J Plant Growth Regul (2012) 31:342–350
DOI 10.1007/s00344-011-9242-8

Blue Light Inhibition of Tuberization in a Day-Neutral Potato

K. R. Fixen · S. C. Thomas · C. B. S. Tong

Received: 16 June 2011 / Accepted: 15 October 2011 / Published online: 26 November 2011

Abstract In tests on the effects of light quality on potato tuberization, continuous blue light was found to consistently inhibit tuberization of tissue-cultured plantlets of *Solanum tuberosum* ssp. *tuberosum* cv. 'Norland'. Other tested cultivars, including sports of 'Norland', formed tubers under continuous blue light. Microarrays identified *BL*, *GA7ox*, and *Nudix* genes as exhibiting altered expression in response to blue light treatment. Quantitative RT-PCR (qRT-PCR) showed that *GA7ox* RNA increased in 'Norland' but not in 'Sangre' plantlets in blue light compared to darkness. RNA levels of genes identified in the literature as having roles in potato tuberization were also measured using qRT-PCR. Levels of *GA20o1x*, but not *GA2ox*, RNA increased in response to blue light in 'Norland' plantlets. *BEL5* RNA content was greater under blue light compared to darkness for both 'Norland' and 'Sangre' plants. Levels of *FT* were not significantly different in blue light compared to dark-treated 'Norland' plants, but were low in blue light-treated compared to dark-treated 'Sangre' plants. Addition of ancymidol to 'Norland' plants exposed to blue light overcame blue light inhibition of tuberization. Ancymidol prevents the oxidation of *ent*-kaurene to *ent*-kaurenoic acid, thus inhibiting gibberellin biosynthesis. These data suggest that blue light may increase GA accumulation in 'Norland' plants, as has been shown to occur in *Arabidopsis* plants. The novel effect of blue light in inhibiting tuberization of 'Norland' plants suggests that this system could be a useful tool in further elucidating the mechanisms of day-neutral potato tuberization.

Keywords *Solanum tuberosum* · Gibberellins · GA20ox1

Introduction

Tubers are storage organs that make important contributions to the survival of various plant species and to the humans who depend on them for food. Among the many plant species that produce tubers are potatoes (*Solanum tuberosum* L.), which are the fourth most important contributor to the human food supply worldwide (Douches and others 1996). Tuberization in potatoes is a complex developmental process that is affected by genetic, environmental, nutritional, and biochemical factors.

Tubers are modified stem tissue that develop from diagravitropic stems called stolons (Fernie and Willmitzer 2001). Stolons originate from lateral buds that develop at the base of the main stem and elongate underground. When conditions are favorable for tuberization and the plant is capable of forming tubers (tuber induction; Ewing and Struik 1993), stolon elongation stops (evocation; Martínez-García and others 2002), the subapical region of the stolon swells (tuber initiation), and the stolon pith and cortex cells enlarge and divide, forming tubers. In in vitro systems, tubers can develop from axillary buds, such as those from stem cuttings, but in the field, they normally develop underground from stolons. Although tubers generally

Electronic supplementary material The online version of this article (doi:10.1007/s00344-011-9242-8) contains supplementary material, which is available to authorized users.

K. R. Fixen · S. C. Thomas · C. B. S. Tong (✉)
Department of Horticultural Science, University of Minnesota, 1970 Folwell Avenue, Saint Paul, MN 55108, USA
e-mail: c-tong@umn.edu

Present Address:
K. R. Fixen
Department of Microbiology, University of Washington, 1705 NE Pacific Street, Box 357242, Health Science Building Rm 327, Seattle, WA 98195-7242, USA

develop and grow underground, their initiation and development can be affected by light (Garner and Allard 1923). Light perception occurs in the leaves where a signal is produced and transmitted underground, resulting in the formation of tubers (Jackson and others 1993).

Much of the work examining light effects on tuberization has focused on photoperiodic effects in potato. Some species and subspecies of potatoes will tuberize only if the plants are grown under short days (for example, more than 14 h of darkness). Jackson and others (1996) studied the effects of phytochromes on tuberization in the short-day species, *S. tuberosum* L. ssp. *andigena*. By introducing a phytochrome B (*phyB*) antisense construct into *S. tuberosum* ssp. *andigena*, they were able to produce transformants that formed tubers even under long-day conditions. When shoots of antisense *phyB* plants were grafted onto non-transformed *S. tuberosum* ssp. *andigena* rootstock, the resulting plants also developed stolons and tubers from the rootstock (just above the root mass), even under long-day conditions (Jackson and others 1998), but only if all leaves and shoots were removed from the rootstock before grafting. Neither the reciprocal graft with non-transformed *S. tuberosum* ssp. *andigena* shoot on *phyB* antisense rootstock nor control plants with *S. tuberosum* ssp. *andigena* shoots grafted onto *S. tuberosum* ssp. *andigena* rootstock tuberized under long days. These grafted plants formed only stolons and no tubers. The authors concluded that *phyB* controls the levels of a graft-transmissible substance in leaves that inhibits tuberization.

Interspecific grafting has also been useful in showing that signals that induce flowering are interchangeable with those inducing tuberization, as have interspecific transgene experiments. Constitutive overexpression in potato of the *Arabidopsis CONSTANS* (*CO*) gene, which hastens flowering in response to long days, delayed tuberization under short-day conditions compared to wild-type plants (González-Schain and Suárez-López 2008). In *Arabidopsis*, *CO* activates *Flowering Locus T* (*FT*), inducing flowering (Turck and others 2008). Expressing *Heading date 3a*, the short-day rice ortholog of *FT*, in short-day potato stimulates tuberization (Navarro and others 2011).

