Final Report

Elucidating the mechanisms of senescent sweetening in stored potato tubers to improve storage regimes and identify candidate genes

Ref: 1100020

Reporting Period: Oct 2016 - Oct 2019

Report Authors: Jose Barrera-Gavira, Robert D Hancock

Date report submitted: 21 May 2020

Additional copies of this report and a list of other publications from the Potatoes Sector can be obtained from:

Publications

AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel: 02476 692051
E-mail: Potatoes.Publications@ahdb.org.uk

Our reports, and lists of publications, are also available at ahdb.org.uk

CONTENTS

1. SUMMARY

1.1. Aim

Senescent sweetening is a phenomenon observed in potato tubers following long-term storage in the presence of sprout suppressors. It has significant impact on potato quality, in particular for the processing industry where sugar accumulation results in dark fry colour and the presence of undesirable chemical components. Unlike cold-induced sweetening, senescent sweetening is irreversible and therefore stored crops need to be monitored and utilised prior to sugar accumulation. Furthermore, while mechanisms of cold-induced sweetening are well understood the biological causes of senescent sweetening remain obscure.
The objectives of the project were therefore to adopt biochemical and molecular approaches to elucidate the mechanisms of senescent sweetening and to identify potential genes underlying the sweetening trait. Such knowledge was intended to assist store managers in the prediction of senescent sweetening and to provide breeders with candidate genes for the development of markers to accelerate the production of senescent sweetening resistant cultivars.

1.2. Methodology

Over the first two years of the project, a senescent sweetening resistant and a sweetening sensitive cultivar were stored for up to 11 months under industry standard conditions. Tubers were regularly sampled and sugar content and fry colour determined to identify the transition to senescent sweetening. Changes in primary metabolites associated with sugar and starch metabolism were determined using gas chromatography/mass spectrometry and changes in the expression of >40000 genes were determined using microarray technologies.
In the final year of the project nine cultivars were stored and tested for sugar accumulation and fry colour. Changes in expression of key genes identified in years 1 and 2 were determined using RT-PCR.

1.3. Key findings

While the precise date of sugar accumulation varied year-to-year, indicating the influence of the seasonal growing environment, the resistant cultivar did not exhibit sugar accumulation in any year while the susceptible cultivar did exhibit sugar accumulation following a period of storage in every year indicating a strong genetic influence. With the exception in differences in sugar accumulation, very few other primary metabolites exhibited consistent significant differences between cultivars. Similarly, changes in gene expression over the storage period were similar between cultivars. However, the susceptible cultivar exhibited a marked decline in expression of a plastid localised glucose phosphate translocator (GPT2) and genes associated with starch synthesis at the onset of sugar accumulation. Starch is only synthesised in the plastid and the substrate for starch synthesis, glucose-6-phosphate is imported from the cytosol by GPT2. As the potato tuber goes through futile cycles of starch degradation and resynthesis, these data suggest that senescent sweetening is associated with a reduction in starch resynthesis caused by a reduced capacity to transport substrate back into the plastid that leads to sugar accumulation in the cytosol. Monitoring of GPT2 expression in a broader range of cultivars in year 3 suggests that the mechanism is common across a broad range of germplasm.

1.4. Practical recommendations

GPT2 is key for the control of senescent sweetening in stored potato tubers and is therefore a primary genetic target for the breeding and selection of sweetening resistant cultivars. An assessment of genetic diversity in GPT2 and its upstream promotor region (that controls expression) is recommended to identify genetic markers associated with the capacity to maintain GPT2 expression over long term storage. These could provide valuable tools for the selection of sweetening resistant genotypes bypassing the need for expensive storage testing. Changes in GPT2 expression additionally represent a valuable tool for the early identification of sweetening onset, allowing store managers to utilise material prior to quality loss.

2. INTRODUCTION

The UK potato processing industry is worth £3.9 billion at retail and supports more than 20,000 jobs in the industry directly, on farms, in transport and in manufacture. To achieve year round potato supply in the UK approximately 1.5 million tonnes of potato tubers are stored for up to 8 months each season. Control of potato quality during storage represents a significant problem for the industry and a key issue remains the capacity to inhibit sprouting while preventing loss of processing quality as a result of reducing sugar (primarily glucose and fructose) accumulation leading to problems of dark fry colour and acrylamide accumulation in processed products.

Tubers destined for processing are stored at relatively high temperatures in the presence of sprout suppressors hence cold induced sweetening no longer represents a significant problem (Cunnington, 2008). However, in longer term storage (> 5 months) the physiologically distinct issue of senescent sweetening represents a significant and underexamined issue for the processing industry (Colgan *et al*., 2012).

Only a small number of studies to date have specifically addressed the issue of senescent sweetening. Colgan *et al*. (2012) and Carvalho (2018) demonstrated cultivar dependent variation in the onset of senescent sweetening indicative of a genetic component. However, environmental factors were also apparent with variations in the onset of senescent sweetening dependent not only on the growing season but also on the growing location within a season. The impact of physiological age of tubers may also play a role in the onset of sweetening. Colgan *et al*. (2012) citing an earlier report indicated that tubers harvested from chitted mother tubers or those that were planted earlier in the season exhibited an earlier onset of sugar accumulation in storage. Similarly, data reported by Driskill *et al*. (2007) suggests that physiologically older tubers exhibit a greater propensity for senescent sweetening. On the other hand, Carvalho (2018) did not observe any differences in sugar accumulation of stored tubers harvested from chitted or unchitted seed.

The biochemical processes associated with senescent sweetening are little examined and poorly understood. Several studies indicate an increase in oxidative stress and the accumulation of reactive oxygen species in stored tubers. For example, Kumar and Knowles (1993) demonstrated an increase in the level of malondialdehyde, a marker of lipid oxidation during tuber storage. This was associated with an increase in the ratio of oxidised to reduced gluatathione, an increase in glutathione transferase and glutathione reductase activity, and an increase in plasma membrane ATPase activity (Kumar & Knowles, 1996). The authors suggested a scheme in which increased oxidative load during tuber aging resulted in enhanced lipid peroxidation requiring greater glutathione transferase activity for removal of lipid hydroperoxides. The resultant increase in glutathione oxidation induces glutathione reductase activity and creates a sink for reducing power in the form of NADPH. Simultaneously the need to synthesise glutathione *de novo* creates an ATP sink requiring increased respiration which was proposed to promote starch turnover and reducing sugar accumulation.

The suggestion that tuber aging results in an increased oxidative load was supported by Carvalho (2018) who observed increases in both hydrogen peroxide and superoxide using 3,3'-diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) staining, respectively following tuber storage. Furthermore, tuber aging was associated with an increase in oxidation, glycation and deamidation of proteins, all associated with oxidative stress (Kumar *et al*., 1999). Mitochondrial proteins had a particularly high level of oxidative modifications (Salvato *et al*., 2014). Contrary to the scheme suggested by Kumar and Knowles (1996) described above these data may suggest impairment of mitochondrially localised respiration thereby potentially reducing a metabolic activity responsible for sugar catabolism. Such a metabolic dysfunction could be particularly significant when combined with increased starch degradation and a mechanism by which this might occur was revealed following transmission electron microscopy of amyloplast membranes which exhibited increasing levels of disorganisation over the storage period (Sowokinos *et al*., 1987). Similar results were obtained following scanning electron microscopy of freeze dried amyloplasts where fractures in the outer membrane became increasingly visible

following extended periods of storage (Carvalho, 2018). These data suggest that break down of amyloplast membranes during storage could result in greater accessibility of starch granules to amylolytic enzymes thereby accelerating starch turnover and leading to the accumulation of reducing sugars.

Potato tuber reducing sugar content is primarily determined by the competing pathways of starch synthesis and degradation, glycolysis and respiration, and sugar storage. Significantly, these competing processes occur in different subcellular compartments with starch synthesis and degradation occurring in the amyloplast, glycolysis occurring in the cytosol, respiration in the mitochondrion and storage in the vacuole. The degree of reducing sugar accumulation is therefore ultimately controlled not only by the enzymes required for the operation of the various metabolic pathways but also the transporters required to transport pathway intermediates between cellular organelles.

Changes in tuber carbohydrate metabolism during the onset of senescent sweetening are surprisingly underexamined. Glucan water dikinase (GWD) phosphorylates starch granules stimulating starch hydrolysis by amylases (Edner *et al*., 2007). Carvalho (2018) observed an increase in the abundance of transcripts encoding two isoforms of GWD following storage in the sweetening susceptible cultivar Lady Rosetta but not in the sweetening resistant cultivar VR808. These data indicate a potential role for GWD activity in accumulation of reducing sugars during the senescent sweetening transition. Vacuolar invertase converts the non-reducing sugar sucrose to the reducing sugars glucose and fructose and its activity is strongly associated with the onset of cold-induced sweetening (Matsuura-Endo *et al*., 2004). Furthermore, silencing of vacuolar invertase has been shown to suppress cold-induced sweetening, almost completely blocking sugar accumulation in the most strongly silenced lines (Bhaskar *et al*., 2010). Silencing of vacuolar invertase has also been shown to slow sugar accumulation during senescent sweetening (Wiberley-Bradford & Bethke, 2018) however, in this case sugar accumulation was only delayed by a few weeks (as opposed to months for cold-induced sweetening) suggesting that although vacuolar invertase exerts some control over reducing sugar accumulation in senescent sweetening other biochemical factors are likely to be important.

There is a clear dearth of knowledge regarding the mechanisms of sugar accumulation, the genes underlying the senescent sweetening trait or the pre- and post-harvest factors that promote it. The objectives of the present research were therefore to identify the mechanisms of senescent sweetening and the key genes underlying the sweetening trait. A key hypothesis was that senescent sweetening is associated with aging-related oxidative stress. A mechanistic understanding of the senescent sweetening process was anticipated to provide information that would i) define predictive markers of senescent sweetening induction, ii) underpin strategies for control by optimising storage regimes and iii) underpin breeding programmes for the development of senescent sweetening-resistant varieties.

3. MATERIALS AND METHODS

3.1. Plant Materials, Growing and Storage Conditions

For the 1st year (season 2016/2017) of this study, tubers of two different potato cultivars, Arsenal and VR 808, were obtained from PepsiCo. Tubers were either untreated or treated by misting with CIPC to inhibit sprouting. When treated, CIPC was applied post-curing at 13°C. Tubers were stored at 9°C in the dark in a cold storage unit (Porkka, UK) at The James Hutton Institute and sampled at the intervals described. For untreated tubers, sprouting buds were removed by hand every two weeks to avoid the development of carbon sinks. The 1st sampling point was in October 2016 and last sampling point in August 2017.

In year 2 (season 2017/2018) tubers of cultivars Arsenal and VR 808, were obtained from PepsiCo. All tubers had been treated with CIPC as described for year 1. Tubers were stored at The James Hutton Institute as previously described and sampled for tuber quality at the intervals described. The 1st sampling point was in November 2017 and last sampling point in October 2018.

In the final year (season 2018/2019) tubers of 9 different cultivars were obtained from PepsiCo, all of them CIPC-treated. The varieties used during this year are Pirol, SH C 909, VR 808, Lady Rosetta, Brooke, Arsenal and Shelford. An additional variety was included that the breeders do not wish to disclose so is described as 'unknown' throughout the report. Tubers were stored at 9°C at The James Hutton Institute and sampled for tuber quality. The 1st sampling point was in December 2018 and last sampling point in September 2019.

During the experimental years all potato cultivars were grown in Shropshire, with the exception of the Shelford variety used during year 3 where tubers grown in two locations, Shropshire and Yorkshire were under study for this variety.

Tubers were sampled following bisection transversally and twice longitudinally at a 90˚ angle. Tuber samples were then taken from opposite eights and comprised periderm, cortex, vascular ring and outer core to capture the maximum range in sugars across the tuber. In the 1st and 2nd years of the experiment opposite eighths were taken from individual replicate tubers. In the last year three tubers were sampled per variety for each sampling occasion.

All the samples were snap frozen in liquid nitrogen and each set of replicates was either stored at -80°C or subject to 72 hours freeze drying at 0.700 mbar (ALPHA 1-4 LSC, CHRIST Freeze Dryers, Germany) before grinding to a fine powder using an electric grinder (DCG39, DE'LONGHI Electric Grinder, UK).

