Drought-induced regulatory cascades and their effects on the nutritional quality of developing potato tubers

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Abstract

Potato is among one of the most important food crops, yet maintaining plant productivity in this drought-sensitive crop has become a challenge. Competition for scarce water resources and the continued effects of global warming exacerbate current constraints on crop production. While plants' response to drought in above-ground tissues has been well documented, the regulatory cascades in developing tubers have been largely unexplored. Using the commercial Canadian cultivar 'Vigor', plants were subjected to a drought treatment under high-tunnels causing a 4 increase in canopy temperature when compared to the well-watered control. Tubers were sampled for RNAseq and metabolite analysis. Approximately 2600 genes and 3898 transcripts were differentially expressed by at least four-fold in drought-stressed potato tubers, with 75 % and 69 % being down-regulated respectively. A further 229 small RNAs were implicated in gene regulation during drought. The comparison of protein homologues between Solanum tuberosum L. and Arabidopsis thaliana L. indicates that downregulated genes are associated with phenylpropanoid, carotenoid and patatin biosynthesis. This suggests that there are nutritive implications to drought stress occurring during the potato tuber bulking phase in sensitive cultivars.

Introduction

Potatoes are the fourth most consumed food crop worldwide and are an efficient source of energy, vitamins and minerals in the human diet (King & Slavin, 2013). High consumption rates and moderate concentrations of dietary antioxidants have led potatoes to be the third largest source of total phenolics in the American diet (Chun et al., 2005). Diets rich in phenolics have been implicated in the prevention of an array of degenerative diseases and concentrations of these compounds vary greatly based on cultivar, highlighting the potential for the targeting breeding of potato (*Solanum tuberosum* L.) to enhance global human health (Andre et al., 2007). In potato tubers, the primary polyphenol is chlorogenic acid with the remaining components comprising of carotenoids, anthocyanins and flavonoids. While the phenolic content of tubers is largely genotype dependent, the phenolic profiles are also driven by environmental conditions present at different locations, during growth and tuber bulking, and throughout storage. All of which are parameters that could be manipulated to manage concentrations of the desired phytonutrients (Ezekiel, Singh, Sharma & Kaur, 2013; Payyavula, Navarre, Kuhl, Pantoja & Pillai, 2012; Rosenthal & Jansky, 2008).

Substrate entry into the general phenylpropanoid pathway in eudicots is driven by phenylalanine ammonia-lyase (PAL), an enzyme that regulates the deamination of phenylalanine to yield the cinnamic acid from which monolignols, flavonoids and anthocyanins are produced (Deng & Lu, 2017). PAL activity responds to a variety of developmental and environmental cues with transcriptional regulation occurring by way

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of MYB, LIM and KNOX transcription factors (Zhang & Liu, 2015). Furthermore, independent MYB transcription factors play a prominent role in the regulation of anthocyanin and flavonoid biosynthesis genes such as flavonol synthase (FLS), flavanone 3 - hydroxylase (F3H) and flavonoid 3' – hydroxylase (F3'H), while expression of genes such as dihydroflavonol reductase (DFR) require MYB transcriptional complexes. MicroRNAs (miRNAs) and small interfering RNAs (siRNAs) have notable functions in regulation of the phenylpropanoid biosynthesis in eudicots as well, through the targeting of MYBs. The most notable being miR858, miR828 and TAS4 (Chen, Zeng, Liu & Xia, 2018; Deng & Lu, 2017). The miRNA expression and putative targets have been previously identified in potato leaves (Zhang et al., 2014), however regulatory cascades present in potato tubers are still unknown.

In addition to phenolics, concentrations of available, essential amino acids effect the nutritional value of potato tubers. Up to 50 % of amino acids in tubers are aspartic acid and glutamic acid with the remaining portion made up of leucine, valine, alanine, lysine and arginine for a total nutrition value of potato protein being comparable to an egg white (Pęksa et al., 2013). Genotypes for improving protein quality have been identified among non-traditional potato cultivars (Bártová, Bárta, Brabcová, Zdráhal & Horáčková, 2015). Essential amino acids function both as substrates for secondary metabolism and as a source of energy (Galili, Amir & Fernie, 2016). As a result, concentrations fluctuate in response to environmental stressors with increased abundance observed due to both protein degradation and *de novo* synthesis. The transcriptional regulation of amino acid biosynthesis is highly complex and their function during stress response is still unclear (Galili et al., 2016).