Gibberellin (GA) activity has been shown to decrease in *S. tuberosum* ssp. *andigena* plants in response to short-day treatment (Railton and Wareing 1973). *S. tuberosum* ssp. *andigena* plants overexpressing the *GA20 oxidase1* gene required longer exposure to short days to tuberize, whereas plants with suppressed expression tuberized earlier than control plants (Carrera and others 2000). Accumulation of *GA20ox1* mRNA is induced by blue light in antisense *PHYB* plants, as well as in wild-type *S. tuberosum* cv. 'Desirée' plants (Jackson and others 2000). On the other hand, plants overexpressing the *GA2ox1* gene tuberized earlier, and plants with suppressed expression of this gene tuberized later than control *S. tuberosum* cv. Karnico plants (Kloosterman and others 2007).

Two transcription factors, *POTH1* and *BEL5*, have been associated with tuberization. Potato plants engineered to overexpress *POTH1* exhibited enhanced rates of tuber growth and decreased GA_1, GA_8, and GA_{20} accumulation (Rosin and others 2003). Promoter activity of the *BEL5* gene in *S. tuberosum* ssp. *andigena* was enhanced by blue light compared to darkness (Chatterjee and others 2007).

In addition to keeping time using light, plants sense light quality to control developmental processes such as germination, flowering, and stem growth. The effects of blue light and the blue light receptors (especially cryptochromes and phototropins) on hypocotyl elongation, floral induction, cotyledon expansion, stomatal opening (Lin 2000), seedling de-etiolation (Liscum and others 2003), anthocyanin accumulation (Chatterjee and others 2006), phototropism, chloroplast relocation (Briggs and Christie 2002), shade avoidance (Vandenbussche and others 2005), and *Chlamydomonas* sexual life cycle (Huang and Beck 2003) have been well documented.

Blue light has been found to alter expression of *GAox* genes. Foo and others (2006) reported that under blue light, cry1, along with phyA, regulates GA levels by suppressing RNA accumulation of *GA3ox1* and upregulating levels of *GA2ox2* RNA in the inhibition of elongation of pea seedlings. Similar results were discovered in de-etiolating *Arabidopsis thaliana* seedlings under blue light (Zhao and others 2007). However, Zhao and others found that continuous blue light increased *GA2ox1* expression and suppressed *GA20ox1* and *GA3ox1* expression, without a significant change in GA_4 levels.

In potatoes, blue light increases the number of axillary buds (Seabrook 2005) and the root-to-shoot ratio (Aksenova and others 1994). Use of yellow Plexiglas filters to eliminate blue light resulted in increasing stem length and haulm fresh weight, and decreased the incidence of leaf intumescences (Seabrook and Douglass 1998).

There are few reports of how light of different wavelengths affects tuberization apart from photoperiodic effects. Blue light promoted more tuberization than red light under 16-h photoperiods (Seabrook 2005). Macháčková and others (1997) reported that isopentenyl transferase transformants of potato tuberized under an 18 h photoperiod and produced more tubers when treated with blue or red light than with white light. Aksenova and others (1994) found that blue light and kinetin promoted tuberization of *S. tuberosum* cv Miranda plants.

In this article we describe blue light inhibition of day-neutral potato tuberization. By studying day-neutral genotypes, the effects of light quality on tuberization can be studied apart from photoperiod effects.

Materials and Methods

Plant Material

Tissue-cultured potato (*S.tuberosum* L.ssp. *tuberosum*) plants were obtained from the USDA-ARS Inter-Regional Potato Introduction Station in Sturgeon Bay, Wisconsin. Plants were propagated under sterile conditions in medium containing Murashige and Skoog basal salts (4.3 g/l), Nitch and Nitch vitamins (0.12 g/l, PhytoTechnology Laboratories, Shawnee Mission, KS), 3% (w/v) sucrose, and 2.5% (w/v) Phytagel (Sigma-Aldrich, St. Louis, MO). Plants were grown under continuous fluorescent white light (49 μmol m^{-2} s^{-1}). After 2 weeks in propagation medium, plants were transferred to tuber-inducing medium containing Murashige and Skoog basal salts, Nitch and Nitch vitamins, 6% sucrose, and kinetin (2.5 mg/l), and placed under different light conditions. A summary of all light experiments is given in Table 1.

Light Treatments

The varieties 'Desirée' and 'Norland' were placed in EGC growth chambers (Model GC-8, Environmental Growth Chambers, Chagrin Falls, OH) with four tungsten halogen and eight fluorescent (F48CW/VHO) bulbs producing average irradiance levels of 425 μmol m^{-2} s^{-1}. Plantlets were exposed at 20°C to either 8 h light/16 h dark, or 16 h light/8 h dark conditions. Three to four Magenta boxes with three plants per box were used for each experiment. 'Desirée' and 'Norland' plants were also exposed to continuous darkness, or red, far-red, or blue light at room temperature. In these experiments, total irradiances of the red, far red, and blue lights were 49, 3, and 3 μmol m^{-2} s^{-1}, respectively. The red bulbs (575–700 nm) were Sylvania F48T12/2364//HO filtered through an Encapsulite red tube guard (Lighting Plastics of Minnesota, St. Louis Park, MN), and the far-red bulbs (700–850 nm) were Sylvania F48T12/232/HO) filtered through an Encapsulite FR tube guard. Red-to-far-red ratios of the red and far-red lights were 0.06 and 4.9, respectively. Blue light (450–550 nm) with irradiance of 3–6 or 27 μmol m^{-2} s^{-1} was used. The low-irradiance blue bulbs were GE Cool White F40CWEX bulbs covered with blue tube guards, and the high-irradiance blue bulbs were Science-Lite 64805 bulbs (CESI, Rockville, MD). For short-day treatments, plants were exposed to 8 h light/16 h dark, and the reverse was used for long-day treatments. Total irradiance was measured using a LI-COR LI-189 light meter (LI-COR, Lincoln, NE). Light quality was measured using an Apogee Instruments SPEC UV/PAR spectroradiometer (Apogee Instruments, Logan, UT). All other potato varieties were exposed to darkness or low irradiance (3–6 μmol m^{-2} s^{-1}) blue light. All plants were exposed to light treatments for 6–8 weeks. Experiments utilized at least three replicates and were repeated at least twice.