3.2. Fry Test Process

Tuber slices in a range 1.26 mm – 1.48 mm thickness were fried at 177°C (L30PFS12, LOGIK Professional Deep Fryer, UK) for 3 minutes. Tubers were sliced using a mandoline (Bron Coucke Mandoline Vegetable Slicer, France) and slice thickness was measured using a digital caliper (RS PRO 150mm Digital Caliper, UK). A long-life vegetable cooking oil (KTC (Edibles) Ltd, UK) was used for frying. Grade of darkening in fry colour was estimated using ImageJ based on the grey scale in each crisp (Schneider *et al*., 2012).

3.3. Biochemical Analysis

3.3.1. Extraction and quantification of sugars

Sugars were extracted and analysed using an adaptation to the method described by Viola *et al*. (2007). Prepared lyophilised tuber powder (50 mg) was extracted in 1 ml of 80% (v/v) ethanol at 80°C for 1 hour with periodic vortexing. Samples were then centrifuged at 16,000 g for 10

minutes at 1°C, the supernatant was decanted, and the extraction was repeated. The two supernatant fractions were combined, reduced to the aqueous phase by evaporation at 40°C under reduced pressure in a centrifugal evaporator (miVac Duo Concentrator, Pump and Speed Trap, GeneVac, UK), The aqueous phase was then frozen, lyophilized and finally resuspend in 20 volumes of distilled H_2O. Sugar quantification was achieved by HPAEC-PAD using a Dionex 3m x 150 mm PA20 column with an eluant of 10mM NaOH at a flow rate of 0.5 ml min^{-1} (Huang *et al*. 2016). The column was washed with 100 mM NaOH and requilibrated with 10 mM NaOH between sample injections.

3.3.2. H_2O_2 extraction and quantification

Frozen (-80ºC) tuber samples were ground to a powder in liquid nitrogen using a mortar and pestle and extracted in ice-cold 5% $HClO_4$ at a ratio of 1 ml 50 mg FW^{-1} using an adaptation of the method described by Queval *et al*. (2008). Following extraction, the homogenate was centrifuged at 14,000 g for 10 minutes at 4°C and the supernatant was neutralized with 5 M K_2CO_3. Insoluble $KClO_4$ was removed by centrifugation and H_2O_2 was immediately quantified by fluorimetry in a microplate reader (VarioskanTM LUX, Thermo ScientificTM) using the commercially available Amplex Red Hydrogen Peroxide/Peroxidase assay kit according to the manufacturer's instructions (Invitrogen Ltd, Paisley, UK).

3.3.3. Malondialdehyde (MDA) determination

MDA content was determined according to Hodges *et al*. (1999) from powdered freeze-dried samples extracted in 20 volumes of 80% (v/v) ethanol.

3.3.4. Total polyphenols extraction and quantification

Total polyphenols were estimated using a modification of the enzymatic method described by Stevanato *et al*. (2004). Powdered freeze-dried samples (0.1 g) were extracted in 10 volumes of 50% (v/v) methanol containing 0.1% formic acid. The homogenate was centrifuged at 4,000 g for 15 minutes. Total polyphenol content was determined in 10 µl of supernatant in a reaction mixture containing 145 µl potassium phosphate buffer pH 8.0, 20 µl 30 mM 4-aminophenazone, 20 µl 20 mM H_2O_2 and 5 µl 100 U ml^{-1} horse radish peroxidase (EC 1.11.1.7). Following 5 minutes incubation in the dark at 25°C, absorbance at 500 nm was recorded in a plate reader (MultiskanTM GO, Thermo ScientificTM) and total polyphenols estimated by reference to a standard curve constructed using catechin.

3.3.5. Extraction and quantification of enzyme activities

3.3.5.1. Kinetic enzyme activity assays of plant ascorbate-glutathione cycle

Frozen tuber samples were ground to a powder in liquid nitrogen using a mortar and pestle and extracted in ice-cold 50 mM MES-KOH buffer pH 6.0 containing 40 mM KCl, 2 mM $CaCl_2$ and 1 mM L-ascorbate (AsA) at a ratio of 4 ml g FW^{-1} following an adaptation to the method described by Murshed *et al*. (2008). Soluble protein content of sample extracts were quantified using the Bio-Rad protein assay using bovine serum albumin (BSA) as a standard. Activities of ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase were estimated in flat bottom 96-well plates as described (Murshed *et al*., 2008).

3.3.5.2. Enzyme activity assays of carbohydrate metabolism

1 g of frozen tuber samples were ground to a powder in liquid nitrogen using a mortar and pestle and extracted in 4 volumes of ice-cold 200 mM HEPES-NaOH buffer pH 7.5 containing 3 mM $MgCl_2$, 1 mM EDTA, 2% glycerol, 1 mM benzamidine, 5 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulphonyl (PMSF) and 5% polyvinylpolypyrrolidone (PVPP) following an adaptation to the method described by Jammer *et al*. (2015). Extracts were clarified by

centrifugation and then used either crude or following desalting using a PD-10 gel filtration column (Amersham Biosciences, UK) depending on the enzymes assayed (Figure 3-1).

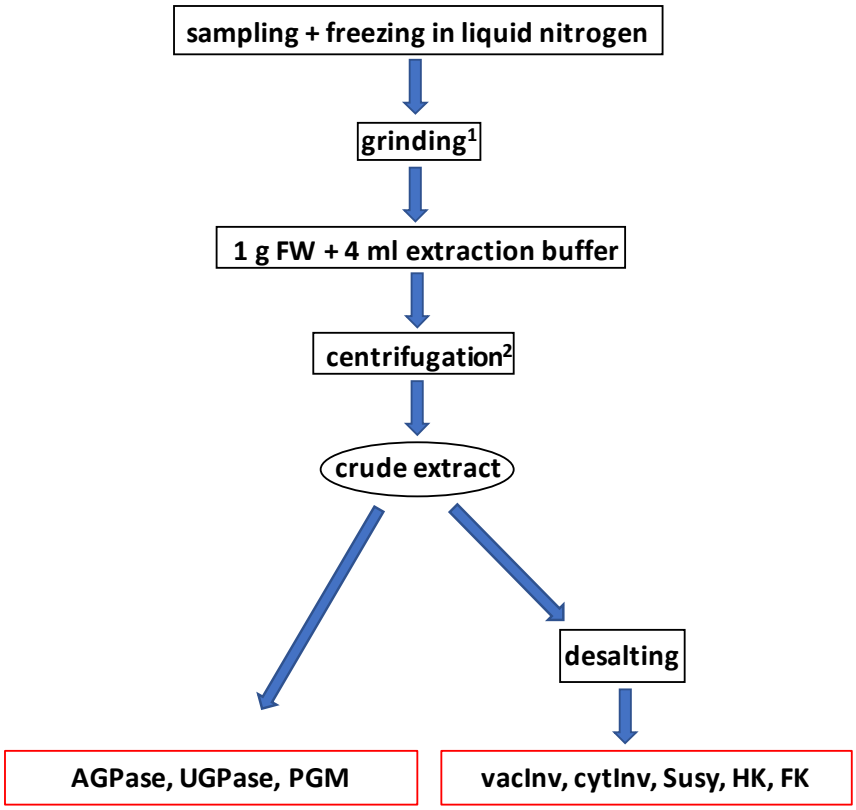

Figure 3-1. Flowchart of the universal protein extraction and desalting protocol. All steps were performed on ice. [1]In liquid nitrogen using a pre-cooled mortar and pestle. [2]Centrifugation at 20,000 g for 20 minutes at 4°C. AGPase, ADP-glucose pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase (EC 2.7.7.9); PGM, phosphoglucomutase (EC 5.4.2.2); vacInv, vacuolar invertase (EC 3.2.1.26); cytInv, cytoplasmic invertase (EC 3.2.1.26); Susy, sucrose synthase (EC 2.4.1.13); HK, hexokinase (EC 2.7.1.1); and FK, fructokinase (EC 2.7.1.4).

Kinetic enzyme activity and invertase activity assays were established in a total reaction volume of 0.16 ml or 1.2 ml, respectively. All enzyme activity assays were performed in flat bottom 96-well plates (NuncTM, MicroWellTM, Thermo ScientificTM), and absorbance measured using a spectrophotometer (MultiskanTM GO, Thermo ScientificTM). Samples and controls were analysed in triplicate. Soluble protein content of the supernatants was quantified (Bio-Rad protein assay) using BSA as a standard. All specific activities were calculated using the extinction coefficient of NADPH at 340 nm (6.22 mM^{-1} cm^{-1}). Reactions were performed with a mixture of buffer components, substrate(s), auxiliary substance(s), and auxiliary enzymes (Figure 3-2):

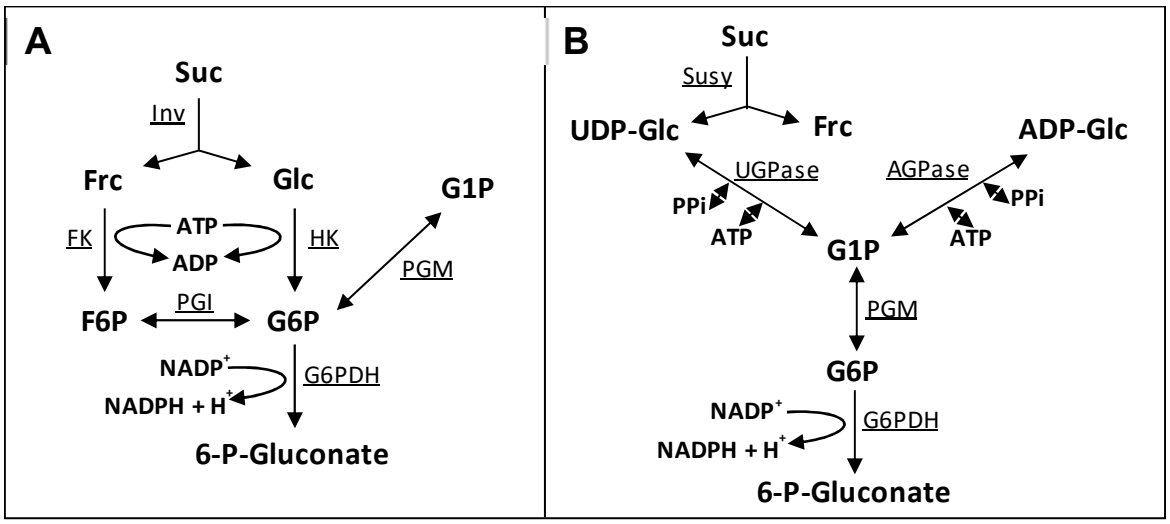

Figure 3-2. Reaction schemes to determine the selected enzyme activities. **A.** Inv, invertase; FK, fructokinase; HK, hexokinase; PGI, phosphoglucoisomerase (EC 5.3.1.9); PGM, phosphoglucomutase; and G6PDH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49). **B.** Susy, sucrose synthase; UGPase, UDP-glucose pyrophosphorylase; AGPase, ADP-glucose pyrophosphorylase; PGM, phosphoglucomutase; and G6PDH, glucose-6-phosphate dehydrogenase.

i) ADP-glucose pyrophosphorylase (AGPase): aliquots of untreated crude extract (25 μl) were incubated with 0.44 mM EDTA, 5 mM $MgCl_2$, 0.1% BSA, 2 mM ADP-Glc, 1.5 mM PPi, 1 mM NADP, 2 mM 3-PG, 0.432 U of PGM, and 1.28 U of G6PDH in 100 mM Tris-HCl at pH 8.0. For control reactions, ADP-Glc was omitted. The increase in absorbance at 340 nm due to conversion of NADP to NADPH was monitored.

ii) UDP-glucose pyrophosphorylase (UGPase): aliquots of untreated crude extract (25 μl) were incubated with 0.44 mM EDTA, 5 mM MgCl2, 0.1% BSA, 2 mM UDP-Glc, 1.5 mM PPi, 1 mM NADP, 2 mM 3-PG, 0.432 U of PGM, and 1.28 U of G6PDH in 100 mM Tris-HCl at pH 8.0. For control reactions, UDP-Glc was omitted. The increase in absorbance at 340 nm due to conversion of NADP to NADPH was monitored.

iii) Phosphoglucomutase (PGM): aliquots of untreated crude extract (25 μl) were incubated with 10 mM $MgCl_2$, 4 mM DTT, 0.1 mM G1,6bisP, 1 mM G1P, 0.25 mM NADP, and 0.64 U of G6PDH in 20 mM Tris-HCl at pH 8.0. For control reactions, G1P was omitted. The increase in absorbance at 340 nm due to conversion of NADP to NADPH was monitored.