Drought stress is one of the primary concerns in potato with the projected increases in aridity. Potato is adapted to temperate climates with optimal tuber growth occurring at temperatures between 15 – 20 °C. Temperatures above this range, coupled with periodic drought, resulted in reduced yields and increased tuber physiological defects (Rykaczewska, 2015). Symptoms of drought in potato include reduced leaf size, increased chlorophyll content, reduced stomatal conductance and wilting, however, rooting depth and plant recovery have been shown as the best indicators of plant susceptibility to drought (Boguszewska-Mańkowska et al., 2018; Wishart et al., 2014). Through comparisons between genotypes with differing tolerance to drought, novel potato drought-responsive genes and transcript markers for drought tolerance in potato leaves have been identified (Pieczynski et al., 2018; Sprenger et al., 2018). Gene responses in developing potato tubers to drought conditions are not well-documented outside of targeted metabolic pathways (Andre et al., 2007).

This study aimed at identifying drought-associated changes in developing potato tubers (i.e., tuber bulking phase) and their impacts on nutritional quality. The assessment of transcriptional changes of genes with metabolic functions and quantification of amino acid concentrations aim to guide production and harvest practices in optimising nutritional value of the crop. Analysis of small RNAs seek to identify components of the drought regulatory cascade in potato tubers which, to our knowledge, has yet to be explored.

Material and Methods

Experimental design and plant growth conditions

This study was conducted during the summer of 2017 at the Agriculture and Agri-Food Canada's Canada-Saskatchewan Irrigation Diversification Centre, Outlook (51°29' N, 107°03' W, 541 m), Saskatchewan. The cultivar 'Vigor' is a cross between 'Agria' and 'Wischip' made at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre, was evaluated for its performance under soil moisture stress during tuber bulking phase. Prominent characteristics of the cultivar are its yellow-fleshed tubers and pigmented (red-violet) flowers. Plants were grown under optimum soil moisture conditions at 70 % field capacity (FC) and restricted soil moisture conditions at 35 % FC under two high-tunnels using drip irrigation. Treatments were imposed at the start of the tuber bulking phase for gradual exposure to drought stress to mimic natural field conditions (Figure 1; Figure 2). High-tunnels were opened from all sides but covered with plastic film on top to mimic the open field condition while preventing rainfall (Figure 2A). Plots laid out in a randomized complete block design containing four replicates with guard rows on either side. Each plot consisted of 12 hills. The two end hills were considered as guard hills for yield estimation purposes. Seed

pieces were spaced 1 m between-row and 20 cm within-row and were planted on May 30, 2017. The crop was raised using standard management practices (i.e., fertility, irrigation, pest control etc.). Pre-plant basal fertilizer included urea (46-0-0), mono-ammonium phosphate (11-52-0), and potash (0-0-60). Two applications of ammonium sulphate (21.5-0-0-24) were given at 4 and 7 weeks after planting. Soil moisture was monitored using Watermark sensors (Figure 2B). Plots were harvested on October 2nd 2017, and graded according to commercial grade standards.

Physiological measurements

Physiological measurements were taken at the end of the tuber bulking phase (90th day after planting) with readings recorded consistently between 11:30 a.m. and 12:30 p.m. Canopy temperature was assessed with an Infra-Red thermal imaging camera (FLIR T530, FLIR Systems. Wilsonville, OR, USA), and leaflet chlorophyll content (CCI) was recorded using a chlorophyll content meter (CCM-200- Apogee Instruments, Logan, UT USA). Also, the quantum yield of dark-adapted leaflets (F_v/F_m) was measured using the portable fluorometer FluorPen FP 100 (PSI, DRASO Czech Republic). Detachable clips were used to dark-adapt the leaflets for 20 min, and F_v/F_m value was measured on the adaxial surfaces of the top leaflet of the 3rd and 4th leaflet from the top of each sampled plant (three plants per replication).

