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ORIGINAL PAPER

Treatment of potato tubers with the synthetic cytokinin 1-(α-ethylbenzyl)-3-nitroguanidine results in rapid termination of endodormancy and induction of transcripts associated with cell proliferation and growth

Michael Campbell · Jeffrey Suttle · David S. Douches · C. Robin Buell

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Abstract Perennial plants undergo repression of meristematic activity in a process called dormancy. Dormancy is a complex metabolic process with implications for plant breeding and crop yield. Endodormancy, a specific subclass of dormancy, is characteristic of internal physiological mechanisms resulting in growth suppression. In this study, we examine transcriptional changes associated with the natural cessation of endodormancy in potato tuber meristems and in endodormant tubers treated with the cytokinin analog 1-(α-ethylbenzyl)-3-niroguanidine (NG), which terminates dormancy. RNA-sequencing was used to examine transcriptome changes between endodormant and non-dormant meristems from four different harvest years. A total of 35,091 transcripts were detected with 2132 differentially expressed between endodormant and non-dormant tuber meristems. Endodormant potato tubers were treated with the synthetic cytokinin NG and transcriptome changes analyzed using RNA-seq after 1, 4, and 7 days following NG exposure. A comparison of natural cessation of dormancy and NG-treated tubers demonstrated that by 4 days after NG exposure, potato meristems exhibited transcriptional profiles similar to the non-dormant state with elevated expression of multiple histones, a variety of cyclins, and other genes associated with proliferation and cellular replication. Three homologues encoding for CYCD3 exhibited elevated expression in both non-dormant and NG-treated potato tissues. These results suggest that NG terminates dormancy and induces expression cell cycle-associated transcripts within 4 days of treatment.

Keywords Potato · Dormancy · Endodormancy · Transcriptional analysis · Cytokinin · 1-(α-ethylbenzyl)-3-nitroguanidine · Sprouting

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M. Campbell (✉)
Penn State Erie, The Behrend College, School of Science, 4205 College Drive, Erie, PA 16563, USA
e-mail: mac17@psu.edu

J. Suttle
US Department of Agriculture, Agricultural Research Service, Northern Crop Science Laboratory, 1605 Albrecht Blvd. N, Fargo, ND 58102-2765, USA

D. S. Douches
Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA

C. R. Buell
Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA

Introduction

After rice and wheat, potatoes are the third largest agricultural commodity (Birch et al 2012). Unlike grain crops, potatoes are stored in a fully hydrated and highly perishable form. At harvest, potato tubers will not sprout and are in a state of endodormancy that is induced and maintained by internal physiological and biochemical mechanisms. The length of tuber dormancy is genetically determined but can be significantly affected by pre- and postharvest environmental conditions (Burton 1989).

Control of tuber sprouting is a critical aspect of successful potato storage management. Aside from potatoes grown for the organic market, suppression of postharvest sprouting in commercial storages is accomplished through the use of chemical sprout-control agents such as chlorpropham (CIPC). CIPC does not extend tuber dormancy but inhibits

sprout growth by interfering with spindle function during mitosis (Vaughn and Lehnen 1991). Societal and health concerns regarding the use of synthetic chemicals directly on food stuffs, and the cost to growers and distributors of treating tubers with sprout inhibitors, demands that genetic alternatives be developed to prevent sprout growth.

The genetic manipulation or extension of the endodormant state, either through breeding or direct gene transfer, has significant potential for increasing storage of potato tubers without the added costs and concerns of chemical treatments. However, development of genetic solutions to postharvest sprout control requires a thorough understanding of the cognate molecular and physiological processes regulating dormancy progression. Thus, a genetic or transcriptional analysis of genes associated with the natural transition from the endodormant to the non-dormant, or growing competent state, would be valuable for the understanding and manipulation of sprouting.

The initiation and maintenance of tuber dormancy is associated with the sustained synthesis and action of the phytohormone abscisic acid (ABA) (Suttle et al 2012). Release from dormancy and the transition to an active meristem is regulated by an increase in both cytokinin and gibberellin content (Hartmann et al 2011; Rentzsch et al 2012; Suttle 2007). Previous studies using microarray technology have demonstrated that transcript changes occur as potato meristems transition from the endodormant to the non-dormant state (Campbell et al 2008; Kloosterman et al 2008). Expression of deoxyuridine triphosphatase (dUTPase) was shown to increase in potato just prior to spouting (Senning et al 2010). Analysis of expressed sequence tags (ESTs) derived from potato tubers indicated that a transcript (*ARF1*) encoding for ADP-ribosylation factor was increased after termination of tuber dormancy (Liu et al 2012). All of the above studies demonstrate a link between hormone regulation and transcription control of gene expression associated with sprout growth in potatoes.

Control of postharvest sprouting by manipulating tuber endodormancy has significance for increased storage, yet terminating endodormancy is important to growers and breeders who have an interest in controlling planting regimes. Cytokinins have been shown to terminate potato dormancy and induce sprout growth (Hemberg 1970; Suttle 2004). Treatment of dormant potato tubers with the synthetic cytokinin agonist 1-(α-ethylbenzyl)-3-nitroguanidine (NG) resulted in the termination of dormancy and resumption of active growth (Suttle 2008). Plant responses to cytokinin involve a two-component signaling system (Hwang and Sheen 2001). More specifically, receptor binding of the hormone induces a histidine kinase and the triggering of a phosphorylation cascade that result in the transcriptional regulation of target genes (Heyl et al 2012) (Schmulling 2013).