iv) Fructokinase (FK): aliquots of desalted crude extract (25 μl) were incubated with 5 mM $MgCl_2$, 5 mM fructose, 2.5 mM ATP, 1 mM NADP, 0.8 U of PGI, and 0.8 U of G6PDH in 50 mM BisTris at pH 8.0. For control reactions, fructose was omitted. The increase in absorbance at 340 nm due to conversion of NADP to NADPH was monitored.

v) Hexokinase (HK): aliquots of desalted crude extract (25 μl) were incubated with 5 mM $MgCl_2$, 5 mM glucose, 2.5 mM ATP, 1 mM NADP, and 0.8 U of G6PDH in 50 mM BisTris at pH 8.0. For control reactions, glucose was omitted. The increase in absorbance at 340 nm due to conversion of NADP to NADPH was monitored.

vi) Sucrose synthase (SuSy): for determination of SuSy activity, two reactions were performed, (A) including 1 mM UDP detecting both SuSy and cytInv background activity, and (B) without 1 mM UDP to detect the cytInv background activity only. SuSy activity was calculated by subtracting cytInv background activity (B) from total activity (A). For both reactions, aliquots of desalted crude extract (25 μl) were incubated with 1 mM EDTA, 2 mM $MgCl_2$, 5 mM DTT, 250 mM sucrose, 1 mM UDP (omitted for reaction B), 1.3 mM ATP, 0.5 mM NADP, 0.672 U of HK, 0.56 U of PGI, and 0.32 U of G6PDH in 50 mM Hepes-NaOH at pH 7.0. For control reactions, sucrose was omitted. The increase in absorbance at 340 nm due to conversion of NADP to NADPH was monitored.

vii) Invertase activity (Inv): invertase activity was assayed in an end-point assay modified from the method of Viola and Davies (1992). Aliquots (600 μl) of desalted crude extract were

incubated at 30°C for 1 hour with 5 mM $MgCl_2$, 25 mM sucrose, 2.5 mM ATP, 1 mM NADP, 1.5 U of HK, 0.5 U of PGI, and 1.2 U of G6PDH in 25 mM acetate buffer at pH 5.2 or pH 6.8 for vacInv or cytInv, respectively, in a total reaction volume of 1.2 ml. For control reactions, sucrose was omitted. Aliquots (100 µl) were taken every 20 minutes and the increase in absorbance at 340 nm due to conversion of NADP to NADPH was monitored.

3.3.6. Metabolite profiling by gas chromatography/mass spectrometry

Gas chromatography/mass spectrometry (GC/MS) analysis was performed as described by Foito *et al*. (2013). Dried material was extracted in a two-phase solvent system as described to generate polart and non-polar fractions. Polar fractions were oximated and derivatised as trimethylsilyl (TMS) derivatives. The non-polar phase was subjected to methanolysis and TMS derivatisation. Metabolite profiles for the polar and non-polar fractions were acquired following separation of compounds on a DB5-MSTM column (15m×0.25mm×0.25µm; J&W, Folsom, CA, USA) using a Thermo Finnigan (San Jose, CA, USA) DSQII GC/MS system as described (Foito *et al*., 2013). The samples were analysed in a randomized order, while quality control samples as well as blanks were incorporated at the beginning and the end of the sequence. Data were then processed using the XCALIBUR software (Thermo Fisher Scientific, Waltham, MA, USA). Peak areas relative to internal standard (response ratios) were calculated following normalization to 100 mg extracted material.

3.3.7. ^{14}C labelling and fractionation of labelled tissue extracts

Tuber discs (diameter 5 mm, thickness 1-2 mm) were cut from fresh potato tuber slices, washed three times in 50 mM MES-KOH (pH 6.5) containing 300 mM mannitol and then incubated (500 mg in a volume of 1 ml in glass vials shaken at 100 rpm) for 3 hours in 50 mM MES-KOH (pH 6.5) containing 300 mM mannitol and 0.148 MBq D-[U-^{14}C]glucose. Vials were sealed with a rubber stopper which held a paper filter moistened with 200 µl of 10% (w/v) KOH to trap $^{14}CO_2$. After incubation material was washed three times for 5 min in 5 ml of 50 mM MES-KOH (pH 6.5) containing 300 mM mannitol prior to successive extraction in 5 ml of 80% (v/v) ethanol. The supernatant was dried down under vacuum (miVac Duo Concentrator, Pump and Speed Trap, GeneVac, UK) and passed through both an anion-exchange cartridge (SAX; HPLC Technology, UK) and cation-exchange cartridge (SCX; Phenomenex® Strata, UK) as described by Souleyre *et al*. (2004). Material not retained by the ion exchange cartridges was considered as the neutral material while the separated anionic and cationic fractions were recovered from the cartridges by eluting with 5 M HCl and 5 M KOH, respectively. The insoluble plant material was separated into starch and non-starch components as described in Runquist and Kruger (1999). All fractions were analysed using scintillation counting.

Glucose, fructose and sucrose were separated by high-performance liquid chromatography (HPLC) using a Metacarb 87C 300x7.8 mm column (MetaChem Technologies Inc., Torrance, CA) with 0.6 ml min-1 ultrapure water as the mobile phase at a temperature of 70°C (Davies *et al*., 2005; Viola *et al*., 2007). Radiolabelled sugars were detected using a Radioflow detector LB 509. Radioactivity in the CO_2, anionic, starch, other insoluble and neutral fractions was determined by liquid scintillation counting (Tri-Carb 3100TR Packard) after dilution into ScintLogic HiCount cocktail (Lablogic Systems Ltd, Sheffield, UK).

3.3.8. Statistical analysis

Data analysis and graphical outputs were performed using Microsoft Excel 2013. Statistical analysis was undertaken by two-way ANalysis Of VAriance (ANOVA, parametric test) with potato cultivar and storage time as parameters in order to identify statistically significant differences between profiles using a significance level (P-value) ≤ 0.05 using GENSTAT (v. 18.1, VSN International Ltd, Hemel Hempstead, UK). When different treatments were present in the experiment, ANOVA was used with parameters cultivar, time of storage, and treatment (either untreated or CIPC-treated). For GC/MS data in year 3, a principal component analysis (PCA) was performed to observe differences in metabolic composition among the nine potato

cultivars. In addition, Fisher's protected least significant difference (LSD) test was carried out for each cultivar independently in order to determine significant differences between time points in quantification of sugars, ascorbate-glutathione cycle enzymes activities and H_2O_2 as well as MDA content determination.

3.4. Molecular Protocols

3.4.1. RNA extraction

RNA was extracted from lyophilised material as described by Ducreux *et al*. (2008). RNA samples were purified, and genomic DNA contamination was removed using Qiagen RNeasy columns (Qiagen, UK) and DNase I, RNase-free (Thermo Scientific, UK) according to the manufacturer's protocol. RNA quality was tested using an RNA 6000 nano chip on an Agilent 2100 Bioanalyzer (www.chem.agilent.com). RNA samples were aliquoted in 20 μg (1 μg μl-1) batches and stored at -80°C.

3.4.2. Analysis of RNA

3.4.2.1. Quantification of RNA by spectrophotometry

Concentration of RNA was estimated using a NanoDrop® ND-1000 full-spectrum UV/Vis spectrophotometer (NanoDrop®, USA).

3.4.2.2. RNA quality determination by gel electrophoresis

RNA was separated and analysed by electrophoresis on ethidium bromide stained agarose gels.

3.4.3. Enzymatic manipulation of nucleic acids

3.4.3.1. cDNA synthesis

cDNA was synthesised using a TaKaRa CloneTech RNA to cDNA EcoDryTM Premix (Double Primed) beads kit. 5 μg of DNase I treated total RNA in a final volume of 20 μl RNase-free H_2O was added to the lyophilized EcoDryTM Premix. The mixture was then mixed by pipetting and heated at 42°C for 60 minutes prior to deactivating the enzyme at 70°C for 10 minutes.

3.4.3.2. qRT-PCR (Universal Probe Library)

Primers and probe sequences were designed using The Roche Universal Probe Library Assay Design Centre (https://lifescience.roche.com/en_gb/brands/universal-probe-library.html) (Table 3-1). All qRT-PCR reactions were performed using an Applied Biosystems StepOne Plus Real Time PCR system. 50 ng total cDNA was used as template in all reactions, which were composed of 12.5 μl 2x FastStart Universal Probe Master Mix (Rox) (Roche, UK), 10 μM Universal Probe, and 20 μM of both forward and reverse primer before being made up to a final volume of 25 μl with ddH_2O. Thermocycling conditions were as follows, denaturation stage of 95°C for 10 minutes, 40 cycles of 95°C for 15 sec and 60°C extension for 1 min. Samples were run in triplicate, using *elongation factor-1-α* (*EF1α*) as an endogenous control. Calculations of relative expression levels were performed using the Pfaffl method (Pfaffl, 2001).

Table 3-1. Primer/probe sequences used in this study.

Gene	PGSC ID	Forward	Reverse	Probe
StEF1α	PGSC0003DMT400059830	CTTGACGCTCTTGACCAGATT	GAAGACGGAGGGGTTTGTCT	AGCCCAAG
GPT2	PGSC0003DMT400013500	CACAATCGATACCAATCGACA	GAGTCCAATCTTGAGCTTCTGAG	CAGCAGCC

3.4.4. Microarray processing

A custom Agilent microarray was designed to the predicted transcripts from assembly v.3.4 of the DM potato genome as described (Hancock *et al*., 2014). A single-channel replicate block microarray design was utilised. RNA labelling and downstream microarray processing were as recommended in the One-Color Microarray-Based Gene Expression Analysis protocol (v.6.5; Agilent) using the Low Input Quick Amp Labelling Kit (Agilent).

3.4.4.1. Microarray data analysis

Following microarray scanning using an Agilent G2505B scanner, data were extracted from images using Feature Extraction (FE) (v.10.7.3.1) software and aligned with the appropriate array grid template file (033033_D_F_20110315). Intensity data and QC metrics were extracted using the FE protocol (GE1_107_Sep09). Entire FE datasets for each array were loaded into GeneSpring (v.7.3) software and data were normalised using default one-colour Agilent settings: (i) intensity values less than 0.01 were set to 0.01; (ii) data from each array was normalized to the 50th percentile of all measurements on the array and; (iii) the signal from each probe was subsequently normalized to the median of its value across the entire dataset). Quality control of the datasets were performed using Principal Components Analysis (PCA) to confirm that there were no outlying replicate samples and that dye labelling had no associated bias. Spot flags from FE (present or marginal) were used to remove probes with no consistent expression. Data were combined from replicate samples and for both cultivars Arsenal and VR 808 accessions in a new interpretation. Statistical filtering was performed using volcano analysis (P-value < 0.01, fold-change > 2x) for year 1 data. Data were visualised using PageMan (Usadel *et al*., 2006) and a gene tree heatmap in GeneSpring using default Pearson correlation. In addition, one-way ANalysis Of VAriance (ANOVA, parametric test) using storage time as parameter was used in both years to identify statistically significant expression profiles at a false discovery rate (P-value) ≤ 0.05. Strict multiple testing correction (Bonferroni) was applied to ensure low false discovery rates. Filtered gene lists were clustered into two groups using the K-means algorithm with default settings (100 iterations, Pearson correlation) in GeneSpring.

4. RESULTS

4.1. Assessment of sugar accumulation and processing quality during storage

4.1.1. Overall effect of varieties and seasons

A three-way ANOVA for season 1 (2016/2017) was carried out to determine whether there were significant differences in sugar content between samples using cultivar, time of storage and treatment (CIPC-treated or untreated) as factors. A two-way ANOVA was carried out for the rest of the seasons using factors cultivar and time of storage. For the three seasons, Fisher's LSD test was carried out for each cultivar independently in order to determine significant differences between time points.

During the first year of this study, differences between cultivars were observed in glucose ($P < 0.001$), fructose ($P < 0.001$), and sucrose ($P = 0.01$). Moreover, cultivars exhibited significant changes dependent on time for glucose ($P < 0.05$), fructose ($P < 0.005$), and sucrose ($P < 0.001$) content. Arsenal exhibited an initial accumulation of glucose followed by a trough and then a second accumulation after 26 weeks of storage with similar behaviour observed for fructose. On the other hand, VR 808 had much lower levels of reducing sugars which fluctuated a little but with no clear pattern. On the contrary, patterns of sucrose accumulation were similar with both cultivars showing peak-trough-peak behaviour (Figure 4-1).