Tuber sampling for amino acids, abscisic acid and transcriptome

In both high-tunnels, from each replication, a tuber was collected from the middle of the plot on September 13, 2017 (106 DAP). The tubers were washed in running water, followed by distilled water, cut into cubes with a new razor blade evading skin and immediately frozen in liquid nitrogen and stored at -80 °C until further use.

Amino acid profiling and abscisic acid content

Amino acid was extracted from 10 mg powder of freeze dried tissue samples following Inaba et al. (1994) with some modifications. Briefly; 1 mL of 80 % (v/v) ethanol solution (40 °C) was added to each sample, shaken for 30 min at 40 °C and the supernatant was recovered by centrifugation (4000 rpm for 10 min) at 4 °C. The pellets were re-extracted under same conditions with additional 500 µL of 80 % (v/v) ethanol solution (40 °C). The supernatants were combined and stored at -20 °C until further use. Amino acids were derivatized following Waters AccQTag Reagent Kit (Waters, Milford, Massachusetts, USA; Cohen & Michaud, 1993). Briefly; 10 µL aliquot of sample was mixed with 70 µL Borate Buffer and 20 µL AccQFluor Reagent which was reconstituted in Acetonitrile. AccQFluor reagent was reconstituted as follows: 1 mL of AccQ Fluor Reagent diluent was transferred to a vial containing AccQ-Fluor reagent powder and vortexed for 10 s before heating at 55 °C for a maximum of 10 min or until dissolved. The derivatized mixture was transferred to auto sampler vial and incubated at 55 °C for 10 min. High-performance liquid chromatography (HPLC) was conducted, as described in Waters AccQTag chemistry package instruction manual, with excitation wavelength of 285 nm and emission wavelength of 320 nm on a Waters Amino Acid Column – 3.9 × 150 mm using a 2475 scanning fluorescence detector (Waters, Milford, Massachusetts, USA). The column was set at 37 °C with a 5 µL of injection volume. Waters AccQTag buffer (100 mL AccQTag Buffer concentrate + 1000 mL deionized water), Acetonitrile and deionized water were used as mobile phase A, mobile phase B and mobile phase C respectively. Concentration of each amino acid (pmol / µL) from a sample was calculated using peak area values of the chromatogram against the calibration curve of serial dilution (10, 25, 50, 100, 150 pmol / µL) of known amino acid standards. The values were converted to µmol / gm using the extraction volume and weight of initial sample.

Abscisic acid content was determined following Yan et al. (2016). Samples were centrifuged to remove debris, and the pellet was washed twice. The supernatant was evaporated in a SpeedVac, reconstituted in 1 mL of 1 % (v/v) acetic acid. Abscisic acid (ABA) was purified by solid phase extraction using Oasis HLB, MCX and WAX cartridge columns (Waters). The solvent was removed under vacuum and subjected to the LC-ESI-MS/MS analysis (Agilent 6410 TripleQuad LC/MS system). An LC (Agilent 1200 series) equipped with a 50 × 2.1 mm, 1.8-µm Zorbax SB-Phenyl column (Agilent) was used with a binary solvent system comprising 0.01 % (v/v) acetic acid in water (Solvent A) and 0.05 % (v/v) acetic acid in acetonitrile (Solvent

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B). Separations were performed using a gradient of increasing acetonitrile content with a flow rate of 0.2 mL min^{-1}. The gradient was increased linearly from 3 % B to 50 % B over 15 min. The retention time of ABA was 14 min.