The availability of a genome sequence for potato (Potato Genome Consortium 2011) permits the use of RNA-sequencing (RNA-seq) and expansion of previous expression profiling studies that used microarray technology (Campbell et al 2011) or the sequencing of ESTs (Ronning et al 2003; Senning et al 2010) to determine transcriptional changes associated with both natural and chemically forced dormancy progression. We demonstrate here that deep sequencing of transcripts for mRNA reveals that transcript profiles shift as dormancy terminates in the meristematic tissue of a potato tuber following cessation of natural dormancy and after termination of dormancy with NG.

Materials and methods

Plant tissue and RNA isolation

Two types of tubers were used in these studies. For studies examining changes in transcript abundances during natural dormancy progression, potato plants (*Solanum tuberosum* cv Russet Burbank) were grown during the years 2005, 2007, 2009, and 2010 in the Red River Valley of North Dakota using standard commercial practices. Tubers were harvested in the fall of each growing year and stored at 4 °C and relative humidity of ca. 95 %. Samples of tubers were placed at 20 °C at 2-week intervals for each harvest and the dormancy status was determined visually. A tuber was considered sprouted if it had any sprouts ≥2 mm in length. The dates tubers reached the non-dormant status for each harvest year are listed in Table 1.

For each harvest year, tuber buds (meristems) were excised from dormant and non-dormant tubers using a 1-mm curette and quick frozen in liquid nitrogen (Campbell et al. 1996). Tissues were stored at −80 °C. RNA was isolated from frozen meristems using an Ambion mirVana isolation kit using a Plant RNA isolation Aid (http://www.invitrogen.com). RNA was quantified by spectrophotometry (BioSpec-nano, Shimadzu) and quality checked visually by examination of the integrity of rRNA bands separated on a 2 % agarose gel. RNA quality was also checked using a Bioanalyzer (http://www.genomics.agilent.com). For dormant and non-dormant samples, three replicates from each harvest year were used for cDNA construction. Four different harvest years were analyzed as biological replicates resulting in matched pairs for dormant and non-dormant samples. The total number of reads from dormant and non-dormant meristems can be found in Table 1.

Commercially grown greenhouse minitubers (cv Atlantic) were used in the studies examining the effects of chemically forced dormancy exit on transcript abundance. Intact dormant potato minitubers (3–5 g tuber^{-1}) were injected with 5 μL DMSO±10 μg NG and incubated at 20 ° C. At the indicated times, a 1–2-mm-thick disc (5 mm diameter) of periderm containing the entire apical bud complex was excised from

Table 1 Dates for the harvest of dormant and corresponding non-dormant potato meristems and number of RNA reads used for transcriptome analysis

Dormant sample			Non-Dormant Sample		
Tissue harvest date	Initial number of RNA reads sequenced	Reads remaining after Fastx "Filter by Quality"	Tissue harvest date	Initial number of RNA reads sequenced	Reads remaining after Fastx "Filter by Quality"
11/30/05	22,321,582	17,835,879	2/24/06	16,891,071	13,458,255
10/22/07	13,564,362	10,811,954	3/13/08	13,640,088	10,917,309
11/20/09	33,494,287	30,216,546	3/22/10	36,556,253	32,483,310
11/26/10	33,964,952	29,724,941	3/4/11	34,126,663	30,664,302

each mini-tuber and frozen in liquid nitrogen. RNA was isolated as described above at 0 time, 4 days after NG exposure, and 7 days after NG exposure. Treatments were replicated three times and RNA was isolated as described above.

RNA was treated with DNAse and Illumina TruSeq mRNA libraries (San Diego CA) were constructed from each sample. Libraries were sequenced on an Illumina Genome Analyzer II (50 nucleotide reads, single end). The total number of reads isolated from tubers treated with NG and followed over 7 days can be found in Table 2.

Mapping of transcripts

RNA sequences were analyzed using the Galaxy software suite (Goecks et al 2010). Raw sequence data was first processed using FASTQ Groomer (Blankenberg et al 2010) and edited to remove adapter sequences.

Transcript reads were aligned to the double monoploid DM1-3 516 R44 (DM1-3), *S. tuberosum* Grp. Phureja line ($2n$=24) using Tophat (Trapnell et al 2009). The reference genome file for mapping was PGSC_DM_v3_2.1.11 and was provided by the Sol Genomics Network (http://solgenomics.net). Differential expression of transcripts was determined using CuffDiff (Trapnell et al 2012) and was a result of pooling transcript data from dormant and non-dormant samples over the four harvest years. Total reads from all samples were filtered for quality using Galaxy where 100 % of all reads met a minimum phred score of 20. RNA-seq samples from dormant and non-dormant meristems were mapped using CuffDiff v.2.02 and RNA samples isolated from potato tubers treated with NG were mapped with CuffDiff v2.1.1. Differential expression for a given transcript was based on differences in fragments per kilo base exon model per million mapped reads (FPKM) between the dormant and non-dormant transcript pools using the CuffDiff function (Trapnell et al 2012). RNA-seq samples from NG-treated tubers were processed as described above. Dispersion and similarity of NG time course samples were analyzed using the R package CummeRbund (Trapnell et al 2012).