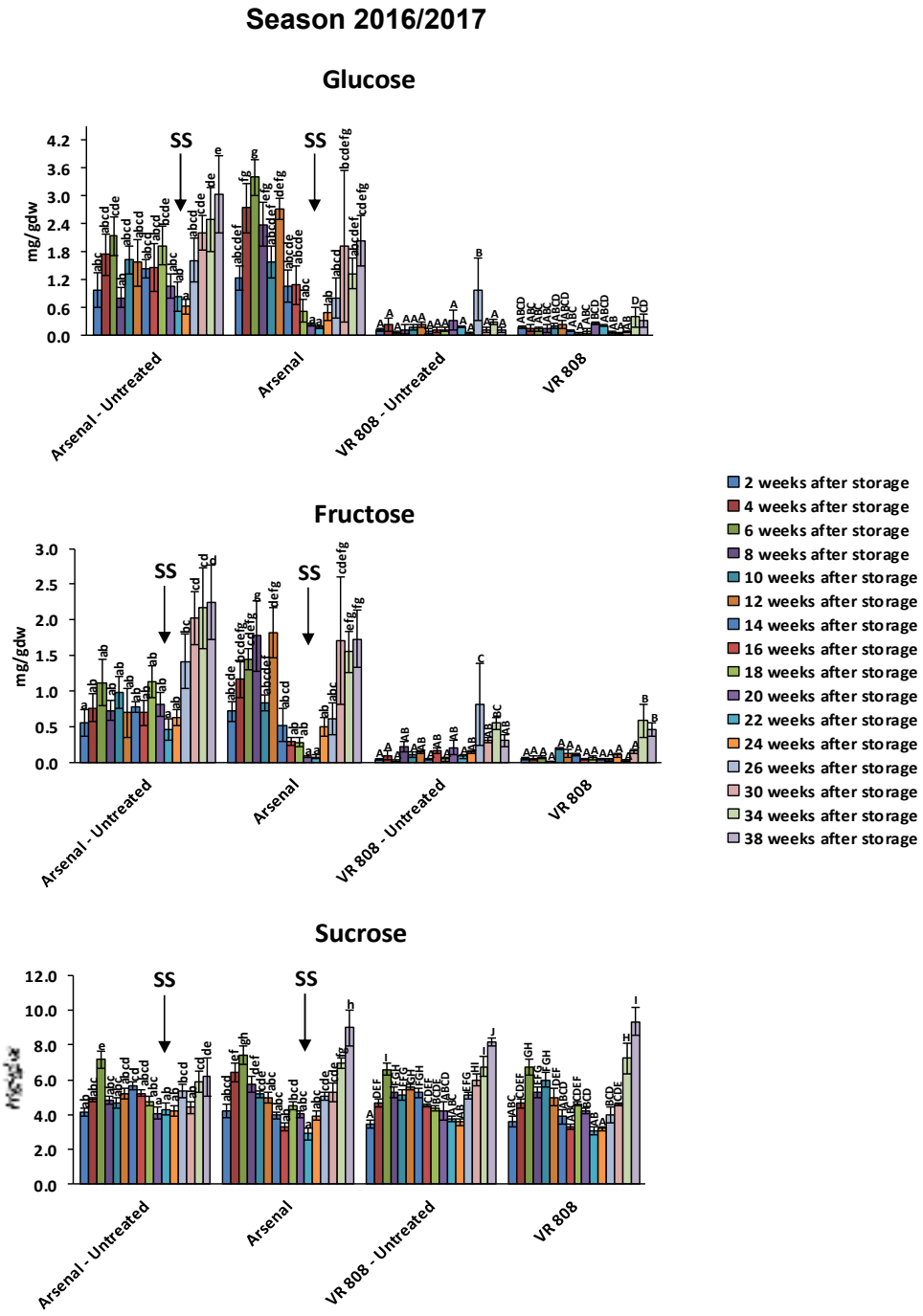

Figure 4-1. Sugar content in potato tubers during the season 1 (2016/2017) of storage at 9°C. Results showed an increase of sugar levels during long-term storage with remarkable differences for glucose and fructose concentration between two cultivars. Values are means ± SE (five biological replicates from one experiment). Mean values with different letters are significantly different according to the Fisher's LSD test. Abbreviations: SS, Onset of senescent sweetening as determined by sustained rise in reducing sugars and darkening of fry colour.

In the second year, cultivars Arsenal and VR 808 exhibited differences in both glucose and fructose content ($P < 0.001$), and sucrose ($P < 0.01$) related to cultivar and time of storage. Arsenal and VR 808 presented similar behaviour compared to the previous year with Arsenal exhibiting storage associated reducing sugar accumulation while both varieties exhibited accumulation of the non-reducing disaccharide sucrose. However, an overall lower content of sugars and a later onset of sweetening (43 weeks after storage) were exhibited during this season. Arsenal showed a small increase of glucose content at 5 weeks after storage followed by a decrease and no changes until the onset of sugar accumulation at 43 weeks after storage,

with the exception of one significant increase at 27 weeks after storage, caused by a single replicate containing high levels of reducing sugars. Similar behaviour was observed for fructose. In contrast, VR 808 exhibited undetectable levels of glucose until 53 weeks after storage, when a little increase was observed. Regarding fructose content, VR 808 showed fluctuations with no clear pattern. Arsenal started accumulating reducing sugars after 43 weeks of storage whereas VR 808 had undetectable levels for glucose and much lower levels of fructose compared to Arsenal, showing no accumulation of reducing sugars. Nonetheless, both cultivars exhibited similar sucrose content and behaviour over time, with an accumulation occurring at 43 weeks of storage (Figure 4-2).

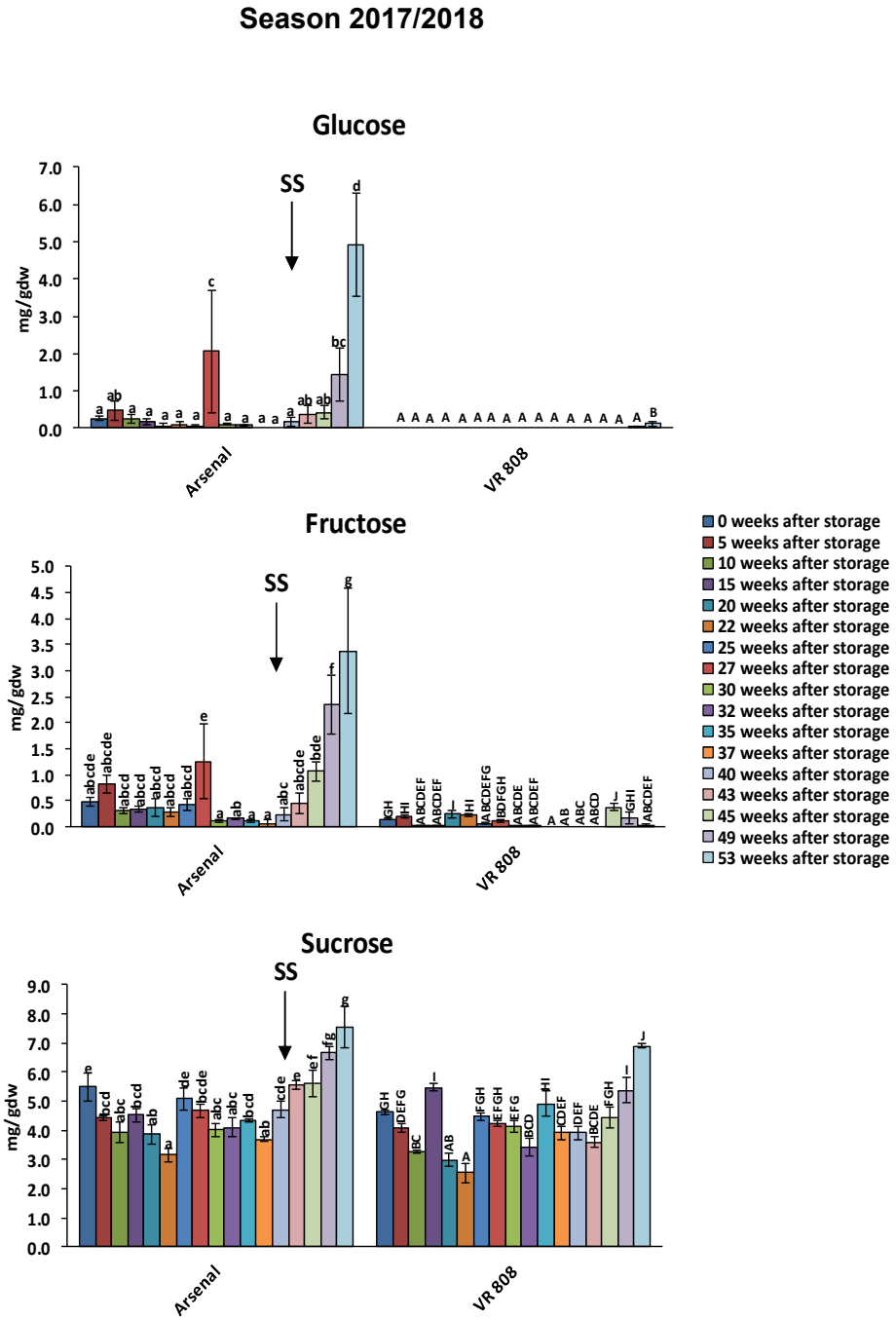

Figure 4-2. Sugar levels in potato tubers stored at 9°C over the season 2 (2017/2018) of study. Cultivars exhibited similar behaviour to the previous season. However, tubers presented a later onset of senescent sweetening. Values are means ± SE (five biological replicates from one experiment). Mean values with different letters are significantly different according to the Fisher's LSD test. Abbreviations: SS, Onset of senescent sweetening as determined by sustained rise in reducing sugars and darkening of fry colour.

The later accumulation and lower content of reducing sugar during storage observed in season 2 (2017/2018) were also observed in all cultivars of season 3 (2018/2019). Glucose, fructose, and sucrose levels presented changes dependent on time ($P < 0.001$). In addition, sugar profiles showed a significant effect of the variety in glucose ($P < 0.05$), fructose ($P < 0.001$) and sucrose ($P < 0.001$) content. All cultivars exhibited a decrease of glucose content at the beginning of the storage, then showed no clear pattern until 43 weeks after storage, when a significant increase was observed with the exception of Brooke. Lady Rosetta, Shelford (Shropshire), and Arsenal were the varieties with the highest levels of glucose. On the contrary, fructose accumulated in all cultivars at 43 weeks after storage, with Lady Rosetta and SH C 909 presenting the highest and lowest content, respectively. Although accumulation of sucrose was no observed in any case, SH C 909 and VR 808 had lower sucrose levels compared to the rest of the cultivars (Figure 4-3).