Transcriptome and small RNA sequencing

Total RNA was extracted from 100 mg of tuber tissue partitioned from the sample taken for metabolite analysis using the RNeasy plant mini kit (Qiagen, United States). RNA quality and concentration were verified using an Agilent Bioanalyzer (Agilent, United States). TruSeq RNA and small RNA sequencing libraries were constructed following the standard preparation guide (Illumina). All eight RNA samples (four replicates each of drought treated and untreated) were multiplexed in a lane of a flow cell, and paired-end sequencing (125 cycles) was performed on an Illumina HiSeq 2500 instrument. Similarly, for small RNA sequencing, all 8 samples were multiplexed in a lane of a flow cell and single-end sequencing was carried out on Illumina HiSeq 2500.

RNA read pre-processing

Before read mapping and expression quantification, all RNA reads were filtered using Trimmomatic (version 0.36; Bolger, Lohse & Usadel, 2014) by (i) removing adapter sequences, (ii) trimming leading and trailing low quality sequences, (iii) removing sequences when the average quality per base dropped below 15 within a 4-base wide sliding window, and (iv) keeping only those pairs where both reads were longer than 75 bp.

RNA Read mapping

Clean reads were aligned to the Potato reference genome (SolTub_3.0, EnsemblPlants) with STAR (v2.5.2b) and isoform expression was quantified with the RSEM (v1.3.3) algorithm (Li & Dewey, 2011). The expected read counts generated by RSEM algorithm were rounded off and fed into DESeq2.

Small RNA read mapping and analysis

The quality of small RNA sequencing reads was assessed using FASTQC program (v0.11.8; Andrews, 2015). Reads were quality filtered and adapter trimmed using cutadapt (v2.8; Martin, 2011). The alignment of filtered reads to the Potato reference genome (SolTub_3.0, EnsemblPlants), and annotation and quantification of small RNAs was carried out using ShortStack (v3.8.5; Johnson et al., 2009). The psRNATarget (Dai et al., 2018) was used to predict the miRNA and small RNA target genes.

Differential RNA expression analysis

Raw read counts obtained from RNAseq were normalized and assessed for differential expression using the Statistical Software 'R' version 3.6.0 and the package DESeq2 (R Core Team, 2019; Love, Huber & Anders, 2014). Log2 fold change threshold of 2 and a 5 % false discovery rate were used as cutoff values for continuing to annotation steps. The same technique was repeated for discovery of differentially expressed small RNA, with target gene identification done using psRNATarget (Dai, Zhuang & Zhao, 2018). Gene annotations for *Solanum tuberosum* and *Arabidopsis thaliana* were obtained from the Ensembl Plants database (http://plants.ensembl.org). *Arabidopsis* homologs with > 50 % identity to the original potato gene were input into the online DAVID Bioinformatics Resources version 6.8 (https://david.ncifcrf.gov/) for function annotation clustering and KEGG pathway mapping analyses.

Results

Physiological responses

A high degree of variability existed within the four control and four treatment plots for the agronomic and physiological traits measured for this study (Table 1). Both crop yield and tuber number per plot were not found to be significantly different from one another although differences in plot averages were observed. Calculated values from spectral measurements, such as CCI and F_v/F_m, showed no significant differences. However, canopy temperatures measured in plots maintained at 35 % FC were 3.9 higher than control plots with soil moisture maintained at 70 % FC (Table 1).

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Amino acid fluctuations in tubers in response to soil moisture deficit

Of the eight essential amino acids, lysine, phenylalanine, isoleucine and leucine were found to be more abundant in drought-stressed tubers. The largest differences were observed in the concentrations of leucine, phenylalanine and isoleucine which increased by 3×, 2× and 1.9× respectively (Figure 3A). Quantities of branched-chain amino acids, a group that includes leucine, isoleucine and valine, were therefore significantly higher under drought treatment. Histidine and valine were the most abundant essential amino acids in developing potato tubers (Figure 3A). The majority of non-essential amino acids had similar concentrations in developing tubers regardless of the treatment. Only glutamic acid showed a marked increase of 5.75 μmol g^{-1} under reduced soil moisture conditions. Concentrations of cysteine, proline and serine were highest among all amino acids measured (Figure 3B).

