)	64	26.51 ± 0.80 a	45.75 ± 2.75 c
Gravitropism			
90° (2 h)	59	26.12 ± 0.73 a	64.58 ± 3.24 c
90° (3 h)	77	26.06 ± 0.53 a	72.71 ± 1.16 d
90° (3 h) + Static (2 h)	57	24.99 ± 0.35 a	14.14 ± 1.29 a
90° (3 h) + Clinostat (2 h)	55	24.81 ± 0.70 a	22.18 ± 1.76 b

Means followed by the same letter are not statistically different according to Duncan's multiple-range test

n Number of plantlets

plantlets can be classified as the second positive response. For the second positive PT to be induced, two requirements must be met: (1) the amount of light applied unilaterally must be in excess of the fluence threshold, and (2) this amount of light must be applied for a period of time in excess of the time threshold (Janoudi and Poff 1990). The 2 h of irradiation for light-grown and 21 h for etiolated plantlets needed for induction of PT in potato (Tables 1, 5) are certainly enough to satisfy the time threshold, which, for all of the previously studied plant species, is 15–60 min long (Ellis 1987; Hasegawa and others 1989; Janoudi and Poff 1990). Also, the fluence of BL needed to induce PT in the potato plantlets (Fig. 1, estimated at 20–30 μmol/m^2) is above the fluence thresholds for the second positive PT of 1–6 μmol/m^2 that has been recorded for light-grown or red-light-preirradiated plants of buckwheat, radish, tobacco, and *Arabidopsis* (Ellis 1987; Hasegawa and others 1989; Janoudi and Poff 1990). Because of the limitations of available equipment, we were not able to decrease the fluence rate to levels lower than 0.024 μmol/m^2 s which would have allowed us to estimate the threshold for the second positive PT with more precision. Still, we are fairly certain that the threshold is in the range of 20–30 μmol/m^2 (Fig. 2), which is higher than the fluence threshold for most of the other plant species (Ellis 1987; Hasegawa and others 1989; Janoudi and Poff 1990). Another discrepancy with previously reported data is in the shape of the curve describing the fluence requirement for the second positive PT. The curve for potato rises slowly and spans five orders of magnitude, whereas curves describing the fluence requirement for the second positive PT of buckwheat, radish, tobacco, and *Arabidopsis* span only one or two orders of magnitude (Ellis 1987; Hasegawa and others 1989; Janoudi and Poff 1990). These differences may be the consequence of species-specific expression of the *PHOT2* gene believed to be responsible for mediation of the second positive PT (Pedmale and others 2010).

A PT bending potential of light-grown SNE potato plantlets was examined at various times during the day and it showed high and regular daily fluctuations. These experiments have shown that in the first half of the day, PT bending can be completed within 120 min (Table 1). There was a prominent daily maximum of bending potential at 1 p.m., when shoots on average performed the full bending movement in just 75 min (Fig. 4b). Also, a decline in the PT bending potential was registered later in the afternoon. For the treatments that started at 5 p.m. and later, the full PT bending could not be realized by 2 h of irradiation. After



photostimulation, plantlets irradiated unilaterally for 2 h starting at 5 and 11 p.m. bent on average only 33.84° and 27.78°, respectively (Table 1). These differences can be the results of different bending rates and/or different duration of the lag phase throughout the day. We are presently performing a detailed study of the kinetics of the PT and GT responses.

◀**Fig. 4** **a** Unstimulated light-grown plantlets exhibiting uniform growth. All of the SNE explants were excised from a single shoot and arranged in succession, with the basal explant on the *left* and subapical on the *right*. **b** Light-grown plantlets in the jar exhibiting PT at the end of 2 h of unilateral BL stimulation that started at 1 p.m. **c** At the end of 2 h of straightening in darkness (following 2 h of PT stimulation), light-grown plantlets are often S-shaped because straightening, just like the PT bending, starts from the shoot's top and spreads basipetally. **d** Etiolated plantlets after 2 h of PT stimulation with hooks still present on some of them. **e** Etiolated plantlets exhibiting PT response after 21 h of unilateral BL stimulation (notice disappearance of hooks). **f** Light-grown plantlets at the end of 4 h of GT stimulation usually overshoot the right angle of 90°. **g** At the end of 2 h of straightening in darkness (following 2 h of GT stimulation), light-grown plantlets are often S-shaped, the same as after straightening. Plantlets in this particular photograph were straightening on a clinostat. **h** Etiolated plantlets after 2 h of GT stimulation. **i** Etiolated plantlets after 2 h of GT stimulation and 2 h of straightening on a clinostat. **j** Etiolated plantlets after 4 h of GT stimulation