qt-PCR analysis

Confirmation of the RNA-seq data was accomplished by qt-PCR. The transcripts and primers selected for amplification are found in Table 3. Primer sequences were derived from the reference genome where the specific transcripts were mapped. Primers were designed using Applied Biosystems Primer Express 3.0 software (www.appliedbiosystems.com). The cDNA templates for qt-PCR were generated using a Superscript III First-Strand Synthesis System (invitrogen.com) and oligo dT-primed mRNA isolated from dormant or non-dormant potato meristems. Amplification was performed on a STEPOneTM Real Time System and analyzed with StepOne Software v2.0 (www.appliedbiosystems.com). The $\Delta\Delta$CT values were determined on a log2 scale from three biological replicates and three technical replicates. Transcript expression was normalized and compared to the expression of EF1-α from potato. EF1-α was chosen as an internal control for the qt-PCR experiments because it did not show a large change in expression based on the RNA-seq results.

Results

RNA-seq using the Illumina platform was used to analyze populations of RNAs from dormant and the corresponding non-dormant meristems. These tissues were excised from potatoes collected from four different seasonal harvests to mitigate for transcriptional changes that might be induced by seasonal variation due to weather or day-length and thus not linked to the dormancy per se. Expression changes between different harvest years for specific transcripts were not determined and expression analysis was limited to an expression comparison between dormant and non-dormant samples.

The total number of RNA-seq reads generated from both dormant and non-dormant samples were 103,345,138 and 101,214,075, respectively. The total number of reads from each harvest year and the resulting filtered reads can be found in Table 1. CuffDiff analysis was used to determine transcripts

Table 2 The replicates and number of associated RNA reads isolated from potato tubers treated with 1-(α-ethylbenzyl)-3-nitroguanidine (NG)

Control			1 day after NG treatment			4 days after NG treatment			7 days after NG treatment		
Tissue harvest date	Initial number of RNA reads sequenced	Reads remaining after Fastx "Filter by quality"	Tissue harvest date	Initial number of RNA reads sequenced	Reads remaining after Fastx "Filter by quality"	Tissue harvest date	Initial number of RNA reads sequenced	Reads remaining after Fastx "Filter by quality"	Tissue harvest date	Initial number of RNA reads sequenced	Reads remaining after Fastx "Filter by quality"
7/5/12	48,045,994	41,161,980	7/6/12	35,655,491	30,369,099	7/9/12	50,655,558	39,569,072	7/12/12	44,839,850	33,681,445
7/5/12	57,998,031	43,341,017	7/6/12	59,906,580	46,609,398	7/9/12	N/A	N/A	7/12/12	57,657,436	45,273,391
7/5/12	38,326,017	28,662,237	7/6/12	43,290,578	33,776,112	7/9/12	51,767,740	38,750,001	7/12/12	56,348,592	48,108,890

unique to dormant and non-dormant meristems. CuffDiff analysis revealed that 2132 transcripts had significant differential expression (q value<0.05) between dormant and non-dormant potato tuber meristems based on a Benjamini-Hochberg correction of the FDR values generated (Supplemental Tables 1 and 2) (Trapnell et al 2012). Termination of dormancy resulted in a decrease of 733 transcripts and an increase of 1399 transcripts.

To investigate our RNA-seq results, transcripts were selected based on changes in FPKM levels between dormant and non-dormant potato meristems and qt-PCR analysis confirmed changes in the RNA-seq data for selected genes (Fig. 1). Transcripts were selected for qt-PCR analysis based on expression levels in RNA-seq linking them to an increase in dormancy (membrane protein M26) or an increase in expression during non-dormancy (the remaining transcripts tested). Increasing or decreasing changes in expression were in agreement between qt-PCR and RNA-seq but overall levels of expression could not be directly correlated since expression levels for RNA-seq were based on FPKM while qt-PCR was relative expression compared to the reference gene EF1-α.

Treatment of minitubers with NG resulted in the abundance of 13,493 transcripts changing over the course of 7 days (Supplemental Table 3). Density plots of the log10 (FPKM) generated using CummeRbund show that there was a high degree of similarity in global levels of expression between control treatments and in tubers treatment with NG and then incubated at 1, 4, or 7 days (Fig. 2). By comparing transcript differences between dormant and non-dormant meristems with those altered by NG treatment, it was possible to find transcripts common to dormancy termination. Transcripts that decrease as dormancy terminates either naturally or through NG treatment are found in Supplementary Table 4. Among these, 355 transcripts are putative dormancy regulators such as a MADS-box-like transcription factor, ARGONAUTE-4, auxin-repressed/dormancy-associated protein, and a series of undefined F-box proteins and transcription factors.

