TROPIC RESPONSES OF POTATO SINGLE-NODE EXPLANT CULTURES

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Abstract - A special *in-vitro* protocol was elaborated enabling the production of potato single-node explant plantlets that can be used as objects for tropic studies. In light-grown plantlets, achievement of a full (90°) phototropic (PT) curvature required 75 to 120 min of continuous unilateral blue light irradiation or 120-135 min of gravitropic stimulation (GT). Time-lapse photography revealed that the curves describing PT and GT bending have a sigmoid shape. Continuous BL irradiation was necessary for the induction of continuous PT bending. If the BL was turned off after 30–50 min of PT stimulation, the bending gradually decreased and stopped in darkness after 25.0 ± 2.0 min. Within this period, curvature increased by $15.5 \pm 1.5°$. When the BL was turned off upon completion of PT bending (when the plantlets reached an angle of 90°), the plantlets entered the phase of fast straightening. The 90° PT curvature was significantly exaggerated in darkness by turning the jars from a vertical to horizontal position providing $120.74 \pm 2.5°$ as the final curvature angle after two more hours in darkness.

Key words: Phototropism, gravitropism, *Solanum tuberosum*, Désirée, *in vitro*

INTRODUCTION

Light-grown potato plantlets derived from single-node explants were observed to manifest vigorous photo- and gravitropic movements *in vitro (*Vinterhalter et al., 2010), offering a novel model system for tropic response studies to be established. To meet the needs of these studies, we had to optimize the protocol for the production of experimental material (single-node shoot explants) and to define experimental conditions supporting their fast and reproducible tropic responses.

Under conditions of *in vitro* culture, potato is a rapidly growing species that requires a simple plant growth regulator-free medium to support maintenance at all propagation stages. Apical shoot buds grow continuously, extending the shoot length by the production of new nodes, each one being equipped with a dormant axillary bud. Sub-culturing was done by shoot dissection into single-node explants (SNE) where each explant contained one node carrying an axillary bud. After excision, these explants exhibited fast growth forming a complete, rooted plantlet in just 11-14 days.

Very little is known about the tropic responses of potato as opposed to other plant species. This situation is probably a result of difficulties in the production and germination of true potato seeds in most of the commercial cultivars. Among related species, some data on phototropism is available from studies in tomato done by Srinivas et al. (2004). Our fist trials on phototropism in potato (Vinterhalter and Vinterhalter, 2002) were done with Nichia, Japan high intensity NPSB 300B and 500B gallium nitride LEDs. In the meantime, various light sources based on LED technology became widely available.

In our previous study (Vinterhalter et al., 2010), we presented the scope of phototropic and gravitropic responses of potato SNE plantlets. We showed that throughout the first 9-10 h of the day (photoperiod: 16 WL, 8 darkness) SNE plantlets required up to 2 h to complete a full, 90° bending curvature in response to phototropic stimulation. For the gravitropic response, the initial lag phase was somewhat longer, requiring the plantlets to take 15 min more to perform a complete 90° bending. The phototropic response of potato SNE plantlets was classified as second positive, indicating both high irradiance and continuous irradiation as necessary requirements. Gravitropism appeared as a process opposite to phototropism, although the proper PT vs. GT interaction was not investigated in detail.

Our previous measurements of tropic responses (Vinterhalter et al., 2010) were static, calculated from photographs taken after a fixed 2 h time interval. This type of recording did not allow for the examination of the real-time kinetics and has therefore left many open questions. To obtain better insight into tropic movements, we used time-lapse photography that allowed us to take digital photographs of bending plantlets at short (1 or 2 min) intervals. These photographs were used to measure the angles of curvature of bending potato plantlets.

One of the most important issues we wanted to investigate was whether there is a need for continuous blue light irradiation for continued phototropic bending. We also performed a number of tests eliminating either PT or GT stimulation with the intention to evaluate their interaction and contribution to tropic bending. The results were unexpected and opened up a plethora of new questions concerning PT vs. GT interactions.