Blue Light Experiments

Due to results obtained from the experiments mentioned above, further experiments concentrating on blue light were performed. For all blue light experiments, individual plantlets were placed in culture tubes containing 20 ml of tuber-inducing medium. Preliminary experiments showed that results were similar to those in which three plants were placed in Magenta boxes. Two to four low-irradiance blue light bulbs were used to obtain irradiances of 5–6 μmol m^{-2} s^{-1}. Plants were kept on tuber-inducing medium under continuous blue light or darkness (negative control) for 6–8 weeks. At least three tubes were used per treatment, and all experiments were replicated two to three times.

Based on results from microarray and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) experiments described below, the effects of the gibberellin antagonist ancymidol on blue light inhibition of tuberization were tested. Ancymidol (10 mg ml^{-1}, Sigma-Aldrich) was sterilized using 0.25 μm syringe filters and added to 25 ml tuber-inducing medium to produce 5 μg ancymidol per ml medium, either before plantlets were added or after plantlets had been in tuber-inducing medium and darkness for 3 weeks.

RNA Extraction and Amplification

RNA was extracted from stem tissue, including leaves, previously frozen in liquid nitrogen and stored at –80°C,

Table 1 Summary of experiments

Experiment	Treatments	Irradiance (μmol m^{-2} s^{-1})
1. Comparison of different light treatments	Darkness, and red, far red, and blue light	49 (red), 3 (far red), and 3 (blue)
2. Effect of length of light treatment	White light for 8 h vs. 16 h per day	425
3. Blue light effects on different potato varieties	Blue light	3
4. Effect of length of blue light treatment	Blue light vs. darkness	3–6
5. Effect of ancymidol on blue light inhibition of tuberization	Blue light ± ancymidol	3–6

using RNeasy mini kits (Qiagen, Valencia, CA) according to the manufacturer's recommendations. For qRT-PCR, genomic DNA was degraded after initial purification using RNase-free DNase I (Qiagen) and total RNA was repurified using RNeasy columns. RNA amounts were measured using the Qubit fluorometer and RNA Quanti-It reagent kit (Invitrogen, Carlsbad, CA). RNA for microarray experiments was vacuum-concentrated to complete dryness. For microarray and qRT-PCR experiments, total RNA was reverse-transcribed into cDNA using SuperScript III (Invitrogen) and random hexamers as primers. cDNA for microarray experiments was purified using MinElute columns (Qiagen), and subjected to two rounds of amplification using the Ambion MessageAmp aRNA kit (Ambion, Austin, TX) according to the manufacturer's protocols.

Microarrays

Microarray experiments were used to identify genes that could be involved in blue light inhibition of tuberization. These experiments utilized a direct-comparison, balanced-block design. Gene expression of plants that had been in tuber-inducing medium and subjected to 48 h of low-intensity blue light treatment after 3.5 weeks in darkness was compared to that of dark control plants harvested at the same time (2 days of blue treatment). Three biological replicates for each treatment were used and each experiment was replicated completely in time at least three times.

Direct labeling of RNA and hybridizations were performed by The Institute for Genomic Research (TIGR; now the J. Craig Venter Institute), using their 10 K potato cDNA microarrays (http://www.jcvi.org/potato/sol_ma_protocols.shtml). Each microarray was hybridized with Cy3-labeled cDNA from dark controls and Cy5-labeled cDNA from blue light treatments. Flip-dye experiments were performed with the same cDNA, but with Cy5-labeled control cDNA and Cy3-labeled blue light treatment cDNA.

Microarray data were normalized using the Lowess algorithm with GeneSpring GX 9 software (Agilent, Santa Clara, CA). Data were visualized using M-A plots using R Bioconductor, and with box plots using GeneSpring GX 9. Analysis of variation using the Bonferroni multiple-testing correction was used to identify cDNAs with at least 2× significant differential expression. Differentially expressed cDNAs were manually categorized using TIGR annotations. Those cDNAs of particular interest were checked against GenBank entries using nucleotide BLAST.

qRT-PCR

qRT-PCR was performed on genes identified from microarray experiments and published literature as genes of potential interest. These reactions were done using the IQ SYBR® Green kit (Bio-Rad, Hercules, CA) in 20 µl total reactions in a Bio-Rad CFX96 Real Time Systems thermocycler. Three biological replicates starting from blue light treatments, with independent cDNAs for each treatment, and each PCR was run in duplicate for each gene of interest.