Differential gene expression and regulatory cascades in developing tubers under drought stress

Tubers subjected to soil moisture deficit showed both differential gene and transcript expression, with 75.2 % and 68.8 % being downregulated respectively. One fifth of genes with differential expression were of unknown function. A full summary of the observed changes are listed in Table 2 with a list of all differentially expressed genes provided in Supplemental Table 1. Downregulated genes include those with functions in ABA, auxin and ethylene signaling as well as in auxin, carotenoid and phenylpropanoid biosynthesis. Upregulated genes have roles in amino acid biosynthesis, function as molecular chaperones and are involved in ubiquitin-driven proteolysis. Gene names, functional annotation and the corresponding *Arabidopsis* homologs used for pathway mapping can be found in Table 3. Focusing on the regulation of the phenylpropanoid pathway, fifteen annotated MYB transcription factors were downregulated by more than four-fold under low soil moisture conditions. Upregulated transcription factors include two MYB transcription factors and one LIM transcription factor. No other MYB, KNOX or LIM transcription factors were above the cut-off values of 5 % FDR and a log-fold change greater than 2. Genes with key functions in the phenylpropanoid and carotenoid pathways that are downregulated in potato tubers at 35 % FC are highlighted in Figure 4 and Figure 5 respectively. Raw read counts for gene expression analysis can be found in Supplemental Table 2.

The isolated small RNA were grouped into 87213 clusters with an additional 10209 unassigned sequences. Of these, 103 clusters and 126 unassigned sequences were differentially expressed. Additional summary statistics are listed in Table 4. Differentially expressed small RNA clusters with identified gene targets are listed in Supplemental Table 3. None of the small RNA clusters with target genes listed in Table 3 showed differential expression between the two treatments. Expression of target MYB transcription factor genes were also not correlated to small RNA cluster expression (Supplemental Table 4). Interestingly, the expression of small RNA clusters primarily targeting patatin genes was significantly upregulated and negatively correlated to target gene expression (r = -0.61). These clusters and their targets can be found in Table 5.

Discussion

Optimal potato tuber growth occurs around 20 °C and plants are susceptible to losses in productivity under hot, arid conditions. Such conditions are expected to increase in the coming decade, therefore functional indicators of plant stress and the cascading effects on the developing tubers were evaluated. In this study, the Canadian potato cultivar 'Vigor' was gradually exposed to increasing water deficit to a level of 35 % FC beginning at the start of the tuber bulking phase. As seen previously in the literature, fluorescent measurements were not distinguishable between treatments (Boguszewska-Mańkowska et al., 2018) and thus were not dependable indicators of drought stress in potato plants (Table 1). Canopy temperature was considerably elevated in the drought treatments and has evidence supporting its use for drought stress assessments (Prashar, Yildiz, McNicol, Bryan & Jones, 2013). Gene expression data further corroborated that drought signaling pathways had been activated as there was marked downregulation of an ABA receptor PYR1, downregulation of a series of small auxin up-regulated RNA (SAUR) genes involved in cell expansion and organ elongation in response to environment (Ren & Gray, 2015), and the upregulation of heat shock factor proteins (Sprenger et al., 2016) (Table 3). Notably, outside of common changes to the regulation of heat shock proteins, several genes previously identified as differentially expressed in severely drought-

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stressed potato leaves (Sprenger et al., 2016) were inversely regulated in the mildly-stressed potato tubers collected in this study. This includes the WRKY transcription factor (PGSC0003DMG400001434) and the developmental gene UPA16 (PGSC0003DMG400031742) (Sprenger et al., 2016) which had 2-fold and 78-fold increases in expression compared to well-watered control tubers. Lists of genes implicated in drought stress response in potato leaf tissue (Obidiegwu, Bryan, Jones & Prashar, 2015) and potato stolons (Gong et al., 2015) have been compiled and here we provide those in developing tubers (Table 3; Supplemental Table 1). Documented impacts of water deficit occurring during tuber filling include restrictions of tuber size and possible physiological defects (Obidiegwu et al., 2015). At the plot level, there were no significant differences in yield or tuber number between the treatments (Table 1), however discrepancies could become more prominent in commercial field production.