There were 967 transcripts that increased with NG exposure and in response to natural dormancy termination (Supplementary Table 5). Many of these transcripts are associated with cell replication, division, and growth. For example, histones 1, 2, 3, and 4 are all induced dormancy termination either chemical or naturally. Transcripts associated with cell cycle regulation (cyclins A, B, D, and F) and cell division (Cell division control 20, Cell division protein cdt2, cdc6, and Cdc45) are also increased in response to dormancy termination.

Not surprisingly, comparison of transcripts isolated from meristems at NG time points using a distance matrix shows that the dormant state is more similar to meristems treated with NG for 24 h (Fig. 3). To discern the differences between transcript populations from NG-treated tubers, we utilized CummeRbund and principle component analysis (PCA).

Table 3 Transcripts, primers, and comparison of qt-PCR to RNA-seq results for select transcripts in dormant and non-dormant potato tissues. The expression change for EF1 between dormant and non-dormant tissues was not statistically different in the RNA-seq analysis

Comparison between RNA-seq and qt-PCR changes for select transcripts after natural termination of dormancy					
PGSC gene model	Gene name	Primer seq	Putative function	qt-PCR RQ (log2)	RNA-seq (two-fold)
PGSC0003DMG400008581	Clav CAGCATACACATCAAAGCAGAAGA	CCAGTTAGCCATCCTGTGATGA	Recepto Protein kinase CLAVATA1	0.64431111	5.00467
PGSC0003DMG400000997	ZnF TGTCCCAGCAGAACTAGCTCAA	TTGTTGCAACTCGGGTGAAC	Zinc Finger-DNA binding	3.22680144	5.36781
PGSC0003DMG400004796	Ext CACGACCCAAAAGAGAAACATTT	GGCAAAACACTCCCATCTGATT	Extensin	4.10679416	5.11795
PGSC0003DMG400002857	Rib TGACAAGAAAGCAGCATGTTGTT	AGTCCATGAATCCCAAAATCTAATG	Ribonuclease t2	0.23038535	7.25791
PGSC0003DMG400022659	M26 CTCTCGCCACCCCACATAA	AGCAGCAGGATCGGTGACTT	Membrane Protein M26	−3.6403246	−3.60089
PGSC0003DMG400024725	UTPase ACACTCCGGTGGATCCAAAG	AAATCAGATCAACTCTCCTGAGATCA	Deoxyuridine 5′-triphosphate nucleotidohydrolase	1.72270261	2.08126
PGSC0003DMG400000919	Nod CAAAGGGCCAATGTTCAACCT	TCGCGGTTGAGGCTTCAT	Nodulin family protein	2.13368704	6.97656
PGSC0003DMG400030432	PCNA CAACAGTTGTATTTTGCCTGCAA	GGTGAACGATGCGAACTTTGA	Proliferating cell nuclear antigen	0.97616663	1.64942
PGSC0003DMG400012900	CYCD2/4 GGCCTTCTTTCCACTTAATATCTCA	AAATGGAGAATGCAGGCTATCAC	Cell cycle regulation	2.00334617	1.15166
PGSC0003DMG400023272	EF1 TCCTTACCTGAACGCCTGTCA	GATTGGAAACGGATATGCTCC	elongation factor 1-alpha	Control	0.356924[a]

[a] Expression is not signficantly different between dormant and nondormant meristems

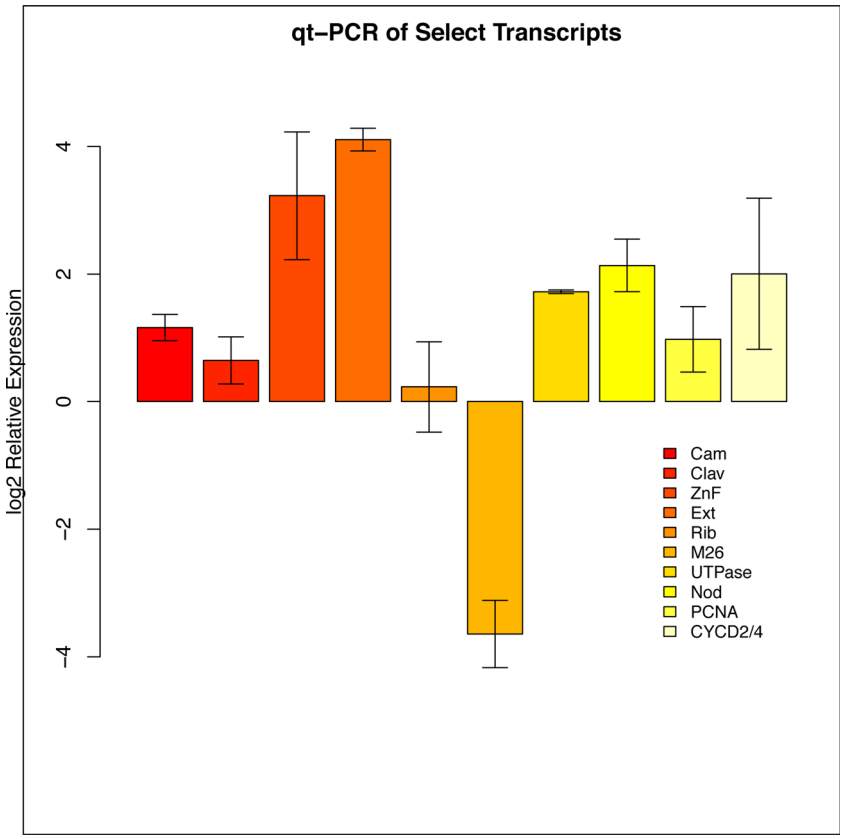

Fig. 1 qt-PCR data for the expression of selected transcripts in non-dormant tissues. Transcripts were compared to the control gene EF1-alpha. The increasing transcripts are associated with meristem identity (*Clav*), cell division (*Cam*, *UTPase*, *Nod*, *PCNA*, *CYCD2/4*) ,proliferative activityin plant tissues (*Ext),* DNA binding (*ZnF),* membrane integrity (*M26),* and RNA stability (*Rib*)