Primers were synthesized by Integrated DNA Technologies (Coralville, IA); the sequences used are given in Supplementary Table 1. PCR was run to validate sequences, and thermal gradients were used to determine optimal annealing temperatures. PCR conditions used for *BEL5* and *POTH1* were denaturation at 94°C for 2 min followed by 29 cycles of (30 s at 94°C, 30 s at 52°C, 1 min at 60°C), and 5 min at 68°C. PCR conditions for *BLR*, *FT*, *GA2ox*, *GA7ox*, *GA20ox*, and *Nudix* were denaturation at 95°C for 5 min followed by 40 cycles of (15 s at 95°C and 1 min at 60°C), and 5 min at 72°C. Melting curves were used to check quality of PCR runs. Dilution series using at least four concentrations were used to validate qRT-PCR runs, and water and RNA controls were included on each plate. Expression levels of genes of interest were calculated using the Pffafl method (Pffafl 2001) relative to *tubulin* and *actin* as internal controls.

Statistical Analyses

Data from all light experiments were analyzed statistically using analysis of variance (ANOVA) and Tukey's HSD with R software (R Development Core Team 2008).

Results

Light Quality Effects on Tuberization

Both 'Desirée' and 'Norland' plants formed tubers under continuous darkness, red light, and far-red light (Fig. 1). Under blue light, 'Desirée' plants tuberized, but 'Norland' plants rarely did. Both 'Desirée' and 'Norland' plants formed tubers under short- and long-day exposures, indicating that they are indeed day-neutral or facultative short-day varieties with regard to tuberization. Plants did not form tubers at the ends of stolons below roots, but above the roots along stem tissue that had turned into stolons (Fig. 2).

ANOVA indicated that Variety ($P = 0.004$) significantly affected tuberization. Light Quality across varieties did not significantly affect tuberization ($P = 0.55$), but the interaction of variety × light quality was significant, with $P = 0.004$. Tukey's HSD analyses showed significant differences between tuberization of 'Norland' plants kept under continuous blue light and tuberization of 'Desirée'

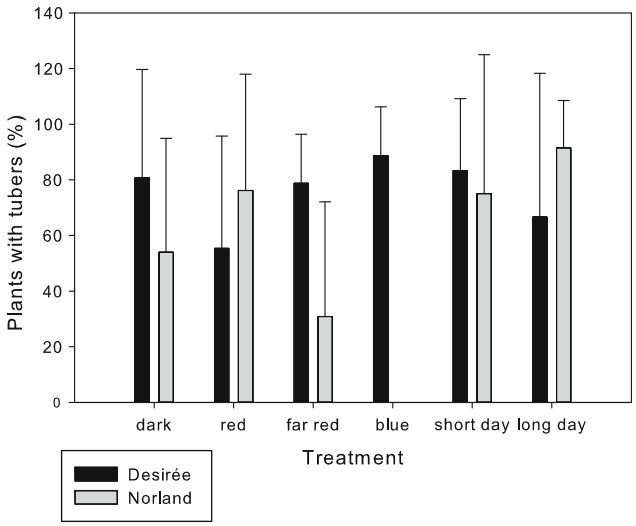

Fig. 1 Percentage of 'Desirée' and 'Norland' plants forming tubers under different continuous light treatments. Plants were grown for 2 weeks in solid medium containing 3% sucrose, and then transferred to liquid tuber-inducing medium with 6% sucrose and kinetin. Both shoots and roots were exposed to various light treatments. Observations were made 8 weeks after plants were put into tuber-inducing medium. Short day = 8 h white light/16 h dark. Long day = 16 h white light/8 h dark. Means are of at least three replicates. *Error bars* indicate standard deviations

plants under blue light, darkness, or far-red light ($P = 0.004$, 0.017, and 0.009, respectively). Tuberization of 'Norland' plants under blue light was different from that of 'Norland' plants kept in darkness ($P = 0.13$) or red light ($P = 0.017$), but not significantly different from 'Norland' plants kept under far-red light ($P = 0.84$).

'Norland' plants in tuber-inducing medium usually did not tuberize when placed under blue light, whether plants were under low- ($\leq$6 μmol m^{-2} s^{-1}) or higher- (27 μmol m^{-2} s^{-1}) irradiance blue light (data not shown), indicating that inhibition of tuberization in 'Norland' plants is dependent on light quality rather than light quantity. Other potato varieties placed in tuber-inducing medium under low-irradiance blue light developed tubers (Fig. 3). Tukey's HSD analysis showed that none of the cultivars differed significantly in their response to blue light.

The percentage of 'Norland' plants that tuberized tended to decrease as length of blue light treatment increased (ANOVA-indicated treatment effect with $P = 0.19$), even if subsequently transferred to darkness (Fig. 4a). This trend was stronger if plants were first treated with darkness after being placed in tuber-inducing medium, then subsequently placed in blue light ($P = 0.03$). The blue light effect was noticeable, even with less than a 7-day treatment of blue light after a 4-week dark treatment (Fig. 4b). Tuberization of 'Norland' plantlets was inhibited even after 2 days under blue light, and this effect was demonstrated with little variation between replicate experiments. There was

Fig. 2 'Red Norland' plants after 8 weeks in tuber-inducing medium in the dark. An *arrow* indicates the site of tuber formation. *White bar* = 1 cm

greater variation in extent of inhibition among replicate experiments after 1-day blue light treatment, so plants harvested after 2 days in blue light were used for microarray experiments.