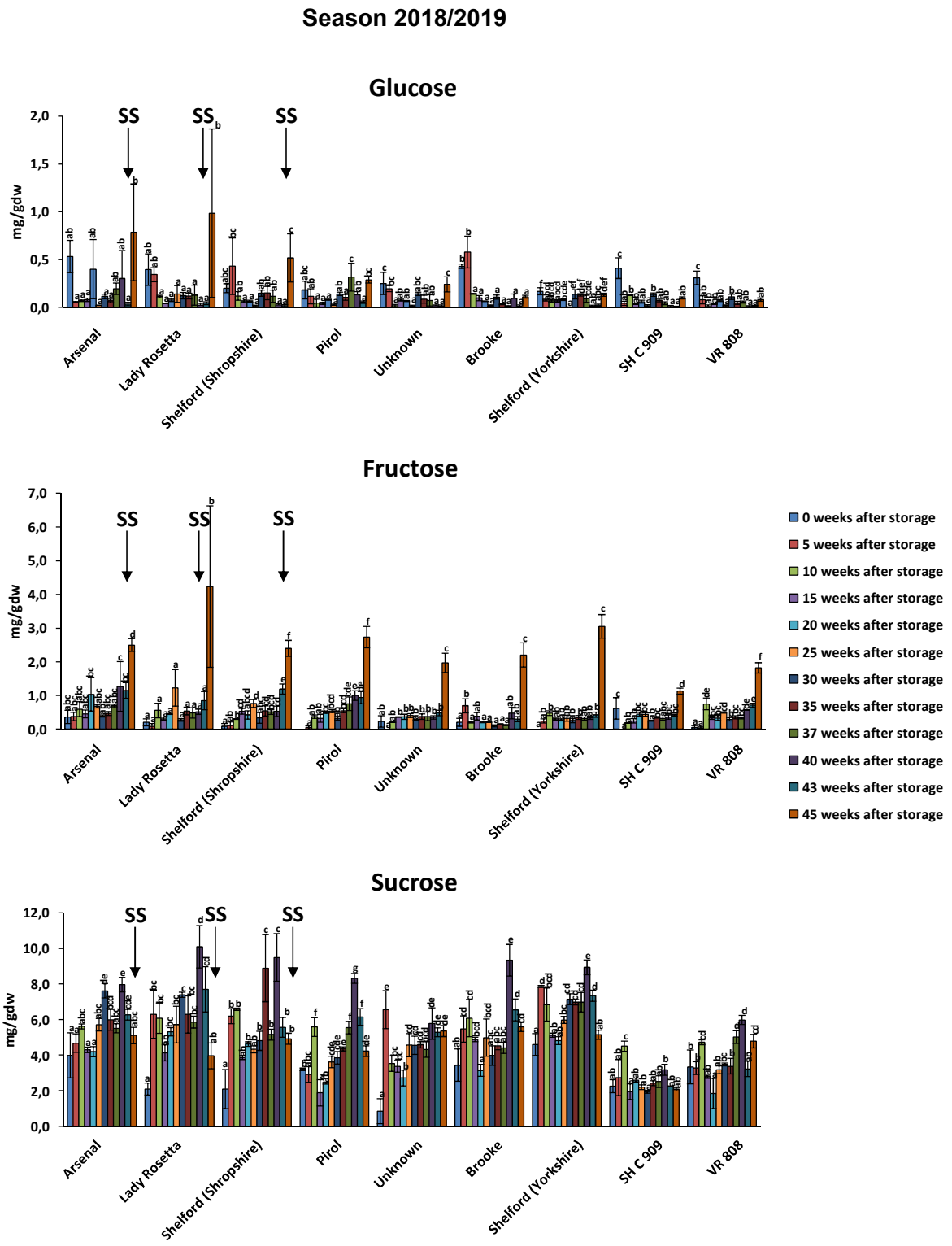

Figure 4-3. Sugar profiles of 9 different varieties during long-term storage for season 3 (2018/2019). Potato tubers showed variability of sugar content depending on the cultivar. Values are means ± SE (three biological replicates from one experiment). Mean values with different letters are significantly different according to the Fisher's LSD test. Abbreviations: SS, Onset of senescent sweetening as determined by sustained rise in reducing sugars and darkening of fry colour.

4.1.2. Effect of CIPC treatment

During year 1, CIPC-treated and untreated cultivars were under study. CIPC treatment had an effect on decreasing glucose ($P < 0.005$), fructose ($P < 0.001$), and sucrose ($P < 0.001$) content in tubers (Figure 4-1). In addition, fructose ($P < 0.05$) content was dependent on CIPC treatment by time interaction. The initial fructose peak in Arsenal was higher in untreated tubers. CIPC-treated tubers had a marginally lower overall sugar content than untreated tubers in which buds were removed possibly due to would-induced catabolic response and/or sink demand in the untreated tubers. Although CIPC had statistically significant effects on sugar content, the overall patterns between CIPC-treated and untreated tubers were very similar. Therefore, it was decided to continue the study using only CIPC-treated cultivars in years 2 and 3.

4.1.3. Effect of growing location

In year 3, Shelford cultivars from two different locations were investigated. No differences ($P > 0.05$) in glucose content were observed between growing location. However, changes dependent on growing location and time were reported for fructose ($P < 0.001$) and sucrose ($P < 0.05$) levels, highlighting the previously reported environmental influence (Kumar *et al*., 2004; Groves *et al*., 2005) on sugar accumulation in stored potato tubers.

4.1.4. Assessment of crisping quality during storage

As sugar content is the key determinant of fry colour and ultimately affects quality of potato crisps, all varieties were subjected to fry quality tests over the 3 years of this study. Crisps from senescent sweetening susceptible tubers presented darker fry colour than those which exhibited more stable sugar profiles during storage. Darkening increased over time following reducing sugars trend. This trend was consistent during the 3 seasons. In addition, untreated cultivars showed a darker fry colour, which was consistent with the sugar data. Quantification of darkening was estimated using ImageJ (Table 4-1) as described in materials and methods. A three-way ANOVA for season 1 (2016/2017) was carried out to determine whether there were significant differences between crisp colour using cultivar, time of storage and treatment (CIPC-treated or untreated) as factors. Fisher's LSD test was carried out for each time point independently in order to determine significant differences between fry colour of crisps from cultivars Arsenal and VR 808. A two-way ANOVA was carried out for the rest of the seasons to determine whether there were significant differences between samples using cultivar and time of storage as factors. Fisher's LSD test was carried out independently for each cultivar (season 2 2017/2018) and time point (season 3 2018/2019) to determine significant differences between means over time and cultivars, respectively.

During season 1 (2016/2017) at 26 weeks after storage (post-sweetening early stage) no differences ($P > 0.05$) in fry colour related to cultivar or treatment (untreated or CIPC-treated) were observed. However, at 38 weeks after storage (post-sweetening late stage) crisps from Arsenal (susceptible to sweetening cultivar) untreated tubers exhibited a significantly darker fry colour related to cultivar ($P = 0.005$) and the interaction between cultivar and treatment ($P < 0.05$). In addition, an increase ($P < 0.05$) in darkening was observed over time depending on the cultivar.

For season 2 (2017/2018) significant changes ($P < 0.001$) in fry colour were reported over time. Differences ($P < 0.005$) in darkening between cultivars were observed. Although crisps from both cultivars exhibited an increase in dark fry colour post-sweetening at 43 weeks after storage, darkening in Arsenal crisps (susceptible to senescent sweetening) was higher than in crisps from VR 808 cultivar (senescent sweetening resistant).

During season 3 (2018/2019), differences ($P < 0.05$) in fry colour between cultivars were found as well as significant changes ($P < 0.001$) over time. Furthermore, each time point was analysed independently in order to find differences between cultivars. At 37 weeks after storage (prior to sweetening), crisps from Shelford (Shropshire) exhibited the highest level of dark fry colour

whereas crisps from Shelford (Yorkshire) showed the lowest. This fact suggested growing location might affect fry colour. At 45 weeks after storage (post-sweetening early stage) no differences ($P > 0.05$) between cultivars were found, probably due to the early stage of sugar accumulation in tubers.

Table 4-1. Quantification of dark fry colour in crisps. Results are presented as percentage of darkening.

Grade of darkening (%) ± SE				
Season 1 (2016/2017)				
Weeks after storage	Arsenal - Untreated	Arsenal	VR 808 - Untreated	VR 808
26	41 ± 2^{a}	36 ± 2^{a}	38 ± 2^{a}	37 ± 2^{a}
38	51 ± 4^{a}	39 ± 3^{b}	35 ± 2^{b}	36 ± 1^{b}
Season 2 (2017/2018)				
Weeks after storage	Arsenal - Untreated	Arsenal	VR 808 - Untreated	VR 808
1	-	31 ± 1^{de}	-	31 ± 1^{cd}
10	-	31 ± 1^{de}	-	30 ± 1^{d}
20	-	33 ± 1^{cd}	-	34 ± 1^{bc}
25	-	27 ± 1^{e}	-	28 ± 1^{d}
30	-	28 ± 1^{e}	-	31 ± 1^{cd}
40	-	31 ± 2^{de}	-	31 ± 2^{cd}
43	-	37 ± 2^{bc}	-	37 ± 1^{b}
49	-	40 ± 2^{b}	-	32 ± 3^{cd}
53	-	57 ± 4^{a}	-	43 ± 2^{a}
Season 3 (2018/2019)				
	Weeks after storage			
	30	37	40	45
Pirol	39 ± 1^{ab}	40 ± 2^{abc}	41 ± 5^{a}	44 ± 8^{a}
SH C 909	39 ± 1^{ab}	42 ± 3^{abc}	40 ± 4^{a}	44 ± 3^{a}
VR 808	38 ± 1^{ab}	37 ± 1^{abc}	42 ± 3^{a}	47 ± 1^{a}
Lady Rosetta	34 ± 3^{b}	39 ± 2^{abc}	39 ± 4^{a}	48 ± 4^{a}
Shelford (Shropshire)	37 ± 3^{ab}	45 ± 3^{a}	44 ± 2^{a}	50 ± 5^{a}
Unknown	45 ± 4^{a}	44 ± 3^{ab}	49 ± 3^{a}	53 ± 2^{a}
Brooke	40 ± 4^{ab}	36 ± 4^{abc}	38 ± 6^{a}	45 ± 4^{a}
Arsenal	39 ± 4^{ab}	35 ± 4^{bc}	41 ± 4^{a}	50 ± 4^{a}
Shelford (Yorkshire)	39 ± 2^{ab}	33 ± 3^{c}	38 ± 4^{a}	46 ± 3^{a}

Mean values with different letters are significantly different according to the Fisher's LSD test. Comparisons: Season 1, cultivars at individual time points; Season 2, time points in individual cultivars; Season 3, cultivars at individual time points.

4.2. Investigation of oxidative stress during long-term storage

4.2.1 Determination of H_2O_2 during long-term storage

In order to determine if an increase of oxidative stress was related to the accumulation of sugars in stored potato tubers over time, H_2O_2 was detected using the Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit. Content of H_2O_2 was measured in tuber samples during storage over the first year. Although H_2O_2 levels showed fluctuations over time, no significant differences were found between time points as estimated using Fishers LSD test (Figure 4-4).

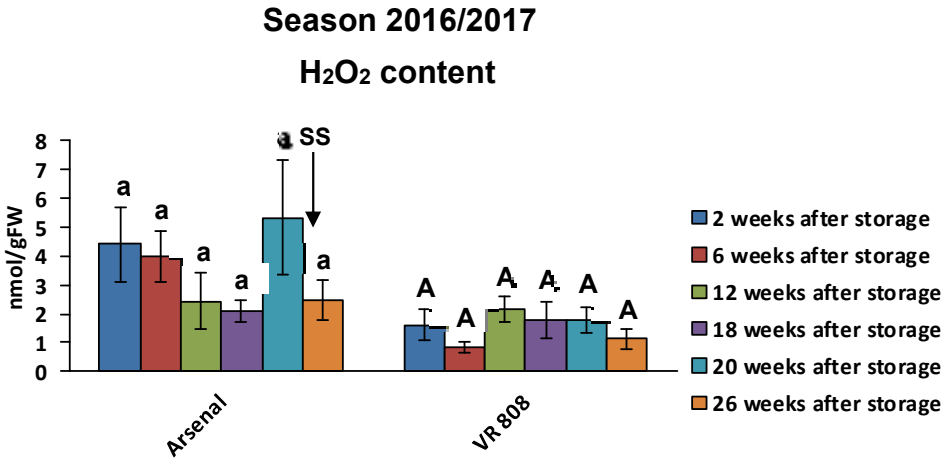

Figure 4-4. H_2O_2 content in sweetened and non-sweetened potato tubers during long-term storage as a marker of oxidative stress. Although differences in H_2O_2 content between cultivars were observed, no significant changes related to reducing sugars increase on onset of senescent sweetening were reported. Arrows indicate the onset of senescent sweetening (SS). Each value is the mean ± SE of measurements from five separate tubers. Mean values with different letters are significantly different according to the Fisher's LSD test, carried out independently for each cultivar and treatment. Differences within Arsenal and VR 808 cultivars are represented by lower- and upper-case letters, respectively.

4.2.2 Quantification of specific activity of ascorbate-glutathione cycle enzymes

Increase in levels of H_2O_2 were not observed in tubers during the storage. However, variations over time were reported. This suggests there may be temporary changes that could be mitigated by antioxidant systems. Therefore, the four enzymes of the ascorbate-glutathione cycle, considered to be the main antioxidant system in plants (Hancock, 2017), were quantified. Results showed fluctuations in the specific activity during storage. Arsenal (susceptible to SS) and VR 808 (SS resistant) showed similar behaviours of antioxidant systems over time. Measurements of specific activities are presented in Figure 4-5.