If the shape of the kinetics curves for the PT response of plantlets stimulated at 5 and 11 p.m. is not different from the curves describing the kinetics of PT at earlier times during the day, then the results from Table 1 present the interesting possibility that some of the factors mediating potato tropic responses may also be associated with a circadian rhythm. The question arising from this discussion is: What would be the ecological and evolutionary advantage for plants to have PT controlled by a circadian rhythm? When exhibiting phototropism in the natural environment, plants are trying to assume the position that will allow their photosynthetic apparatus to have an adequate supply of energy from sunlight. Considering that moonlight does not result in activation of photosynthetic machinery, it is appropriate for plants not to orient their organs in the direction of incoming moonlight thereby saving energy and resources for other processes. Also, tropic responses are not the only processes controlled by circadian rhythms in potato. It is known that tuber formation is controlled by the circadian clock and that under conditions of a long day, the circadian clock can be reset by the action of phytochrome A (Yanovsky and others 2000).

In the potato plantlets irradiated with unilateral BL at 11 p.m., the point of the curvature moved the least from the top of the stem compared to all the tested groups and as a consequence they had the shortest curved apical portion (Table 1). These plantlets also had the lowest PT response. Upon perception of the PT signal, the point of curvature also moved along the stem of responding *Arabidopsis* seedlings as a result of redistribution of growth on the opposite sides of the stem (Orbović and Poff 1991, 1993). It was suggested that in the complicated mechanism responsible for such responses in plants, downward movement of auxins plays a major role (Stone and others 2008). However, in some cases differences in the invoked PT responses can be a trivial result of mechanics as shoots with larger diameters require more differential growth than thinner shoots (Whippo and Hangarter 2006). In the case of the potato SNE plantlets that we used, variation in thickness was negligible (0.87 ± 0.009 mm; $n = 154$) and could not result in a different PT response between short and long plantlets.

There was also a significant difference in bending capacity of light-grown potato SNE plantlets exhibiting GT throughout the day (Table 3). As the day progressed, the GT response increased and was the highest at 5 p.m. (Fig. 4f) but decreased to its lowest levels at 11 p.m. Considering that no experiments involving auxins were done in this study, we cannot postulate their role as final effectors of the transduction chains for either PT or GT of potato SNE plantlets. No matter what the nature of the effector for tropic responses of potato is, the magnitude of its redistribution along and across the plantlets and/or sensitivity of tissue to this molecule(s) due to outside stimuli seems to change throughout the day.

Light-grown potato plantlets that were irradiated with unilateral BL for 2 h and then left for an additional 2 h in darkness in an upright position exhibited a curvature of 19.75° (Table 4; Fig. 4c). Compared to plantlets that were irradiated with BL for only 2 h (Table 1), plantlets left for two more hours in darkness lost almost 70° of curvature through the process of straightening. Straightening also occurred in cultures returned to their original upright position (Table 4) or those placed on a clinostat (Table 4; Fig. 4g) following gravity stimulation. The rate at which plantlets straightened back toward the original position would have to be slightly lower than the rate of either PT or GT bending, considering the time allowed for straightening to take place and final angles recorded. Additionally, the lag phase for the GT response to stimulation brought about by bending of plantlets to unilateral light may be longer than the lag phase of PT. Our results on the kinetics of PT and GT of light-grown potato plantlets revealed exactly that. The lag phase was longer for GT than for PT, and the rate of GT bending was lower than the rate of PT response (Fig. 3). This suggests that the straightening could be a delayed and slow GT response to stimulation arising as the plantlets assume a new position when responding to unilateral stimulation. With plantlets moving back toward a more upright position, the intensity of gravity stimulation decreases and this could be one of the reasons why the straightening is not complete. At some point, weakening gravity stimulation approaches a threshold and the plantlets stop responding to it. From our experiments with a clinostat it is clear that gravity itself is not the only factor mediating straightening, as the plantlets straightened back even in the absence of a unidirectional gravity vector (Table 4). Similar results were recorded for seedlings of *Arabidopsis* (Orbović and Poff 1991). Regardless of the BL stimulation (first or second positive

PT) or pretreatment (with or without the pulse of red light), *Arabidopsis* seedlings always exhibited straightening (Orbović and Poff 1993). Additional studies on both the physiological and the molecular basis of this process are needed before any detailed hypotheses can be suggested about control of straightening.