Aside from yield, effects of drought on parameters such as free amino acids, soluble protein and phenolics were assessed. The gradual drought stress to which the potato tubers were exposed resulted in no significant differences in total free amino acid concentrations, although treatment averages appeared to be divergent with 154.1 $\mu mol\ g^{-1}$ and 207.2 $\mu mol\ g^{-1}$ in the control and drought treatments respectively. Elevated concentration of proline has been shown to indicate stress in potato leaves (Vasquez-Robinet et al., 2008), however similar concentrations were observed in tubers irrespective of treatment. The largest changes occurred in the amino acid profile, where concentrations of branched-chain amino acids leucine and isoleucine increased (Figure 3). This indicated a greater proportion of dietary essential amino acids.

A major fraction (up to 40 %) of the soluble protein in potato tubers consist of a glycoproteins known as patatins that act both as storage proteins and show activity as non-specific lipid acyl hydrolases (LAH) with potential roles in plant defense against biotic stressors (Dhondt, Geoffroy, Stelmach, Legrand & Heitz, 2000; Shewry, 2003). In the case of abiotic stress, it was observed that five patatin genes were downregulated by at least 16-fold with regulation of gene expression likely occurring via increased presence of small RNA (Table 5). A possible consequence is reduced protein content in the resulting potato tubers.

As one of the major sources of plant phenolics in the human diet, potatoes have been targeted in breeding for greater total phenolics and antioxidant capacity (Andre et al., 2007; Reyes, Miller & Cisneros-Zevallos, 2005). Phenolic content is known to show a high degree of environmental plasticity with cooler temperatures during the growing period and storage attributed to higher average accumulation (Payyavula et al., 2012; Rosenthal & Jansky, 2008). Under drought conditions, expression of key enzymes required for the biosynthesis of anthocynanins (DFR), flavonoids (FLS) and chlorogenic acid (HCT) were drastically reduced (Figure 4). Initial flow into the phenylpropanoid pathway through PAL was also reduced, leading to the accumulation of phenylalanine observed in Figure 3. Similar results have been previously observed in the literature (Payyavula et al., 2012). Key enzymes of the carotenoid pathway were also downregulated (Figure 5). Environmental conditions leading to the repression of phenolic biosynthesis could minimize gains achieved in breeding programs. Regulation of the phenylpropanoid pathway gene expression can occur via MYB transcription factors (Zhang & Liu, 2015), fifteen of which were significantly supressed under drought (Supplemental Table 1). There was no evidence to suggest that small RNA played a role in regulating the phenylpropanoid or carotenoid pathways under drought conditions (Supplemental Table 4).

From a nutrition perspective, decreasing soil water availability during tuber filling as a function of a warming climate or as a production practice to induce senescence for an earlier harvest may lead to reduction in tuber quality. While mild drought increases the proportion of essential amino acids, potential losses in protein and phenolic content would outweigh the benefit. While MYB transcription factors may be targeted to reduce effects on the phenylpropanoid pathway, identification of small RNA as the regulator of patatin gene expression suggests it may be difficult to maintain patatin expression in drought-susceptible cultivars using current breeding techniques.

Acknowledgments

physiological measurements.

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Table 1: Agronomic and physiological trait averages for the drought (35 % FC) and control (70 % FC) plots with standard error of the mean presented in brackets. Bold numbers indicate significance between treatments.

Treatment	Yield (g)	Tuber No.	CCI	CCI	Fv/Fm	Fv/Fm	Canopy Temp (°C)	Ca
35 % FC	1381.7 (248.1)	12.8 (0.8)	12.8 (0.8)	19.5 (0.2)	19.5 (0.2)	0.24 (0.05)	0.24 (0.05)	**28.**
70 % FC	1737.3 (198.1)	18.1 (3.5)	18.1 (3.5)	15.4 (1.6)	15.4 (1.6)	0.38 (0.03)	0.38 (0.03)	24.