MATERIALS AND METHODS

Plant material and growth conditions

Shoot cultures of potato (*Solanum tuberosum* L.) cv. Désirée, were grown on plant growth regulator-free MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.64% agar according to the continuous propagation procedure suggested by Hussey and Stacey (1981). Batches of 5 to 6 single-node explants were cultured in Ø 70 mm x 120 mm glass jars (270 ml volume) with 40-50 ml medium and translucent polypropylene closures. Sub-culturing was done at three- to four-week intervals, i.e. always prior to the activation and outgrowth of axillary buds. Shoot tips were used to maintain cultures while the single-node explants (SNE) were harvested for experimental treatments or to increase the number of available shoot tips. Three SNE explants were aligned in a row across baby-food jars with 20 ml of medium, and closed with Magenta B caps. On average, the SNE required 10-14 days in the light to reach the height suitable for tropic experiments. At this stage, the explants had developed adventitious roots and were therefore referred to as plantlets.

Cultures were multiplied and maintained in a growth room with a long-day photoperiod (16h WL/8h darkness) and temperature controlled at 24 ± 2°C. White light in the growth room was provided by Phillips TLD 58w/54) fluorescent lamps providing a fluence rate of ~74 μMm^{-2} $s^{-1°}$ as measured by a LiCor 1400 spectrophotometer with a Quantum sensor. The beginning of the day in the growth room was fixed at 7 AM, with most experiments starting at 11 AM, i.e. 4 h after the beginning of the day.

Light sources and irradiation conditions

Experiments were performed in black-walled cabinets situated in a dark room adjusted to the same temperature conditions as the growth room. There was no other light (safe light) in the dark room apart from the light sources providing unilateral irradiation. Commercial, narrow-beam spot LED lamps produced by Phillips, equipped with a GU10 socket, were used as a source of unilateral blue light for PT stimulation. The lamps provided a fluorescence-rate of 24 μMm^{-2} s^{-1} from a distance of 40-42 cm. During unilateral blue light (BL) stimulation, each culture jar was illuminated by a single, separate LED lamp. Culture flasks were positioned in such a way that the

upright axes of the plantlets were perpendicular to the incoming light. Yellow LED lamps (Vito GU10) providing 2 µMm^{-2} s^{-1} were used for brief background illumination in the dark treatments and GT studies.

Gravitropic stimulation

For gravitropic (GT) stimulation, flasks with SNE plantlets were turned on their side and placed horizontally (at 90°) in darkness on a black-cloth support which prevented the jars from rolling out of the desired position. For time lapse photography studies, the culture flasks with GT stimulated shoots were briefly illuminated for 5-6 s with yellow-colored LED lamps at regular 3 min intervals.

Measurements of plantlets curvature and statistics

For experimental treatments, 45-60 SNE plantlets from 15-20 culture flasks were studied. A pilot test used 8 culture flasks with 24 plantlets. Photographs 3.5 Mpix in size were taken with a Lumix DMC FZ28 digital camera at regular 1 or 2 min intervals for PT responses. Quantitative measurements of the curvature angle and lengths were done with the UTHSCSA Image tool for Windows, 3.0 (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, Texas) from our stored digital images. Figure drawings and statistics were made using Origin8 (Originlab Corp. Northampton, MA, USA).

RESULTS

Graphic presentation of the curvature angles of single, PT-stimulated shoots were sigmoid in shape (Fig. 1). Following a short ~20 min-long initial lag phase, a steady, nearly linear, rapid bending stage was achieved, ending with a progressive slow-down as the curvature approached the right (90°) angle. In most cases the complete right angle curvature was achieved within two hours of PT bending and the graphics differed only by the curvature slope.

There was a considerable variation in the PT responses of individual plantlets. According to the time required to fulfill the right angle curvature, PT bending was classified into the following categories: very fast 45-60 min, fast 60-75 min, moderate 75-90 min and slow 90-120 min or more (Fig. 1 A,B,C and D).

On reaching the full, complete right angle (90°) curvature, a fast and prominent straightening was observed in about 5% of the plantlets, but only from the fast and very fast bending groups. In the majority of shoots, the curvature achieved by PT stimulation was maintained with minor fluctuations for at least 1-2 h. After a full 90° PT bending, straightening was obligatory if the BL was turned off. However, if the light was kept on and the culture flask quickly rotated 90° to the left or to the right, the plantlets after a moderate ~30 min long lag phase started a new, secondary horizontal PT movement, aligning the shoot tips towards the re-positioned BL source. Secondary PT movement required some more time and it was conducted after some 105-150 min.