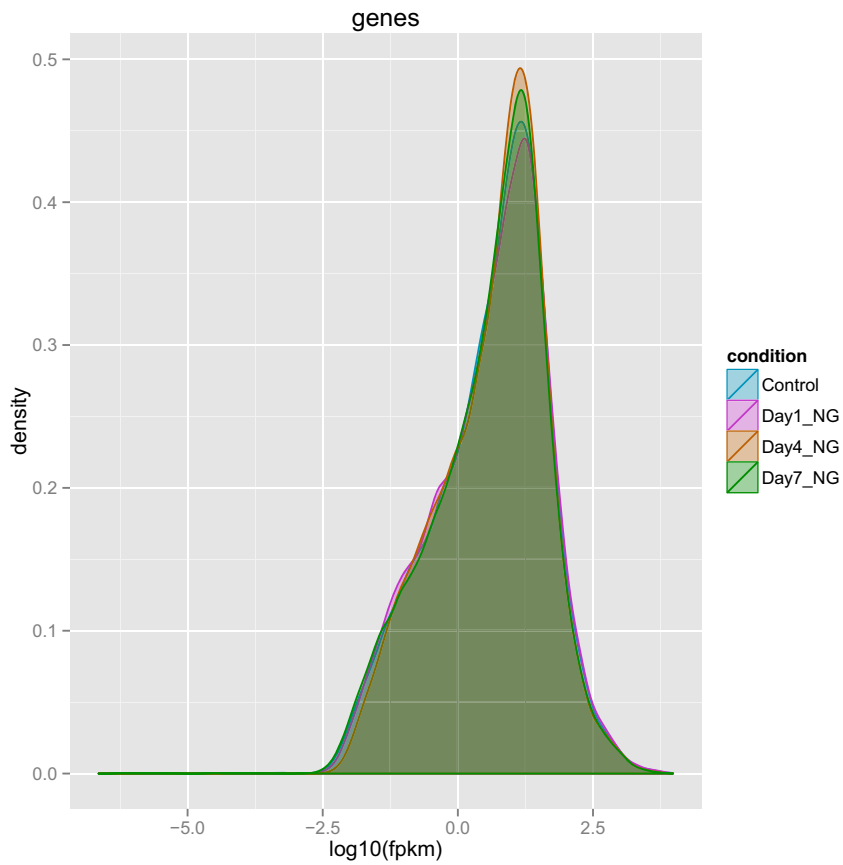

Fig. 2 Density graph of mapped RNA-seq data from tubers treated with 1-(α-ethylbenzyl)-3-niroguanidine (*NG*). *Control* is no treatment and the other samples correspond to the days after NG exposure

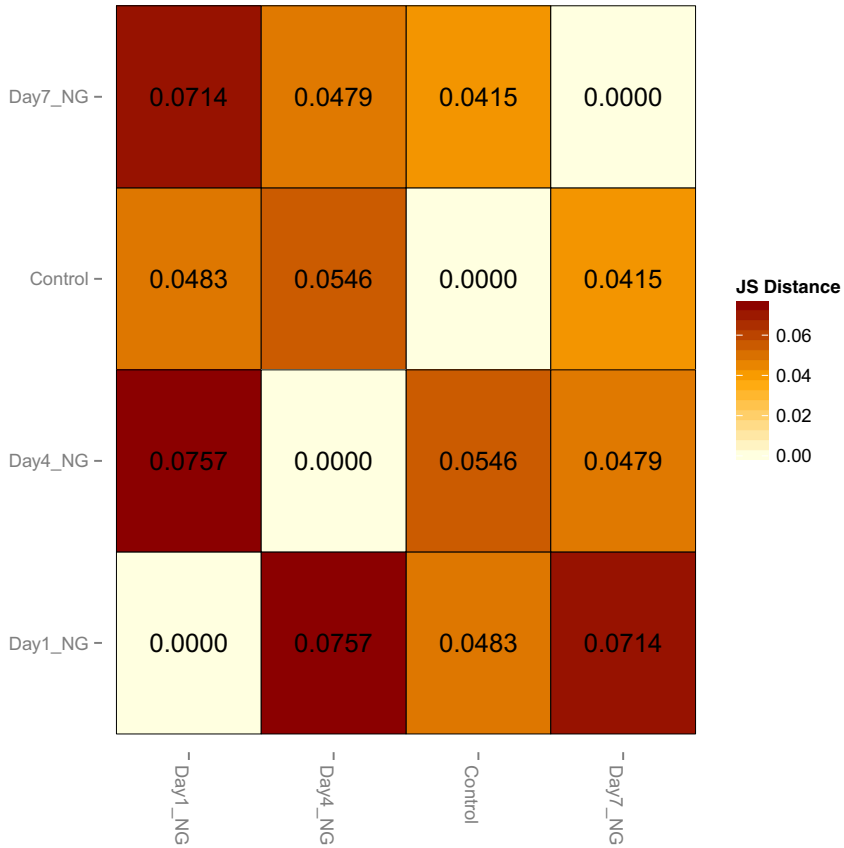

Fig. 3 Distance matrix of mapped RNA-seq data. The greatest difference is found between the "Day1_NG" treatment and "Day4_NG"