qRT-PCR and Microarrays

Microarrays were used to compare RNA accumulation of plants in tuber-inducing medium placed in continuous darkness and plants that had been placed in 2 days of blue light after 3.5 weeks of darkness. The number of cDNAs that were up- or downregulated at least twofold after blue light treatment compared to dark controls was 782 (Supplementary Table 2). Of these cDNAs, 303 (39%) were metabolism-related; 190 (24%) were expressed, hypothetical, putative, or unknown proteins or nulls; 176 (22.5%) were related to photosynthesis or light, including blue light or UV-B and chloroplast cDNAs; 42 (5%) were stress- or

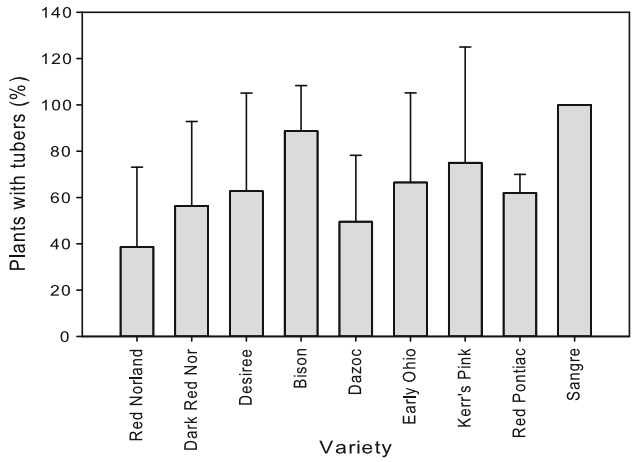

Fig. 3 Percentage of plants of different potato varieties that formed tubers after 8 weeks under low-irradiance (3 μmol m^{-2} s^{-1}) blue light. Experiments used at least three replicates and were repeated at least twice. There were no statistically significant differences among varieties. *Error bars* indicate standard deviations

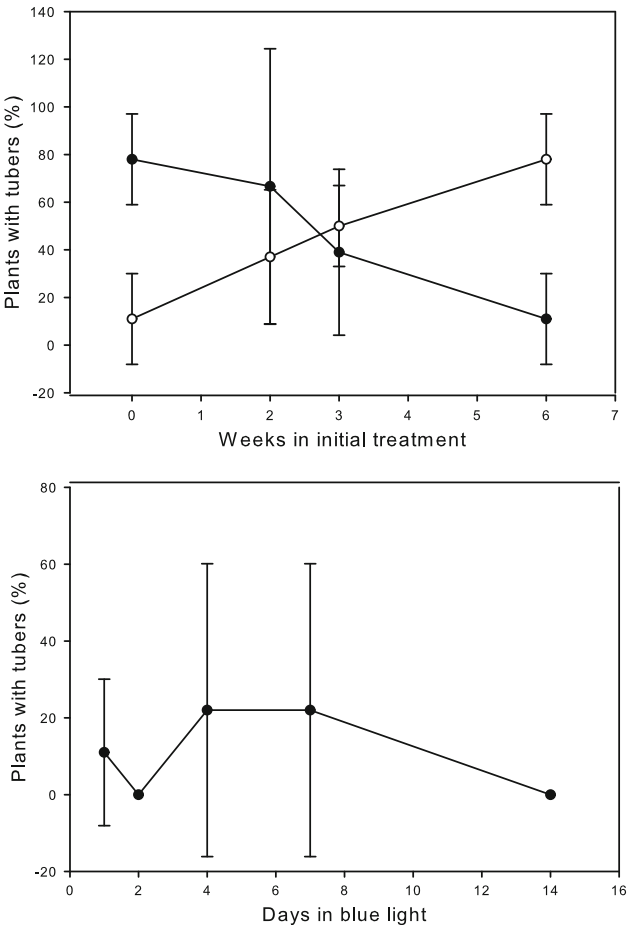

Fig. 4 Percentage of 'Norland' plants forming tubers in tuber-inducing medium under different weeks of low-irradiance (3–6 μmol m^{-2} s^{-1}) blue light treatment. Plants were treated first under blue light, then transferred to darkness (*closed symbols*), or first in the dark, followed by blue light (*open symbols*, *top figure*). Blue light effects were observed within days of exposure (*bottom figure*)

disease-related; 20 (3%) were ribosomal; 16 (2%) were mitochondrial or plastidic, but not including chloroplasts; and 12 (1.5%) were cytochrome P450 cDNAs. There was one of each of 8 (1%) cDNAs related to membranes, plant growth regulators, and transcription factors. The majority of cDNAs were upregulated. Of the metabolic cDNAs, 41 were downregulated, with a mutT/nudix cDNA downregulated sixfold. A photolyase/blue light receptor cDNA was upregulated twofold. Genes for gibberellic acid oxidase7 and an *ent*-kaurenoic acid oxidase were also upregulated twofold.

qRT-PCR was used to verify expression of genes thought to be relevant to tuberization identified from the microarray experiments, and to determine the extent to which expression of genes previously identified with tuberization in *S. tuberosum* ssp. *phureja* were affected by blue light treatment in 'Norland' or 'Sangre' (Table 2). These latter genes included *BEL5*, *POTH1*, *GA2ox*, *GA20ox1*, and *FT*.

Of the genes identified by microarray experiments as up- (*GA7ox* and *BLR*) or downregulated (*Nudix*), only *GA7ox* showed a slight, significant difference in expression between 'Norland' blue and dark treatments when qRT-PCR was used. Large variation between biological replicates negated significant effects due to variety or treatment on *Nudix* and *BLR* expression levels.