After 2 h of continuous unilateral BL irradiation, etiolated plantlets did not bend at all (Table 5; Fig. 4d), as opposed to light-grown plantlets that exhibited almost 90° of curvature (Table 1; Fig. 4b). It took 21 h of BL stimulation for the etiolated plantlets to curve to a 90° angle (Fig. 4e). We did not find such a drastic difference in the required quantity of stimulus for plantlets exhibiting GT. Etiolated plantlets needed just 50 % more time (3 vs. 2 h; shown in Fig. 4h) of gravitropic stimulation than their light-grown counterparts to attain a similar curvature (Table 5). These results suggest that the bending machinery is present and almost as active in the etiolated plantlets as it is in light-grown ones. That would mean that the responsibility for the low PT response to short-term irradiation in etiolated plantlets is mostly in the upstream portion of the transduction chain. The amount of BL used in PT experiments was in the range that far exceeds the requirement for induction of the first positive phototropism for most species, which is why we did not record any bending toward the BL source after the shortest irradiation period of 2 h. According to Poff and others (1994), in the fluence–response relationship curve there is the so-called "zone of indifference" that describes the response of plants to unilateral irradiation that has not reached either the time or fluence threshold for the second positive PT. This zone exists as the consequence of the loss of plant sensitivity to unilateral stimulation due to sensory (in maize coleoptiles; Iino 1988) or effector adaptation (in *Arabidopsis* seedlings; Janoudi and Poff 1990). The fluence–response relationship curve that describes the PT of etiolated potato plantlets appears to have an extended zone of indifference to very high fluences (long irradiation time) as a threshold for the second positive PT. From the experiments we conducted, it is not possible to determine what type of adaptation is taking place in potato plantlets and contributing to such a high threshold for the second positive phototropism. Considering that plantlets eventually did bend toward the BL, it is clear that they do have appropriate photoreceptors. One of the possible explanations for the high threshold for the second positive phototropism could be a low concentration of photoreceptors in the etiolated tissue. However, this is not likely as phototropins are known to be regulated by light (Pedmale and others 2010) and the levels of phototropin 2 increase twofold in irradiation with a high fluence rate (Sakai and others 2001), which is what we used in our experiments. Another possibility is that slow establishment of differential growth on two sides of potato plantlets is the result of a low rate of synthesis of molecules that could act as screening agents. A gradient of quantum density created across unilaterally irradiated plants due to the existence of molecules with the ability to absorb light is crucial for a phototropic response (Poff and others 1994). We noticed that etiolated plantlets looked greener after 21 h of unilateral irradiation than after 2 h (Fig. 4d, e). The process of greening may be necessary for the initiation of PT in etiolated potato plantlets. Results presented here for potato are in accordance with those obtained for buckwheat, radish, and cress (Ellis 1987; Hasegawa and others 1989; Hart and Macdonald 1981a), where light-grown specimens responded to BL stimulation by exhibiting a response of a significantly larger magnitude when compared to etiolated material.

Etiolated potato plantlets behaved the same way as their light-grown counterparts with respect to the process of straightening. After the completion of unilateral stimulation (either PT or GT), they started straightening and within the next 2 h annulled most of the tropic response if they were left in the upright position (Table 5). If plantlets were placed on a clinostat upon completion of stimulation, the process of straightening was slower (Table 5; Fig. 4i).

The maximum GT response of 72.71° (Table 5) was recorded after 3 h of gravitropic stimulation of etiolated plantlets and curvature did not increase with longer exposure to 90° of angle (data not shown). This maximum GT response was lower in magnitude than the maximum PT response (Table 5). The reason for the difference between the maximal GT and PT responses may be the presence of a hook. After 21 h of irradiation by BL, hooks on the plantlets exhibiting PT completely opened up while they were still present on the plantlets doing GT bending (Fig. 4e, j). It has been previously reported that the presence and position of the hook can affect the magnitude of tropic responses. Those *Arabidopsis* seedlings that had the hook oriented in the direction of outside stimuli exhibited significantly higher PT and GT responses in comparison to seedlings with the hook oriented away from the direction of incoming stimulation (Khurana and others 1989). Because the population of potato plantlets exhibiting GT consisted of individuals with hooks oriented toward and individuals with hooks oriented against the direction of stimuli, it would be expected that their average response was lower than the response of populations of plantlets responding to unilateral BL that all had their hooks fully open.

Etiolated plantlets were on average 4–5 days younger than their light-grown counterparts when they were used in experiments. They also did not have their photosynthetic machinery developed at the time of stimulation with the outside stimuli. The question of a difference in the availability of energy resources to perform tropic responses between etiolated and light-grown plantlets can be brought up. However, this should not be a problem as etiolated plantlets had heterotrophic metabolism driven by the (more

than) sufficient amount of sucrose in the medium and were in no way devoid of energy to perform tropisms.

In conclusion, potato plantlets produced from in vitro-grown shoot cultures exhibited vigorous tropic responses and can be used as good experimental material for studies in this field. For both PT and GT responses, a much higher quantity of stimulation was required to evoke a response in etiolated seedlings compared to light-grown seedlings. Upon completing the PT and GT responses, potato plantlets started straightening back; this process can be attributed only partly to the effect of gravity as it also took place in plantlets there were rotated on a clinostat. The magnitudes of both the PT response and the GT response changed with time of day which suggests that some element of these responses may be under the control of the circadian clock.

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