Results from PCA analysis also demonstrated that dormant meristems were more similar to tubers treated with NG and incubated for 1 day (Fig. 4). The analysis also showed that PC2 was largely responsible for the shift in transcript differences highlight in tubers treated with NG and incubated for 4 or 7 days.

Interestingly, the 4-day incubation after NG exposure has less similarity to the no NG control than does tubers treated and incubated for 7 days. When the NG time points are compared to non-dormant tubers, the 4-day NG treatment has the greatest number of unique changes in both up- and downregulated transcripts (Fig. 5).

Discussion

Transcriptome changes associated with varying stages of dormancy release in perennial species have been investigated in *Paoenia ostii*, *Euphorbia esula*, *Castanea sativa* (Campbell et al 2008; Gai et al 2013; Horvath et al 2008; Santamaria et al 2011). Release of dormancy in *Vitus vinifera* with hydrogen cyanamide has been shown to specifically alter transcripts encoding for proteins involved with the ascorbate-glutathione cycle (Pérez et al 2009). Unlike potato, these species respond to both photoperiod and temperature as cues for dormancy cessation. There is some commonality among transcript changes between these species but there is limited analysis of the effects of cytokinin analogs as a means to artificially terminate the dormant state.

The natural termination of tuber dormancy resulted in the downregulation of 733 transcripts. A comparison of NG-induced termination of dormancy and the natural process of dormancy cessation identified 355 transcripts that were downregulated during both processes. Comparing the commonality of transcript suppression by both NG and the natural process of dormancy cessation mitigates for transcriptional changes that occur due to tuber aging or cytokinin exposure that does not relate to the regulation of dormancy (Supplemental Table 4). Both dormancy cessation and NG treatment reduced the transcripts of an AGAMOUS-like MADS-box transcription factor (PGSC0003DMT400010451). In *Prunus persica*, six dormancy-associated-MADS-box (DAM) genes were found to have elevated expression during dormancy (Bielenberg et al 2008). Five of the six DAM genes from *P. persica* were found to be regulated by photoperiod (Li et al 2009). In *Euphorbia esula*, DAM genes were associated with bud meristems and were regulated by both dormancy status and photoperiod (Horvath et al 2010). The AGAMOUS-like MADS-box-like transcription factor in potato that was downregulated as dormancy terminates shares sequence similarity to the DAM genes from *P. persica* and *E. esula* (data not shown).

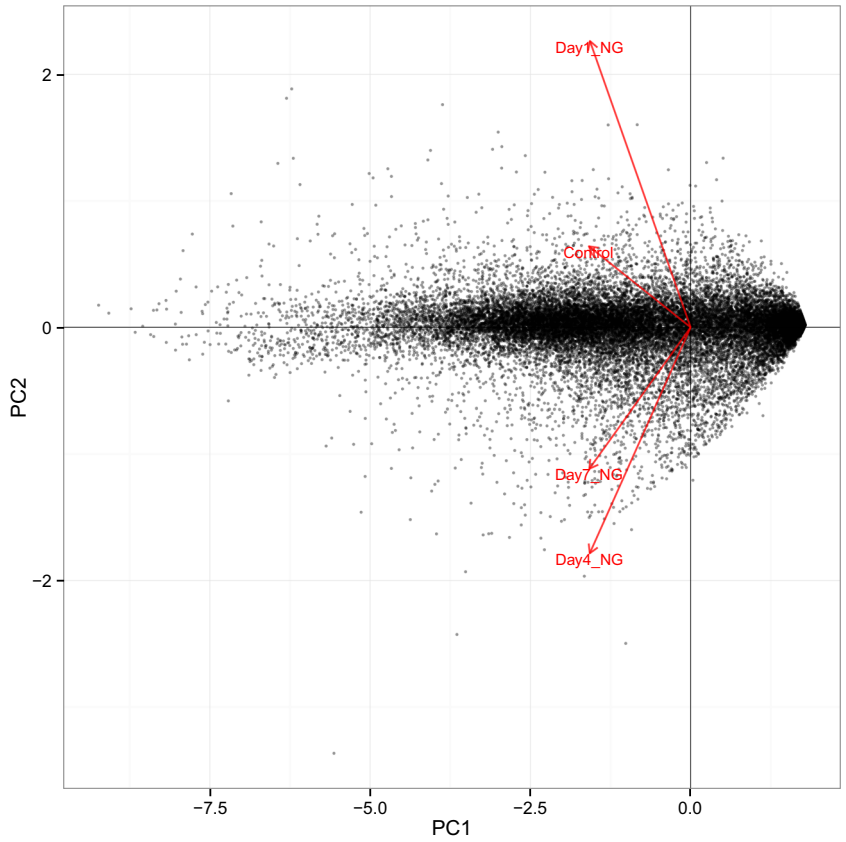

Fig. 4 Principle component analysis of RNA-seq mapped data for samples temporally treated with NG. The large differences demonstrated between RNA-seq data from "Day1_NG" and "Day4_NG" are largely due to the impact of PC2