Of genes previously identified as related to tuberization in short-day potatoes, qRT-PCR experiments showed that RNA levels of *BEL5* and *GA20ox1* were significantly greater in blue light-treated 'Norland' plants compared to those kept under continuous darkness. RNA levels of *BEL5* but not *GA20ox1* were also greater between blue light- and dark-treated 'Sangre' plants. In contrast, *FT* expression was significantly lower in blue light-treated 'Sangre' plants compared to its dark controls, but not or just slightly different in corresponding 'Norland' plants.

ANOVA suggested that levels of expression of *GA7ox* and *FT* were significantly affected by Variety and that *GA20ox1* and *FT* were affected by Treatment across varieties. Variety affected *GA20ox* expression at $P = 0.12$.

Because the microarray and qRT-PCR data showed that *GA7ox* and *GA20ox1* RNA accumulation increased with blue light treatment compared to darkness, experiments testing the effect of inhibiting GA accumulation were performed. Addition of ancymidol, a gibberellin biosynthesis inhibitor, did not lead to a significant increase in tuberization in 'Norland' plants exposed to continuous blue light if added at the start of blue light treatment (Fig. 5). However, if ancymidol was added 3 weeks after plantlets were placed in tuber-inducing medium under blue light, more tubers were induced compared to controls. This

Table 2 Ratios of cDNAs expression from blue light treatments compared to dark controls, relative to *tubulin* or *actin* genes, and abbreviated ANOVA

Gene of interest	Ratio expression blue light/darkness			Variety		Treatment	
	'Sangre' blue	'Norland' blue	'Norland' 2d blue	Sum of squares	Pr (>F)	Sum of squares	Pr (>F)
BEL5	53	9	Not measured	8260	0.46	Not applicable	
POTH1	1	21	Not measured	586	0.002	Not applicable	
GA2ox	0.5	0.6	2.5	7.9	0.28	0.7	0.75
GA7ox	0.5	2.2	1.5	3.5	0.01	0.007	0.88
GA20ox	0.4	11	2.8	456	0.12	727	0.06
BLR	1	0.6	1	0.1	0.44	0.4	0.18
Nudix	0.4	5.7	0.8	13	0.44	32	0.24
FT	0.03	0.4	1.6	1.4	0.003	1.9	0.002

For *BEL5* and *POTH1*, there was no 2d blue treatment, so treatment was not a factor for these genes in the ANOVA table

indicates that by inhibiting GA biosynthesis, tuberization of 'Norland' plants can be restored after exposure to blue light, suggesting that blue light may increase GA levels in 'Norland' plants, which leads to inhibition of tuberization.

Discussion

The blue light inhibition of tuberization in 'Norland' plants is an interesting phenomenon that was not observed with the other tested day-neutral cultivars, including the 'Norland' color sports 'Red Norland' and 'Dark Red Norland' (Fig. 3). Isozyme analysis could not distinguish 'Norland' from its color sports (Douches and Ludlam 1991), so it is interesting that blue light inhibited tuberization in 'Norland' but not its sports. It is unknown what genetic mutations occurred between these cultivars. Sequencing of their genomes may point to an explanation of their differing responses to blue light.

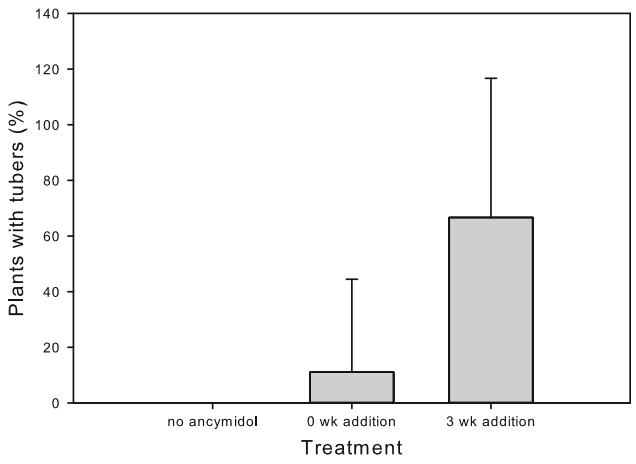

Fig. 5 Addition of 5 mg l^{-1} ancymidol could offset blue light effects if added to media upon transfer to blue light after plants had been in darkness for 3 weeks. Experiments used at least three replicates and were repeated at least twice

Tuberization inhibition in 'Norland' plants could be observed with short exposure to blue light, and increased as exposure length increased (Fig. 4). Results of ancymidol experiments suggest that evocation of tuberization occurred after about 3 weeks of incubation in tuber-inducing medium. After this time, darkness could not reverse blue light effects (Fig. 4).

Interestingly, tuberization of 'Norland' plants may also have been inhibited by far-red light, but not by red light. ANOVA showed that there was no significant difference in tuberization between 'Norland' plants under blue or far-red light. This may have been due to large variation in response to far-red light. If far-red light inhibits tuberization of 'Norland' plantlets, this would be opposite of that described by Batutis and Ewing (1982) who showed the involvement of phytochrome in tuberization, in which a 5-min exposure to red light in the middle of a long dark period decreased the percentage of cuttings that tuberized. This inhibition was reversed by a 2-min treatment with far-red light. In 'Norland' plants, if phytochrome has a role, it is opposite that of short-day potatoes and should be further studied. Regardless, in other day-neutral varieties, neither red nor far-red light inhibited tuberization.