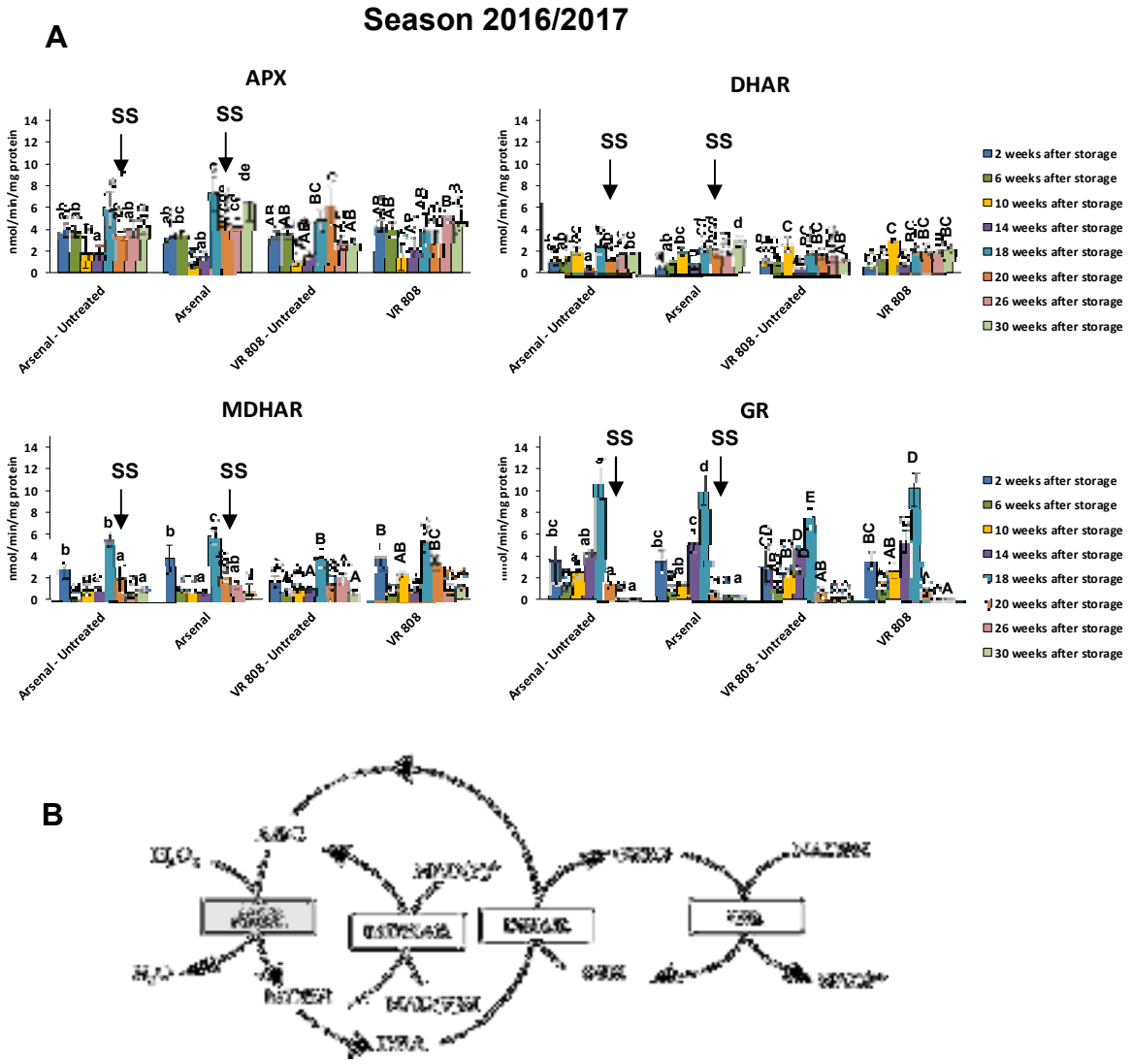

Figure 4-5. Impact of long-term storage on antioxidant systems in potato tubers. **A.** Specific activities of antioxidant enzymes in tubers. Arrows indicate the onset of senescent sweetening (SS). Each value is the mean ± SE of measurements from five separate tubers. Mean values with different letters are significantly different according to the Fisher's LSD test, carried out independently for each cultivar and treatment. Differences within Arsenal and VR 808 cultivars are represented by lower- and upper-case letters, respectively. **B.** Ascorbate-glutathione cycle pathway. Taken from Locato *et al*. (2013). An important role in the plant antioxidant defence mechanism has been attributed to this pathway. First, the hydrogen peroxide (H_2O_2) generated by oxidative stress is scavenged via the oxidation of ascorbate (ASC) by ascorbate peroxidase (APX). This enzyme is involved in the oxidation of ASC to monodehydroascorbate (MDHA), which can be converted back to ASC via monodehydroascorbate reductase (MDHAR). MDHA that escapes this recycling is converted rapidly to dehydroascorbate (DHA) which is converted back to ASC by the action of dehydroascorbate reductase (DHAR). DHAR utilizes glutathione (GSH), which is regenerated by glutathione reductase (GR) from its oxidized form, glutathione disulphide (GSSG).

4.2.3 Determination of lipid peroxidation by measurement of MDA levels

We observed no changes in average cellular levels of H_2O_2 over time relating to the onset of senescent sweetening. However, it is possible that oxidative damage may have occurred due to localised concentrations of this ROS in specific organelles or due to sudden spikes that were subsequently controlled. Hence, MDA was quantified to determine whether oxidative damage was occurring at cellular level.

MDA content in stored tubers were quantified as TBARS as an indicator of lipid peroxidation and overall biomarker of oxidative stress (Fletcher *et al*., 1973; Konze & Elstner, 1978; Dhindsa *et al*., 1981). Fisher's LSD test was carried out for each cultivar and treatment independently in order to determine significant differences between means over time. Levels of MDA in tubers were influenced by cultivar and time of storage ($P < 0.001$). We observed an increase during the beginning of the storage, which is potentially related to oxidative stress occurring due to harvest and manipulation of tubers. For this early accumulation of MDA, Arsenal showed higher levels ($P < 0.001$) in CIPC-treated tubers compared with the untreated tubers (Figure 4-6). Untreated and CIPC-treated tubers from Arsenal (susceptible to SS), and CIPC-treated VR 808 (SS resistant) tubers exhibited no increase of MDA levels related to the accumulation of sugars. MDA content in untreated VR 808 tubers showed a small increase around the time that senescent sweetening was first observed in Arsenal tubers.

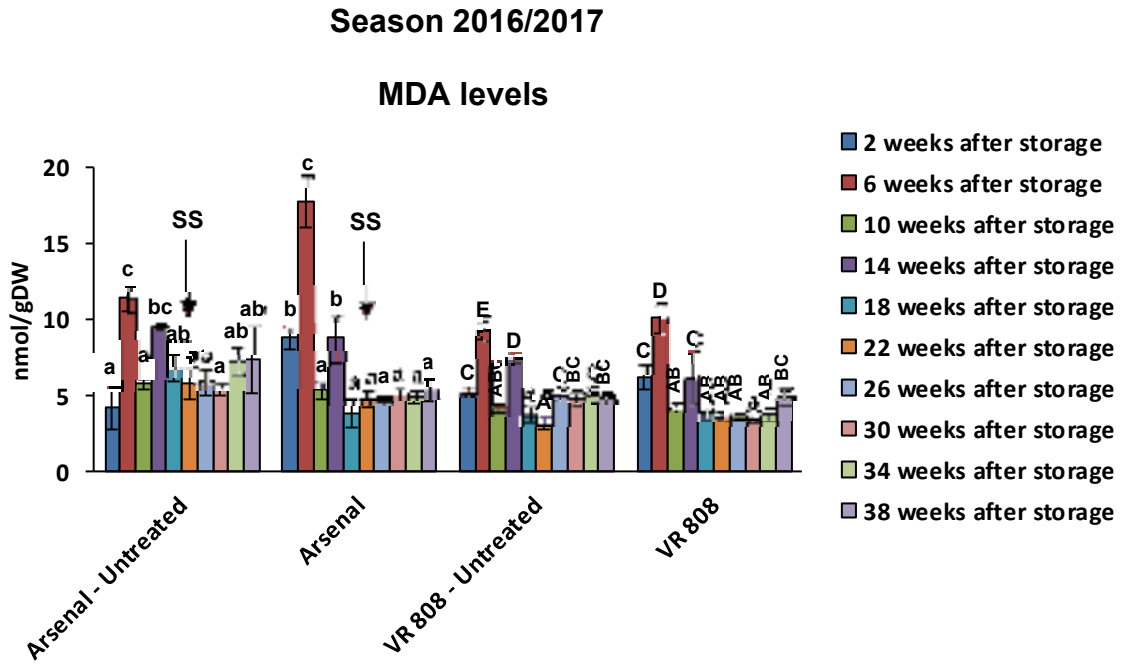

Figure 4-6. Quantification of malondialdehyde (MDA) content during long-term storage as an indirect measurement of lipid peroxidation and marker of oxidative damage. Similar behaviour between all cultivars and conditions were observed. Tubers presented no increase of MDA related to sugar accumulation suggesting senescent sweetening may not be linked to oxidative stress. Onset of senescent sweetening (SS) is indicated by arrows. Each value is the mean ± SE of measurements from five separate tubers. Mean values with different letters are significantly different according to the Fisher's LSD test, carried out independently for each cultivar and treatment. Differences within Arsenal and VR 808 cultivars are represented by lower- and upper-case letters, respectively.

4.3. Metabolome profiles of potato tubers during long-term storage

4.3.1 Metabolome profiling during long-term storage in season 1 (2016/2017)

The metabolite profiles of tubers from Arsenal (sweetening susceptible) and VR 808 (sweetening resistant) were compared in untreated and CIPC-treated tubers during long-term storage, at 2, 4, 6, 12, 20, and 26 weeks of storage (Figure 4-7). Five biological replicates were used for each cultivar and time point. Polar extracts (mainly sugars, organic acids, and amino acids) and non-polar extracts (mainly fatty acids and fatty alcohols) were examined. In the tubers analysed in this study, a total of 123 metabolites were detectable (72 polar and 51 non-polar), of which 76% could be identified.

Metabolites, whose abundance was changed in a statistically significant manner over time, were identified using three-way ANOVA based on cultivar, treatment (CIPC-treated or untreated), and time of storage. Arsenal and VR 808 exhibited differences ($P < 0.05$) in 83 metabolites during the storage period. The levels of 11 metabolites were significantly different ($P < 0.05$) between CIPC-treated and untreated tubers. A total of 123 metabolites were significantly ($P < 0.05$) influenced by the time of storage. Arsenal tubers exhibited a higher concentration of glucose and fructose than VR 808 tubers. In addition, CIPC-treated tubers from both cultivars had a lower concentration of reducing sugars during the onset of the senescent sweetening (at 20, and 26 weeks of storage). Sugar phosphates showed no clear pattern. Amino acids generally showed an initial high peak followed by a rapid decrease and then gradual increase until the end of the storage. Arsenal and VR 808 showed initial higher levels of valine, and urea, respectively. Moreover, a higher concentration of aspartic acid in VR 808 was observed over time. Glycerol and caffeic acid showed a declining concentration in both cultivars. On the contrary, both Arsenal and VR 808 exhibited an increase in galactinol and chlorogenic acid during storage. Arsenal had higher levels of quinic acid than VR 808 during the storage. VR 808 showed higher concentrations of galactaric acid and galactosyl glycerol than Arsenal.

Both cultivars showed a general decrease in saturated and unsaturated fatty acids as well as fatty alcohols during the onset of senescent sweetening (at 20, and 26 weeks of storage). Additionally, Arsenal exhibited higher levels of heneicosanol whereas VR 808 presented higher solanid-5-enol at the onset of sweetening.