For rapid and smooth shoot bending, it is of the utmost importance that the culture flasks, once mounted under the beam of BL, are undisturbed and left alone until the end of PT stimulation. Even small adjustments of the light source or corrections of the flask position in the light beam during the BL irradiation resulted in a lengthening of the initial lag phase of bending, prolonged curvature duration or even early termination of shoot bending (Fig. 1D). Short 10-20 min-long periods of darkness applied between the sampling of the culture flasks from the growth chamber and the beginning of unilateral BL irradiation affected the PT bending response in a similar way. The initial lag phase here was often extended to last for more than 20-30 min and bending was slower, ending much before full, 90° curvature was reached. For this reason, the time required to transfer the cultures from the growth room and mount them under unilateral blue light was reduced and standardized to last up to 3 min, avoiding transport of the culture flasks in dark containers or brief exposure to direct sunlight.

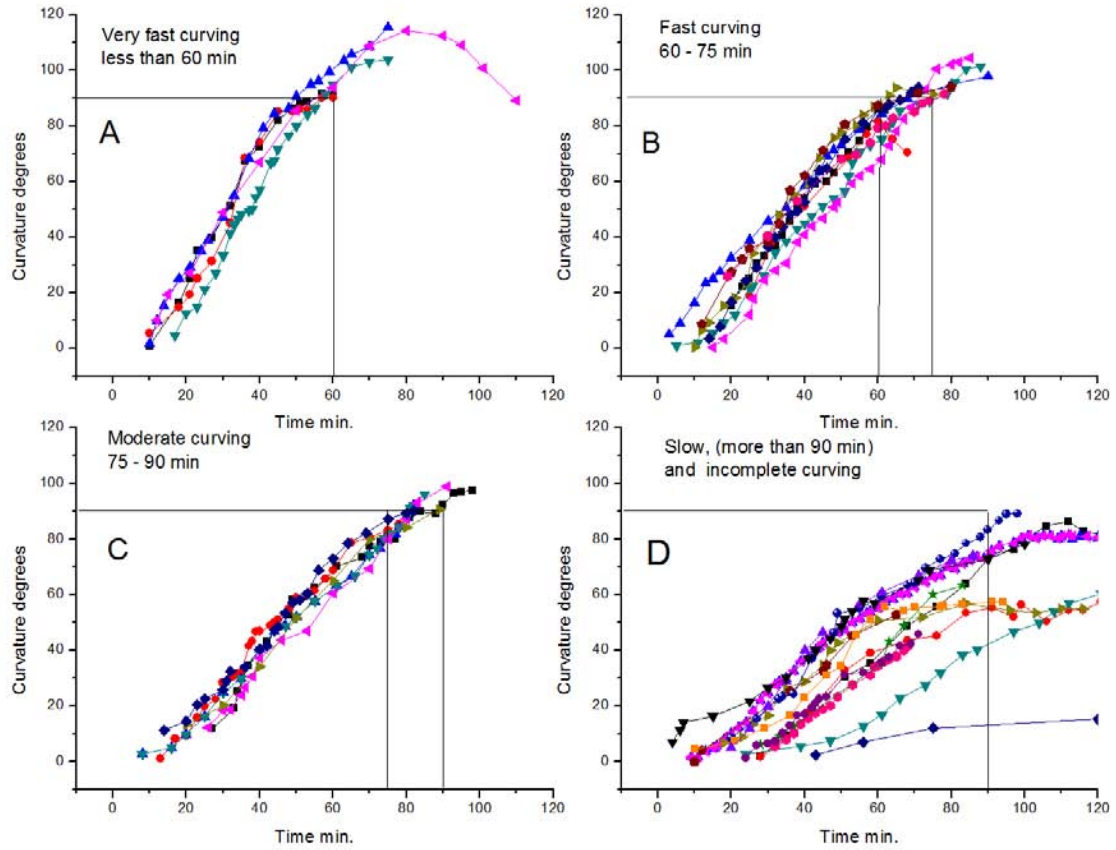Gravitropic stimulation also resulted in fast 90° bending of the apical shoot portions. GT bending