Microarray experiments were used to identify candidate genes involved in the blue light inhibition of tuberization. Genes differentially expressed in blue light compared to darkness identified by microarray experiments included those involved in metabolism, photosynthesis, light responses, and GA biosynthesis, with some similarity to those identified by Folta and others (2003) in *cry1* and wild-type *A. thaliana* seedlings grown in darkness or treated with blue light for 45 min. Based on their microarray results, and because previous data suggested that auxin and GA may have roles in the photocontrol of stem

growth, Folta and others (2003) performed growth studies that indicated that blue light acting through cry1 suppresses stem growth by repressing GA and auxin levels and/or sensitivity.

Of the cDNAs on the potato microarrays, *GA7ox*, *BLR*, and *Nudix* were identified in our study as possible genes of interest. Quantitative RT-PCR verified that of these three genes, only *GA7ox* was slightly upregulated by blue light in 'Norland' but not in 'Sangre' plants, suggesting that GA may be involved in blue light inhibition of tuberization. It was surprising that BL was not significantly upregulated under blue light. Also, Northern blot experiments (data not shown) showed that there were no differences in *cry1* RNA accumulation in 'Norland' plants kept under blue light compared to darkness.

The stimulation of GA synthesis by blue light is supported by the increase in *GA20ox* expression under blue light in 'Norland' but not in 'Sangre' plants, as determined using qRT-PCR (Table 2). This result is not surprising, as papers describing blue light effects on genes related to GA metabolism have previously been published (Folta and others 2003). Also, Jackson and others (2000) found that *StGA20ox1* expression was induced in wild-type potato plants in response to blue light, concluding that *StGA20ox1* is regulated by light.

The ancymidol results described in this article also suggest that blue light acts by increasing or preventing a decrease in GA levels. Gibberellin inhibits tuberization (Carrera and others 2000), and ancymidol inhibits GA biosynthesis by preventing the oxidation of *ent*-kaurene to *ent*-kaurenoic acid (Rademacher 2000). Direct measurements of GA and tests with different ancymidol concentrations in future experiments could verify this hypothesis.

Quantitative RT-PCR was used to examine RNA accumulation of genes other than *GA20ox1* that had been identified as playing roles in tuberization by other researchers. Among these genes were *BEL5*, *POTH1*, and *FT*. Levels of expression of *BEL5* were greater in blue light treatments compared to dark controls in both 'Norland' and 'Sangre' plants, consistent with previous reports that blue light enhanced BEL5 accumulation (Chatterjee and others 2007).

Levels of *POTH1* RNA were greater in 'Norland' kept under blue light compared to darkness. Rosin and others (2003) showed that *S. tuberosum* ssp. *andigena* lines overexpressing *POTH1* produce "more tubers in less time than controls." Overexpression of *POTH1* represses *GA20ox1* expression and leads to decreased GA levels (Rosin and others 2003), so low *GA20ox1* expression in blue light-treated 'Norland' plants was expected. However, *GA20ox1* expression seemed to increase over time under blue light treatment (Table 2).

Expression of *FT* was depressed in blue light-treated 'Sangre' plants compared to dark controls. This result was unexpected, as 'Sangre' is day-neutral and *FT* was not expected to vary in this study because it is related to photoperiod-induced tuberization (Martínez-García and others 2002). Two versions of *FT* cloned from *S. tuberosum* are available in GenBank, with a BLAST identity score of 98%. ClustalW alignment of these sequences results in an alignment score of 72, so they are highly homologous. Primers used in this study were designed using *StFT* accession GU223211, which has been reported to be regulated by *CONSTANS* (Fan and others 2010), so it was assumed that the product amplified by the primers was truly an *FT* gene.

In conclusion, this article describes a novel effect of blue light, that of inhibiting tuberization in the day-neutral potato cultivar 'Norland'. Microarray and qRT-PCR data showed that blue light affects expression of many genes in 'Norland' potato plantlets, including those involved in GA synthesis, that have previously been identified as affected by blue light in other plant systems. Continued investigation of this phenomenon may be a useful tool in further elucidating mechanisms of tuberization in day-neutral potatoes.

Acknowledgments We thank John Bamberg and Max Martin for potato plants; Gary Gardner for advice on light quality and bulbs; Andrea Appel for performing qRT-PCR; Wayne Xu, the University of Minnesota Supercomputing Institute, and Lynn Hartweck for microarray analysis assistance and software; James Bradeen for comments on a draft manuscript; and Lois Fruen and the Breck School for their support of S. Thomas. This project was supported in part by the University of Minnesota Undergraduate Research Opportunities Program and the Minnesota Agricultural Experiment Station.

References

Aksenova NP, Konstantinova TN, Sergeeva LI, Macháčková I, Golyanovskaya SA (1994) Morphogenesis of potato plants in vitro. I. Effect of light quality and hormones. J Plant Growth Regul 13:143–146

Batutis EJ, Ewing EE (1982) Far-red reversal of red light effect during long-night induction of potato (*Solanum tuberosum* L.) tuberization 1. Plant Physiol 69:672–674

Briggs WR, Christie JM (2002) Phototropins 1 and 2: versatile plant blue-light receptors. Trends Plant Sci 7:204–210

Carrera E, Bou J, Garcia-Martinez JL, Prat S (2000) Changes in *GA 20*-oxidase gene expression strongly affect stem length, tuber induction and tuber yield of potato plants. Plant J 22:247–256

Chatterjee M, Sharma P, Khurana JP (2006) Cryptochrome 1 from *Brassica napus* is up-regulated by blue light and controls hypocotyl/stem growth and anthocyanin accumulation. Plant Physiol 141:61–74