The non-dormant buds used in this experiment did not exhibit any visually detectable growth but the increase in transcripts for extensin suggests this stage an early physiological phase toward metabolic preparation for cell expansion because extensins have been shown to be necessary for alterations of cell wall architecture (Lamport et al 2011). The increased (Senning et al 2010) expression of xyloglucan endotransglucosylase in non-dormant meristems is also an indicator of metabolic shifts toward change in cell wall structure as these enzymes are associated with cell wall loosening (Van Sandt et al 2007). Modeling of plant organ growth suggests that changes in cell wall structure and loosening should be linked with an increase in proteins that alter turgor pressure or result in cell wall pH changes that enhance cell expansion (De Vos et al 2012). Non-dormant potato meristems had an increase in transcripts encoding the enzyme pectinesterase, a protein that has been shown to be associated with increase in cell wall H+ (Wolf et al 2012). The induction of dormancy in potato meristems is associated with an elevation of abscisic acid (ABA) content (Suttle et al 2012). ABA has been shown to be inhibitor of expression for transcripts encoding extensin, pectinesterase, and xyloglucan endotransglycosylase (Gimeno-Gilles et al 2009).

The expression of deoxyuridine triphosphatase (dUTPase) has been shown to be an indicator of dormancy release and its expression precedes visible sprout growth in potato tubers

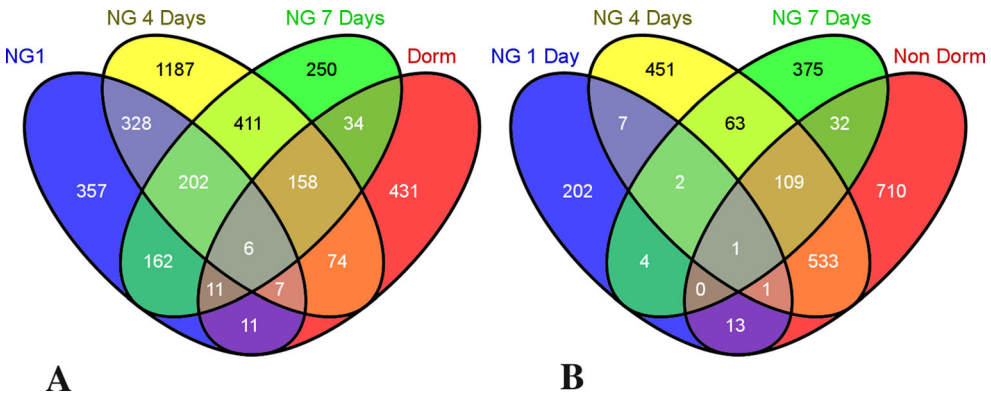

Fig. 5 Venn diagram showing mapped transcripts differences between dormancy status and NG treatments over time. Transcripts upregulated in dormant tissues and altered by NG exposure are found in panel (**a**). Transcripts upregulated in non-dormant tissues altered by NG exposure are found in panel (**b**)

Table 4 Expression of cyclin D3 homologues in potato tubers treated with no NG (Cont.), NG for one day, NG for 4 days, or NG for 7 days. Expression levels were determined using RNA-seq and are based on Fragment Per Kilobase Mapped (FPKM)

Expression changes in *Solanum tuberosum* cyclin D transcripts after NG treatments (values are in FPKM)						
PGSC gene model	Cyclin D-type	Con vs day 1	Con vs day 4	Cont vs day 7	NG day 1 vs NG day 4	NG day 1 vs NG day 7
DMT400064307	CYCD3.1	−0.765	1.73	0.656	2.50[a]	1.42[a]
DMT400066557	CYCD3.2	−0.663	1.29	0.374	1.96[a]	1.04
DMT400020648	CYCD3.3	−1.48	1.56	1.26	3.04	2.73

[a] Statistically significant

(Senning et al 2010). The expression of the transcripts PGSC3000DMT400063599 and PGSC3000DMt400057936 increased two-fold in $\log_2$ FPKM expression by 2.08126 and 2.01524, respectively, after dormancy termination, thus supporting the conclusion of Senning et al. (2010) that UTPase expression is an early indicator of dormancy release.