Fig. 1. Classification of phototropic bending in shoots of potato SNE plantlets: (A) very fast 45-60 min; (B) fast 60-75 min; (C) moderate 75-90 min; (D) slow 90-120 min or more.
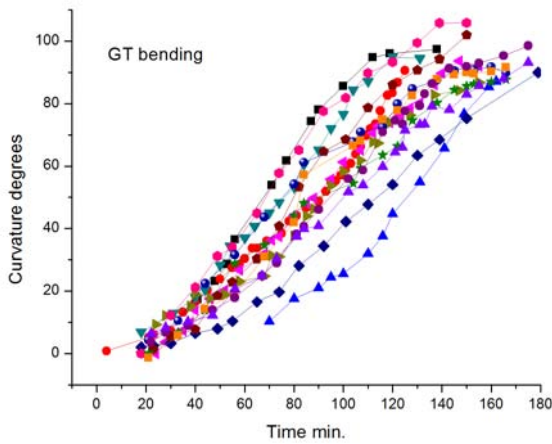

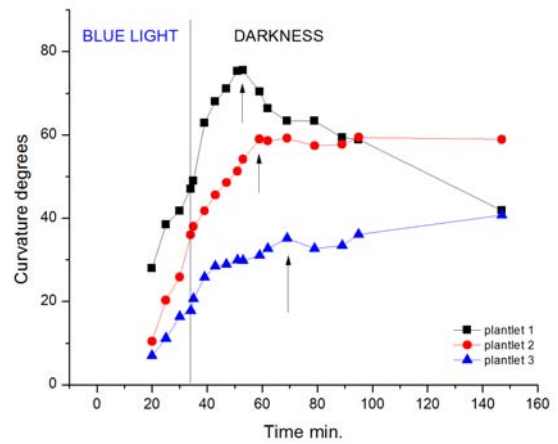**Fig. 2.** Gravitropic bending of potato SNE plantlets.

Fig. 3. Phototropic bending interrupted by darkness after 32 min of unilateral BL irradiation. Culture flask with 3 SNE plantlets.

Fig. 4. Exaggerated PT bending after 120 min of BL irradiation and additional 60 min of darkness with culture flasks overturned to horizontal position. Adventitious roots removed.

was far less variable than PT bending and it could be presented on a single graph (Fig. 2). Graphic presentation of GT bending provided sigmoids similar to those of PT bending (Fig. 2). According to the time-lapse studies, the initial lag phase of GT bending was somewhat longer than in PT, lasting at least 30 min, and full GT bending required 120-135 min. Studies showed some differences in the dynamics of PT and GT bending. Thus, the bending rate in PT-stimulated shoots gradually decreased with time, being the highest shortly after the end of the initial lag phase. In GT-stimulated shoots, the bending rate often increased with time, causing the shoots to slightly overshoot the expected vertical position.

From the earliest trials, it has been obvious that continuous BL irradiation was indispensable for PT bending since short, up to 10 min long exposures to BL did not trigger significant PT bending. We, however, additionally checked the BL contribution, turning the light off after some 30-50 min of PT stimulation (Fig. 3). Turning the light off did not instantly affect the bending process. Bending decreased gradually, starting after some 10-15 min until it stopped 10-25 min later. Thus in darkness bending continued for 25.0 ± 2.01 min, increasing the curvature angle by $15.5 \pm 1.5°$. Interestingly, shoots could maintain the achieved angle for extended periods of time (Fig. 3, plantlet 2) or even increase the curvature angle (Fig. 3, plantlet 3), while some of them entered shallow straightening (Fig. 3 plantlet 1).

We also showed that extended PT shoot bending could occur even in darkness under certain conditions, leading to over-exaggerated bending (Fig 4). Namely, at the end of a standard PT treatment, when

Table 1. Curvature exaggeration in darkness following PT bending with culture flasks overturned to a horizontal position (both PT and GT canceled).

Plantlets	Angle at the end of PT stimulation (degrees)	Highest curvature angle in darkness degrees	Total curvature in darkness (degrees)	Time until highest angle (min)
1	32.26	112.61	80.35	150
2	45.89	101.33	55.44	146
3	50.55	125.23	74.68	110
4	55.96	129.43	73.47	133
5	57	105.1	48.1	112
6	61.28	123.24	61.96	146
7	61.76	110.27	48.51	103
8	64.56	103.81	39.25	85
9	68.72	112.71	43.99	85
10	72.57	111.3	38.73	85
11	73.42	136.16	62.74	100
12	73.74	112.21	38.46	151
13	74.19	112.81	38.62	128
14	74.96	122.6	47.64	120
15	79.94	124.75	44.81	96
16	81.91	145.29	63.38	108
17	83.13	128.87	45.74	112
18	83.28	147.84	64.56	106
19	85.97	106.51	20.54	180
20	87.6	114.14	26.54	78
21	88.16	132.64	44.48	100
22	88.51	120.46	31.95	84
23	88.79	129.86	41.07	88
24	98.99	128.8	29.81	120
Average for 8 flasks	72.21 ± 3.26	120.74 ± 2.57	48.53 ± 3.19	113.58 ± 5.39