Chatterjee M, Banerjee AK, Hannapel DJ (2007) A *BELL1*-like gene of potato is light activated and wound inducible. Plant Physiol 145:1435–1443

Douches DS, Ludlam K (1991) Electrophoretic characterization of North American cultivars. Am Pot J 68:767–780

Douches DS, Maas D, Jastrzebski K, Chase RW (1996) Assessment of potato breeding progress in the USA over the last century. Crop Sci 36:1544–1552

Ewing EE, Struik PC (1993) Tuber formation in potato: induction, initiation, and growth. In: Janick J (ed) Horticultural reviews, vol 14. John Wiley, Hoboken, pp 89–198

Fan C, Yin J, Wang B, Zhang Y, Yang Q (2010) Molecular cloning and expression analysis of a *FT* homologous gene from *Solanum tuberosum*. Agric Sci China 9:1133–1139

Fernie AR, Willmitzer L (2001) Molecular and biochemical triggers of potato tuber development. Plant Physiol 127:1459–1465

Folta KM, Pontin MA, Karlin-Neumann G, Bottini R, Spalding EP (2003) Genomic and physiological studies of early cryptochrome 1 action demonstrate roles for auxin and gibberellin in the control of hypocotyl growth by blue light. Plant J 36:203–214

Foo E, Platten JD, Weller JL, Reid JB (2006) PhyA and cry1 act redundantly to regulate gibberellin levels during de-etiolation in blue light. Physiol Plant 127:149–156

Garner WW, Allard HA (1923) Further studies on photoperiodism, the response of the plant to relative length of day and night. J Agric Res 23:871–920

González-Schain ND, Suárez-López P (2008) CONSTANS delays flowering and affects tuber yield in potato. Biol Plant 52: 251–258

Huang K, Beck CF (2003) Phototropin is the blue-light receptor that controls multiple steps in the sexual life cycle of the green alga *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 100:6269–6274

Jackson SD, Sonnewald U, Willmitzer L (1993) Characterisation of a gene that is expressed in leaves at higher levels upon tuberization in potato and upon flowering in tobacco. Planta 189:593–596

Jackson SD, Heyer A, Dietze J, Prat S (1996) Phytochrome B mediates the photoperiodic control of tuber formation in potato. Plant J 9:159–166

Jackson SD, James P, Prat S, Thomas B (1998) Phytochrome B affects the levels of a graft-transmissible signal involved in tuberization. Plant Physiol 117:29–32

Jackson SD, James PE, Carrera E, Prat S, Thomas B (2000) Regulation of transcript levels of a potato gibberellin 20-oxidase gene by light and phytochrome B. Plant Physiol 124:423–430

Kloosterman B, Navarro C, Blijsterbosch G, Lange T, Prat S, Visser RGF, Bachem CWB (2007) *StGA2ox1* is induced prior to stolon swelling and controls GA levels during potato tuber development. Plant J 52:362–373

Lin C (2000) Plant blue-light receptors. Trends Plant Sci 5:337–342

Liscum E, Hodgson DW, Campbell TJ (2003) Blue light signaling through the cryptochromes and phototorpins. So that's what the blues is all about. Plant Physiol 133:1429–1436

Macháčková I, Sergeeva L, Ondřej M, Zaltsman O, Konstantinova T, Eder J, Ovesná J, Golyanovskaya S, Rakitin Y, Aksenova N (1997) Growth pattern, tuber formation and hormonal balance in in vitro potato plants carrying *ipt* gene. Plant Growth Regul 21:27–36

Martínez-García JF, García-Martínez JL, Bou J, Prat S (2002) The interaction of gibberellins and photoperiod in the control of potato tuberization. J Plant Growth Regul 20:377–386

Navarro C, Abelenda JA, Cruz-Oró E, Cuéllar CA, Tamaki S, Silva J, Shimamoto K, Prat S (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature 478:119–122

Pffafl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:2002–2007

Rademacher W (2000) Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. Annu Rev Plant Physiol Plant Mol Biol 51:501–531

Railton ID, Wareing PF (1973) Effects of day length on endogenous gibberellins in leaves of *Solanum andigena*. I. Changes in levels of free gibberellin-like substances. Physiol Plant 28:88–94

Rosin FM, Hart JK, Horner HT, Davies PJ, Hannapel DJ (2003) Overexpression of a *Knotted*-like homeobox gene of potato alters vegetative development by decreasing gibberellin accumulation. Plant Physiol 132:106–117

Seabrook JEA (2005) Light effects on the growth and morphogenesis of potato (*Solanum tuberosum*) in vitro: a review. Am J Potato Res 82:353–367

Seabrook JEA, Douglass LK (1998) Prevention of stem growth inhibition and alleviation of intumescence formation in potato plantlets in vitro by yellow filters. Am J Potato Res 75:219–224

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna: ISBN 3-900051-07-0. Available at http://www.R-project.org

Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: flowering locus T moves center stage. Annu Rev Plant Biol 59:573–594

Vandenbussche F, Pierik R, Millenaar FF, Voesenek LACJ, Van Der Straeten D (2005) Reaching out of the shade. Curr Opin Plant Biol 8:462–468

Zhao X, Yu X, Foo E, Symons GM, Lopez J, Bendehakkalu KT, Xiang J, Weller JL, Liu X, Reid JB, Lin C (2007) A study of gibberellin homeostasis and cryptochrome-mediated blue light inhibition of hypocotyl elongation. Plant Physiol 15:106–118