Significant changes in tuber bud DNA cytosine methylation and histone acetylation occur during dormancy progression (Law and Suttle 2003, 2004). The gene product of SW12/SNF2 has been found to regulate the methylation state of the genome in *Arabidopsis thaliana* (Jeddeloh et al 1999). Two potato transcripts (PGSC0003DMT400040011 and PGSC0003DMT400034983) show an increase as dormancy terminates and also after NG treatment. Both of these transcripts have amino acid similarity to the *A. thaliana* SW12/SNF2 gene (At5g66750) based on a tBLASTx result of less than e^{-20}. Additional evidence of chromatin modification is indicated by the increased expression of PGSC0003DMT400069460, which encodes for ARGO-4. ARGONAUTE4 in plants has been shown to alter chromatin structure and methylation of histone H3 (Zilberman et al 2003) and functions via interaction with heterochromatic siRNAs.

Endogenous levels and increased sensitivity to cytokinins are known to accompany termination of dormancy in potato tuber meristems (Suttle 2007). In particular, the synthetic cytokinin NG has been shown to effectively terminate dormancy from greenhouse minitubers (Suttle 2008). In the tubers used in these studies, visible sprout growth was observed 4 days after NG treatment. Application of the cytokinin analog NG to dormant potato tubers resulted in transcript changes within 24 h of application. Previous studies have shown that dormant potato tubers are arrested at the G1/S-phase of the cell cycle (Campbell et al 1996) and NG must be moving cells through the G1/S block present during the endodormant state. It has been shown that application of NG to dormant potato tubers induced sprout growth (Suttle 2008). Thus, it would be expected that NG would induce transcripts for cyclins, which are associated with increased cell cycling. However, many of the cyclins that exhibit statistically significant changes in expression in this study are decreasing after 24 h exposure to NG and most of these are linked to the G2/M phase of the cell cycle. For example, the B-type cyclins are associated with regulation of the G2/M phase of the cell cycle (Inze and De Veylder 2006). It is possible that exposure to NG removes the G1/S-phase block associated with dormancy tissue and entry into the cell cycle results in repression of G2/M-phase associated transcripts. The initial exposure of potato tubers to NG results in a downregulation of G2/M phase cyclins and proteins associated the mitotic phase of the cell cycle 24 h after exposure to NG (Supplemental Table 3).

However, 4 days after NG treatment, potato tubers exhibited an increase in transcripts encoding for proteins associated chromatin rearrangement and cell cycle regulation. NG treatments and natural transition to non-dormancy result in an increase for transcripts encoding for multiple cyclins, histones 1, 2, 3, and 4, and other proteins associated with cell proliferation and replication. Of particular note are the CYCD3 homologues. Cytokinins are known regulators of CYD3 expression and activity (Riou-Khamlichi et al 1999; Scofield et al 2013). RNA-seq analysis reveals that following NG exposure there is a temporal change in the expression of three CYD3 homologues in potato (Table 4). One day after NG exposure, there is a slight repression of CYD3 transcripts followed by a rise by day 4. The initial repression of CYCD3 homologues by NG may be a stress response but by day 4, the elevation of CYCD3 expression that the endodormant state has been terminated and cytokinin activation of the cell cycle may have begun. Overexpression of CYCD3 in *A. thaliana* resulted in induction of cell division without elevated cytokinin and resulted in an increase in expression for Histone H4 (Riou-Khamlichi et al 1999). The increased expression of histones H1B, H2A, H2B, H3, and H4 in this study may be indicative of a downstream response of NG treatment if CYCD3 induction predicates histone expression in potato as it does in *Arabidopsis*. Thus, the expression pattern of CYCD3 is a more rapid response in comparison to some of the other transcripts that increase following dormancy cessation and NG.

Downregulation of KIP-related proteins (KRP proteins) results in dedifferentiation and callus-like growth in *A. thaliana* meristems (Anzola et al 2010). *A. thaliana* contains seven genes encoding for KRP homologues and all appear to regulate cell division (reviewed in Komaki and Sugimoto 2012). Cessation of dormancy in potato meristems did not result in changes in KIP-homologues suggesting that repression of cell growth during dormancy is not associated with transcriptional regulation of p27 cell division inhibitors. Earlier reports by (Campbell et al 2011) showed that the sprout inhibitor DMN induces KIP-homologues and thus prevents tuber sprouting not by inducing or maintaining the dormant state but through repression of cell division.

Previous studies investigating transcript changes associated with termination of dormancy with the compound bromoethane demonstrated a large increase in stress-related genes prior to any changes in cell growth or division (Campbell et al 2008). Environmental conditions such as temperature extremes, exposure to elevated CO_2, and induction of anaerobic metabolism can result in rapid termination of sprouting in dormant potato tubers (reviewed in Suttle 2007). Based on expression profiles, the termination of tuber dormancy with NG did not elicit the level of stress response associated with bromoethane-induced dormancy cessation. NG appeared to rapidly increase transcripts associated with cell division and proliferation while bromoethane-induced ABA and stress-associated proteins before the expression of cell proliferative and visible sprouting.

We conclude that there is an overlap in transcript changes between potato meristems that transition naturally from endodormant to non-dormant and tubers that break endodormancy following exposure to the cytokinin analog NG. Additionally, the termination of endodormancy with NG is rapid, occurring within 4 days of exposure based on a shift in transcripts associated with cell division and growth. The different transcriptional responses between NG and bromoethane suggest that the mechanism of dormancy termination may be different between these two compounds. Bromoethane appears to result in dormancy termination via a stress-induced pathway while NG functions as a cytokinin-like growth promoter.

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