Table 2: Summary of differentially expressed genes in potato tubers at 35% FC using a threshold of four-fold difference in expression and a 5% FDR. Homologs in Arabidopsis thaliana were considered if identity was greater than 50%. Clustering was based on Arabidopsis gene names using the DAVID Bioinformatics online resource 6.8 (https://david.ncifcrf.gov/).

Differentially expressed (DE) small RNA summary	Differentially expressed (DE) small RNA summary
No. DE small RNA/ small RNA clusters	229
Small RNA downregulated	31.8%
No. with identified gene targets	84
No. with target gene inhibition via cleavage	88.1%
No. unique target genes	60
Unique target genes with unknown function	20.0%

Table 3: List of differentially expressed genes in drought stressed potato tubers, the corresponding homologs in Arabidopsis thaliana and the pathway in which they participate. Pathway mapping was done based on the Arabidopsis gene names and similarities between the original Solanum tuberosum gene and its homolog are expressed as percentage of identical base pairs in the gene sequences (% ID).

Gene regulation	Pathway	Gene name	Description

Gene regulation	Pathway	Gene name	Description
Down-regulated	ABA signaling	PGSC0003DMG400002100	Abscisic acid receptor PYR1
	Auxin biosynthesis and signaling	PGSC0003DMG400001589	Amino acid transporter
		PGSC0003DMG400024978	Indole-3-acetic acid-amido synthetase GH3.3
		PGSC0003DMG400024997	Indole-3-acetic acid-amido synthetase GH3.6
		PGSC0003DMG400014707	Flavin monooxygenase
		PGSC0003DMG400026087	Flavin monooxygenase
		PGSC0003DMG400003773	SAUR family protein
		PGSC0003DMG400001667	SAUR family protein
		PGSC0003DMG400001614	SAUR family protein
		PGSC0003DMG400001668	SAUR family protein
		PGSC0003DMG400001655	SAUR family protein
		PGSC0003DMG400022233	SAUR family protein- ARG7
		PGSC0003DMG400001615	SAUR family protein
	Carotenoid biosynthesis	PGSC0003DMG400028180	Cytochrome P450-type monooxygenase 97C11
		PGSC0003DMG400024063	Phytoene synthase 1, chloroplastic
	Ethylene signaling	PGSC0003DMG400014204	Transcription factor TSRF1
	Phenyl-propanoid biosynthesis	PGSC0003DMG400003605	Dihydroflavonol 4-reductase
		PGSC0003DMG400014093	Flavonol synthase
		PGSC0003DMG400014152	Hydroxycinnamoyl transferase
		PGSC0003DMG400023458	Phenylalanine ammonia-lyase
		PGSC0003DMG400014223	4-coumarate–CoA ligase 2
		PGSC0003DMG400028929	4-coumarate–CoA ligase 2

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Gene regulation	Pathway	Gene name	Description
Upregulated	Amino acid biosynthesis	PGSC0003DMG400034102	Acetolactate synthase
	Protein folding	PGSC0003DMG400008223	Heat shock factor protein HSF30
		PGSC0003DMG400003219	Small heat shock protein, chloroplastic
		PGSC0003DMG400030341	Small heat shock protein - Class I 17.6kD
		PGSC0003DMG400024707	Small heat shock protein
		PGSC0003DMG402028907	Small heat shock protein 90
		PGSC0003DMG400030426	Small heat shock protein - Class I 17.6kD
	Proteolysis	PGSC0003DMG400006185	Skp1 1
		PGSC0003DMG400006184	Skp1

Table 4: Summary of differentially expressed small RNA in potato tubers at 35 % FC using a threshold of four-fold difference in expression and a 5 % FDR.