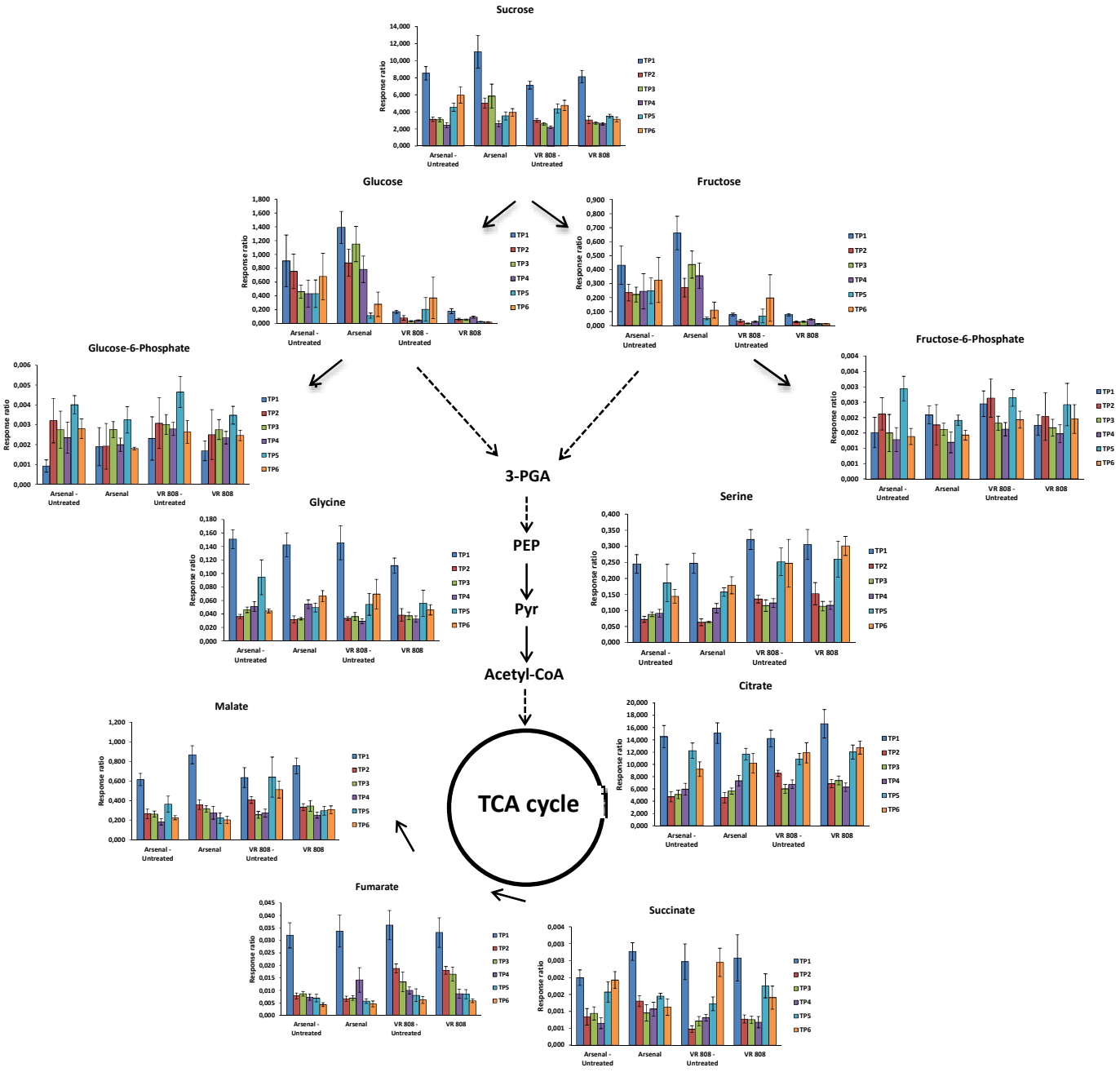

Figure 4-7. Influence of time of storage and CIPC-treatment on selected metabolites in potato tubers during season 1 (2016/2017). TP5 and TP6 represent senescent sweetening transition. Values are means ± SE (five biological replicates from one experiment). Arrows indicate metabolic relationships where dashed arrows indicate multiple metabolic steps. Abbreviations: 3-PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate; TCA, tricarboxylic acid; TP1, 2 weeks of storage; TP2, 4 weeks of storage; TP3, 6 weeks of storage; TP4, 12 weeks of storage; TP5, 20 weeks of storage; and TP6, 26 weeks of storage.

4.3.2. Metabolome profiling during long-term storage in season 3 (2018/2019)

Phytochemical diversity was examined by GC/MS in tubers of cultivars Arsenal, VR 808, Pirol, SH C 909, Lady Rosetta, Brooke, Shelford (both Shropshire and Yorkshire locations) as well as the unknown cultivar at 5, 30, 37, and 43 weeks of storage. Three different biological replicates were used for each cultivar and time point. Polar extracts (mainly sugars, organic acids, and amino acids) and non-polar extracts (mainly fatty acids and fatty alcohols) were examined. In the tubers analysed in this study, a total of 124 metabolites were detectable (74 polar and 50 non-polar), of which 77% could be identified. Metabolites, whose abundance was changed in a statistically significant manner over time, were identified using two-way ANOVA based on cultivar and time of storage. Cultivars showed differences ($P < 0.05$) in 94 metabolites during the storage period. In addition, a total of 106 metabolites were significantly ($P < 0.05$) influenced by the time of storage.

PCA was used to summarise broad-scale variation among the cultivars and time points using all the metabolites simultaneously, and both polar and non-polar compounds independently..

For all data, the first and third Principal Components (PC1 and PC3) together were able to distinguish between all time points analysed (Figure 4-8). The loadings for PC1 are dominated by amino acids, more specifically valine, leucine, and isoleucine. Analysis of the loadings for PC3 showed the separation of time points is driven by the levels of α-glycerophosphate, fucosterol, and galactose.

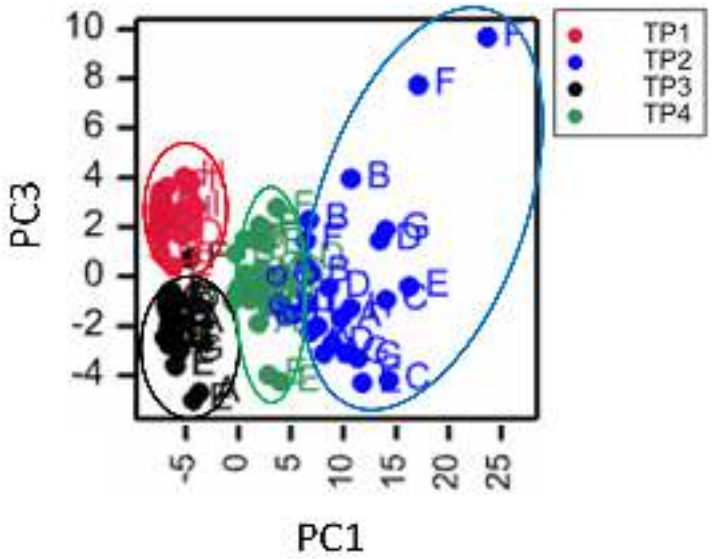

Figure 4-8. Selected score plot from PCA of all metabolites identified by GC/MS (polar/non-polar fraction) during long-term storage, with samples labelled according to time of storage. Plot of PC1 against PC3.Abbreviations: A, Pirol B, SH C 909; C, VR 808; D, Lady Rosetta; E, Shelford (Shropshire location); F, unknown cultivar; G, Brook; H, Arsenal; and I, Shelford (Yorkshire location). TP1, 5; TP2, 30; TP3, 37; and TP4, 43 weeks of storage.

For the polar compounds the first and fourth components separated all four time points into distinct groups (Figure 4-9A); 5, 30, 37, and 43 weeks of storage were separated. The separation of time points for PC1 was driven by glutamine, galactosyl glycerol, glycine, and β-alanine responses. The loading for PC4 are dominated by glutamine, α-glycerophosphate, galactosyl glycerol, galactose, and glycerol. In addition, the sixth and eighth separated the cultivars into two different groups (Figure 4-9B): cultivars from Shropshire location and cultivar from Yorkshire location. Analysis of the loadings showed the separation of cultivars is driven by galactose, α-glycerophosphate, and galactaric acid (for PC6), and galactose, and tyrosine (for PC8).

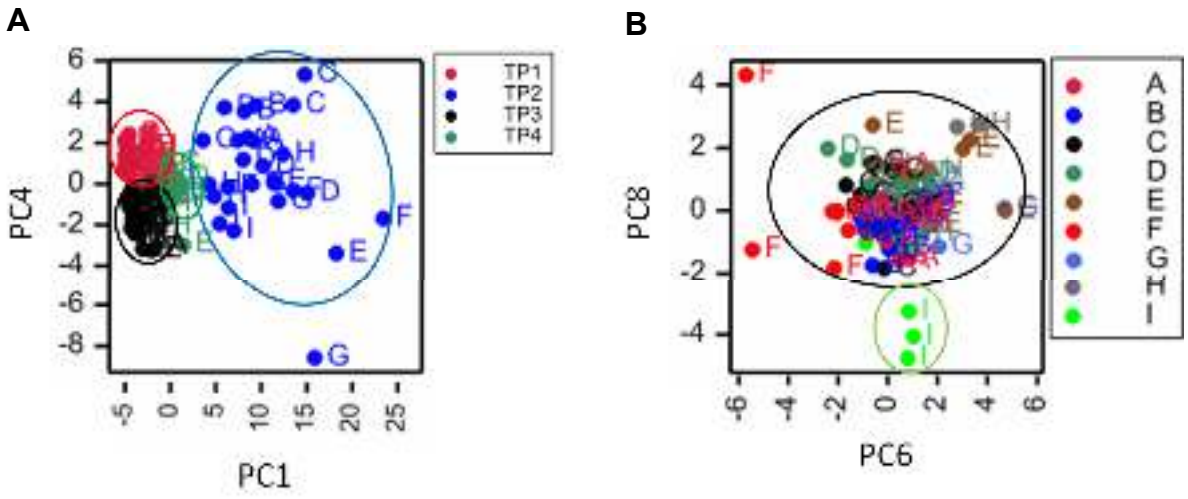

Figure 4-9. Selected score plots from PCA of polar metabolites identified by GC/MS, with samples labelled according to time of storage and cultivar. **A.** Plot of PC1 against PC4. **B.** Plot of PC6 against PC8. Abbreviations: TP1, 5; TP2, 30; TP3, 37; and TP4, 43 weeks of storage. A, Pirol B, SH C 909; C, VR 808; D, Lady Rosetta; E, Shelford (Shropshire location); F, unknown cultivar; G, Brook; H, Arsenal; and I, Shelford (Yorkshire location).

PCA of the non-polar data separate cultivars by location on PC3 vs. PC4 (Figure 4-10A) and time points on PC1 vs. PC2 (Figure 4-10B). The separation for PC3 is driven by fucosterol, and δ-5-avenasterol as well as unknown compounds. The compounds driving the separation by cultivar location for PC4 are fatty alcohols (heneicosanol, tetracosanol, and docosanol). The first and second components separate the time points into three distinct groups: 5 and 37 weeks of storage are separated from 30, and 43 weeks of storage. The loadings for PC1 are dominated by saturated fatty acids, mainly eicosanoic, hexadecanoic, and heptadecanoic acids. The separation for PC2 is driven by unknown compounds.

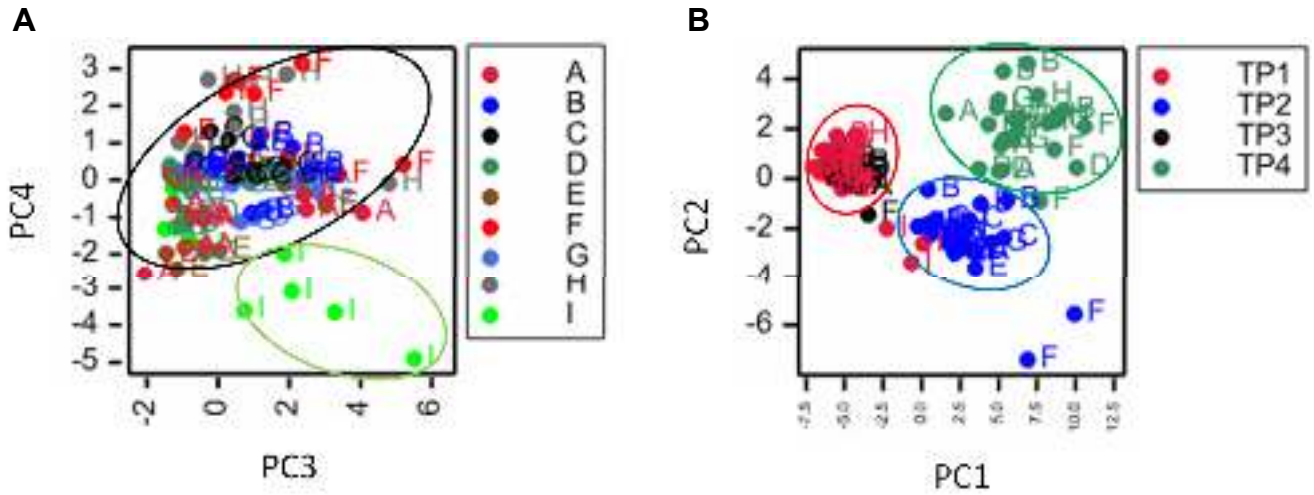

Figure 4-10. Selected score plots from PCA of non-polar metabolites identified by GC/MS, with samples labelled according to time of storage and cultivar. **A.** Plot of PC3 against PC4. **B.** Plot of PC1 against PC2. Abbreviations: A, Pirol B, SH C 909; C, VR 808; D, Lady Rosetta; E, Shelford (Shropshire location); F, unknown cultivar; G, Brook; H, Arsenal; and I, Shelford (Yorkshire location). TP1, 5; TP2, 30; TP3, 37; and TP4, 43 weeks of storage.