the shoots had already attained a horizontal position and their bending rate was negligible, light was turned off and the culture flasks were placed horizontally. In this position the already bent shoot tips were pointing upward, i.e. the tips were not subjected to further GT stimulation. However, following a short ~20 min lag phase, the shoots renewed (extended) bending in darkness in the direction of the former PT response, exaggerating the curvature angle. After 60 min of darkness, bending was 110.2 ± 1.6°. The

maximum bending angle 120.74 ± 2.5° was reached after 113 min of darkness with a total 48.51 ± 3.17° bending in darkness (Table 1).

DISCUSSION

There is no doubt that photo- and gravitropism are the most important tropic movements of higher plants. In the last decade, numerous studies on both topics were done using various experimental approaches, and they have been amply reviewed (Blancaflor and Masson, 2003; Morita and Tasaka, 2004; Molas and Kiss, 2009; Pedmale et al., 2010). Many interactions and signal transduction pathways were established, but the primary step in both tropic responses and their interaction has remained elusive. Most of the research was performed on the *A. thaliana* model system and perhaps it is time to shift our attention to other plant species. The potato is interesting since through its life cycle it develops different shoot types, among which stolons and tubers are known to be insensitive to gravity.

The phototropic bending responses of potato *in vitro* plantlets cultured in light are in general similar to those described for other dicotyledonous species, including cress, lettuce, mustard and radish (Hart and Macdonald, 1981). Our first study on the gravitropic and phototropic responses of potato (Vinterhalter et al., 2010) provided results highly congruent with those from detailed studies done on cress (Hart and Macdonald, 1981b), indicating that potato *in vitro* plantlets can be used in studies of plant tropic responses.

The variability that we registered in the phototropic response of individual potato plants is common in other plant species, including *A. thaliana* (Orbovic and Poff, 1991). However, the selection of culture flasks with uniform growing explants, their fast transport and rapid mounting under BL enabled us to suppress variability and obtain highly reproducible responses.

Small adjustments (corrections) of culture vessel position done at the early stages of BL irradiation were found to be responsible for most of the recorded variability. Standardization of the sampling, transport and mounting of culture flasks enabled us to eliminate shoots falling in the slow curvature response group (Fig. 1D).

The continuous presence of unilateral BL light was indispensable for the fast PT bending of light-grown plantlets that is expected for conditions of second positive phototropic response. Since most of the classic studies in plant photobiology have been done on the first positive phototropic response (using etiolated seedlings) which continues in darkness after a short BL pulse, there are some misunderstandings and difficulties in the comparison of these different experimental approaches. Thus, following a short BL pulse, etiolated seedlings bending in darkness is expected for nothing else but to finally enter straightening as the last stage of a tropic movement as postulated by Firn and Digby (1980). However, under continuous BL irradiation the appearance of straightening in potato SNE plantlets is a rare event that occurs only is some fast bending shoots. On the other hand, turning the BL light off, or replacing the blue light with the light of red LEDs after the completion of the PT bending of potato SNE explants always resulted in straightening starting after a short lag phase. It is difficult to imagine that gravity is not involved in this post-PT straightening when GT is the only remaining tropic stimulus.

We showed that BL-induced PT bending requires a continuous BL presence since in darkness PT-induced bending gradually decreases until it completely stops after some 20-30 min. Similarly, following the full 90° PT bending, GT stimulation in darkness apparently prevails, leading to straightening. Finally, under conditions that lead to exaggerated bending in darkness, GT is the only tropic force available to shoots. In shoots incompletely bent by PT stimulation, it is present in nearly full force. In shoots which have already reached full 90° PT bending, we can only speculate about the residual tropic forces that bring about this curvature exaggeration.

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