Differentially expressed (DE) small RNA summary	Differentially expressed (DE) small RNA summary
No. DE small RNA/ small RNA clusters	229
Small RNA downregulated	31.8%
No. with identified gene targets	84
No. with target gene inhibition via cleavage	88.1%
No. unique target genes	60
Unique target genes with unknown function	20.0%

Table 5: List of differentially expressed small RNA clusters in drought stressed potato tubers that negatively correlate to target transcript expression. Target alignments, gene ID, expression and descriptions are included.
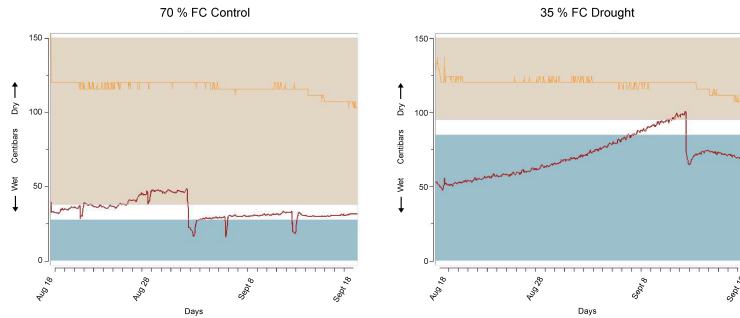
Small RNA Cluster	Log 2 fold change	Target alignment	Target gene	Log 2-
Cluster 34023	5.03	AGCUCAUUAAUCUCUUCGAUA	PGSC0003DMG400009921	-6.24
Cluster 23921	4.68	AGGGUUCAAGAAAAUGCAUUA	PGSC0003DMG400029247	-4.75
Cluster 15144	4.62	AGGGUUCAAGAAAAUGCAUUA		
Cluster 41775	4.49	ACCUCAGGGUUCAAGAAAAUG		
Cluster 83189	5.49	AGGCACUGGCACUACUUCAGA	PGSC0003DMG400017091	-4.25
Cluster 83175	4.98	AGCCAGUAAUAUUCACCAAGU		
Cluster 83174	3.45	AGGCACUGGCACUACUUCAGA		
Cluster 7920	4.95	GGCAGCAAGUUCUUACAUGAC	PGSC0003DMG400008749	-4.06
Cluster 68384	3.01	AUCAUUCCGGGUAUCAUUCUC		
Cluster 83190	2.87	UUCCGGGUAUCAUUCUCGAAU		
Cluster 83166	2.66	UCCGGGUAUCAUUCUCGAAU		
Cluster 68380	5.49	AGGCACUGGCACUAAUUCAGA	PGSC0003DMG400014104	-4.47
Cluster 83164	5.49	AGGCAGCUAAAUGGGGUCCUC		
Cluster 20497	5.38	CUGUUGGUGAUCCGGCGUUA		
Cluster 68397	5.36	GUUGCUACUGUUGGUGAUCCG		
Cluster 83182	4.97	GGCACUACUUCAGAGUUUGAU	PGSC0003DMG401017090	-4.91

A)

B)

Phenylpropanoid pathway

Phenylalanine

PAL

Cinnamic acid

C4H

p-coumaric acid

4CL

p-coumaroyl CoA

Flavonoids

Kaempferol   Flavanones

FLS   *F3H*   *HCT*

Dihydroquercetin ← Dihydrokaempferol → Dihydromyricetin   Coumaroyl shikimate

FLS   *DFR*   *F3´H*   *F3´5´H*   *DFR*   *FLS*   *C3H*

Quercetin   Leucocyanidin   Leucopelargonidin   Leucodelphinidin   Myricetin   Caffeoyl shikimate

DFR   *HCT*

Cyanidin-CRG*   Pelargonidin-CRG*   Petunidin-CRG*   Caffeoyl CoA ⇄ Chlorogenic acid

A3´5´MT   *A3´5´MT*   *HQT*

Peonidin-CRG*   Malvidin-CRG*

Anthocyanins   * CRG indicates a coumaryolrutinosideglucoside

Carotenoid pathway

Geranylgeranyl diphosphate

$\downarrow$ *PSY*

15-*cis*-phytoene

$\downarrow$ *PDS, ZDS,*
Z-ISO, CrtISO

trans-lycopene

LCY-e $\swarrow$ $\searrow$ *LCY-b*

α-carotene   β-carotene

$\downarrow$ $\downarrow$ (dashed)

Zeinoxanthin

$\downarrow$ *LUT1*

Lutein   Neoxanthin