4.3.4. The influence of time of storage on metabolome profile

PCA were performed for each time point independently. Separation of cultivars was observed at 5 and 43 weeks of storage. PCA of 5 weeks after storage separate cultivars by location on PC1 vs. PC5 (Figure 4-11). Analysis of the loadings for PC1 showed the separation is driven by glutamine, galactosyl glycerol, glycine, and β-alanine. For PC5, the separation is driven by unknown compounds as well as pentadecanoic acid. Moreover, PCA of 43 weeks after storage separate cultivars by sugar profile (sweetening resistance or susceptibility) on PC1 vs PC3, and PC2 vs. PC3 (Figure 4-12A); and PC3 vs PC4, and PC3 vs PC5 (Figure 4-12B). The separation for PC1 is driven by amino acids: methionine, lysine, serine, histidine, isoleucine, tyrosine, tryptophan, glycine, valine, threonine, asparagine, and leucine as well as unknown compounds. The loadings for PC2 are dominated by fumaric and malic acids, hexacosanol, tetracosanol, phosphate, threonic acid, ethanolamine, and valine. Analysis of the loadings for PC3 showed the separation is driven by inositol, sucrose, proline, fructose, glucose, phenylalanine, putrescine, and quinic acid as well as noctadecanoic and tetracosanoic acids, octacosanol, and β-sitosterol. The separation for PC4 is driven by unknown compounds, piperidinecarboxylic acid, cinnamic acid, hexadecanoic acid, and asparagine. The loadings for PC5 are dominated by unknown compounds, octacosanol, phenylalanine, galactosyl glycerol, β-sitosterol, octacosanoic acid, and γ-aminobutyric acid.

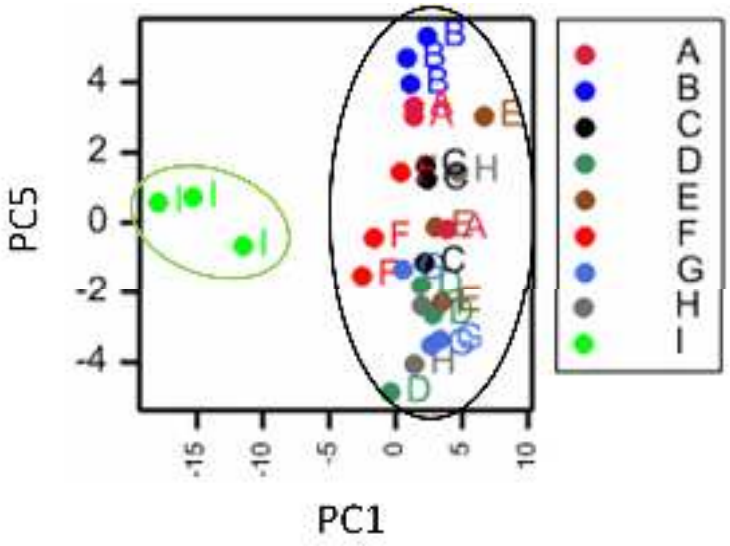

Figure 4-11. Selected score plot from PCA of all metabolites identified by GC/MS (polar/non-polar fraction) at 5 weeks of storage with samples labelled according to cultivar. Plot of PC1 against PC5. Abbreviations: A, Pirol B, SH C 909; C, VR 808; D, Lady Rosetta; E, Shelford (Shropshire location); F, unknown cultivar; G, Brook; H, Arsenal; and I, Shelford (Yorkshire location).

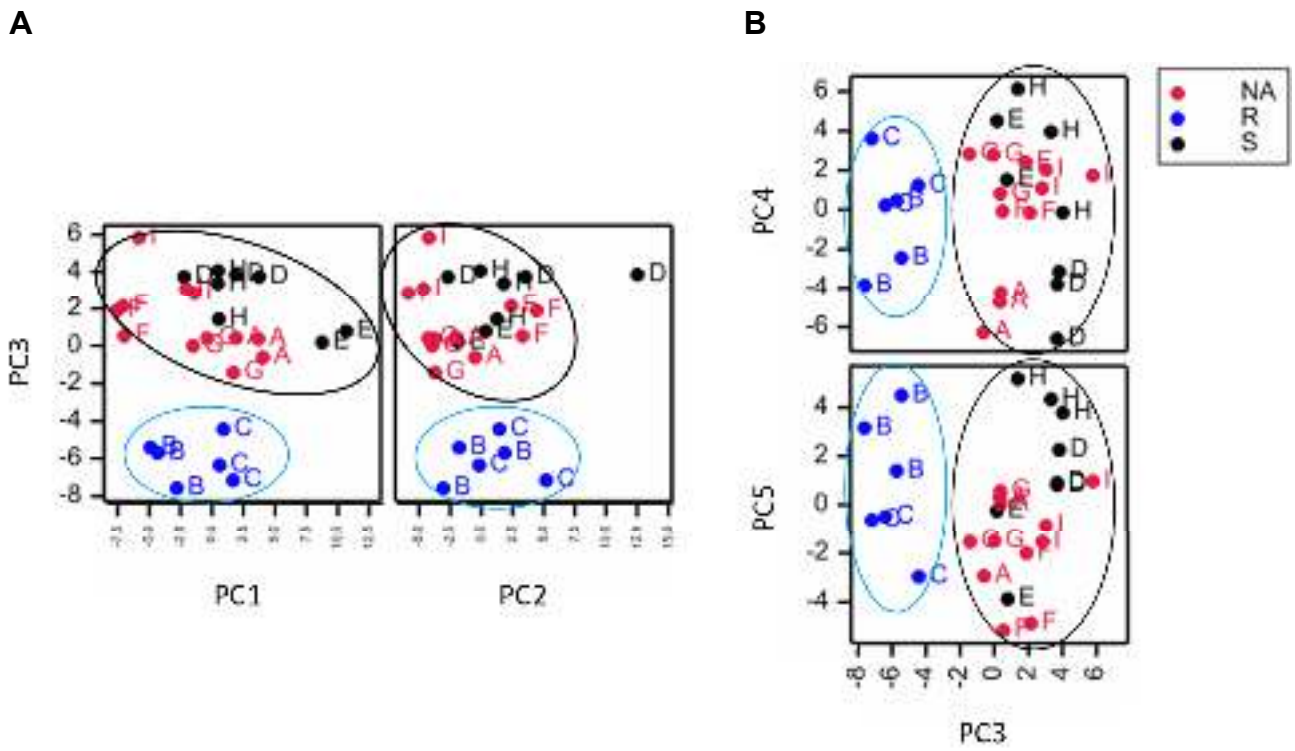

Figure 4-12. Selected score plots from PCA of all metabolites identified by GC/MS (polar/non-polar fraction) at 43 weeks of storage with samples labelled according to senescent sweetening susceptibility. **A.** Plot of PC1 against PC3; Plot of PC2 against PC3. **B.** Plots of PC3 against PC5; Plot of PC3 against PC4. Abbreviations: A, Pirol B, SH C 909; C, VR 808; D, Lady Rosetta; E, Shelford (Shropshire location); F, unknown cultivar; G, Brook; H, Arsenal; and I, Shelford (Yorkshire location). R, resistant to senescent sweetening; S, susceptible to senescent sweetening; and NA (not applicable), cultivars which showed an intermediate sugar content between R and S cultivars.

4.3.5. The influence of growing location on metabolome profile

Tubers of the Shelford variety grown either in Shropshire or Yorkshire are present in this study. In order to identify differences in propensity for sweetening, tuber metabolome profiles from both locations were compared by PCA. The first and fourth components separate the four time points into four different groups (Figure 4-13). The separation for PC1 is driven mainly by amino acids: β-alanine, valine, glycine, leucine, serine, isoleucine, ethanolamine, and aspartic acid. The compounds driving the separation for PC4 are nonacosanol, δ-5-avenasterol, solanid-5-enol, stigmastadienol, fucosterol, mannitol, α-linolenic acid, tetracosanoic, and octadecenoic acids. This result suggests that genotype and storage are more significant than the growing environment in determining the potato tuber metabolome.

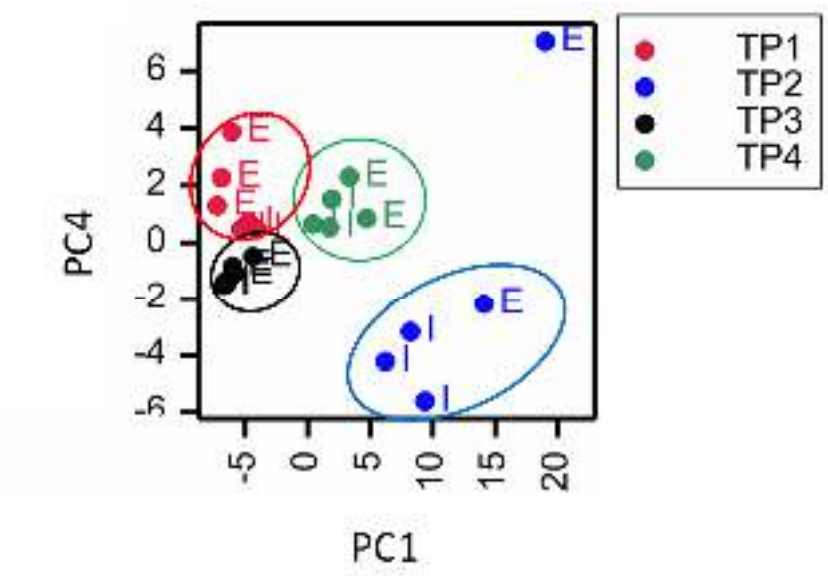

Figure 4-13. Selected score plot from PCA of all metabolites identified by GC/MS (polar/non-polar fraction), with samples labelled according to time of storage and cultivar. Plot of PC1 against PC4. Abbreviations: E, Shelford (Shropshire location); and I, Shelford (Yorkshire location). TP1, 5; TP2, 30; TP3, 37; and TP4, 43 weeks of storage.

Although within each time point group a minor separation of both cultivars is observed, PCA of each individual time point shows no separation based on location.

4.4. Changes in transcript levels associated with long-term storage in potato tubers

4.4.1. Microarray analysis of gene expression

Senescent sweetening (SS) is genotype dependent, but the genetic basis remains uncertain. In order to gain further insights into the genes associated with the accumulation of sugars in potato tubers during long-term storage observed in this study, microarray experiments were designed to identify genes that were differentially expressed during the SS transition for years 1 and 2. One-way ANOVA using storage time as parameter was used in both years to identify statistically significant expression profiles at a false discovery rate (P-value) ≤ 0.05. Data were visualised using PageMan (Usadel *et al*., 2006) and a gene tree heat map in GeneSpring using default Pearson correlation. MapMan is a software tool that supports the visualization of profiling data sets in the context of gene ontologies and gene-by-gene basis on schematic diagrams of biological processes (Usadel *et al*., 2009). The PageMan module uses the same ontologies to statistically evaluate responses at the pathway or processes level.

4.4.2. Gene expression and biological processes influenced by long-term storage during season 1 (2016/2017)

In order to identify genes that may be related to the accumulation of sugars during long-term storage, two time points corresponding to the senescent sweetening transition were selected. The ratio of gene expression at 26 weeks relative to 20 weeks of storage were under study to identify genes that were significantly up- or down-regulated following senescent sweetening. During SS transition, between 20 and 26 weeks after storage, genes associated with several processes were altered in Arsenal and VR 808 tubers (Figure 4-14). Significant changes in expression were observed for a total number of 329 genes in Arsenal and 224 genes in VR 808. Arsenal showed up-regulation in genes associated with photosynthesis. Major carbohydrate metabolism and DOF transcription factors were down-regulated in Arsenal. On the other hand, over-expression of transcripts associated with secondary metabolism was observed in VR 808. Moreover, both cultivars exhibited an up-regulation in genes related to hormone metabolism and ethylene synthesis-degradation.

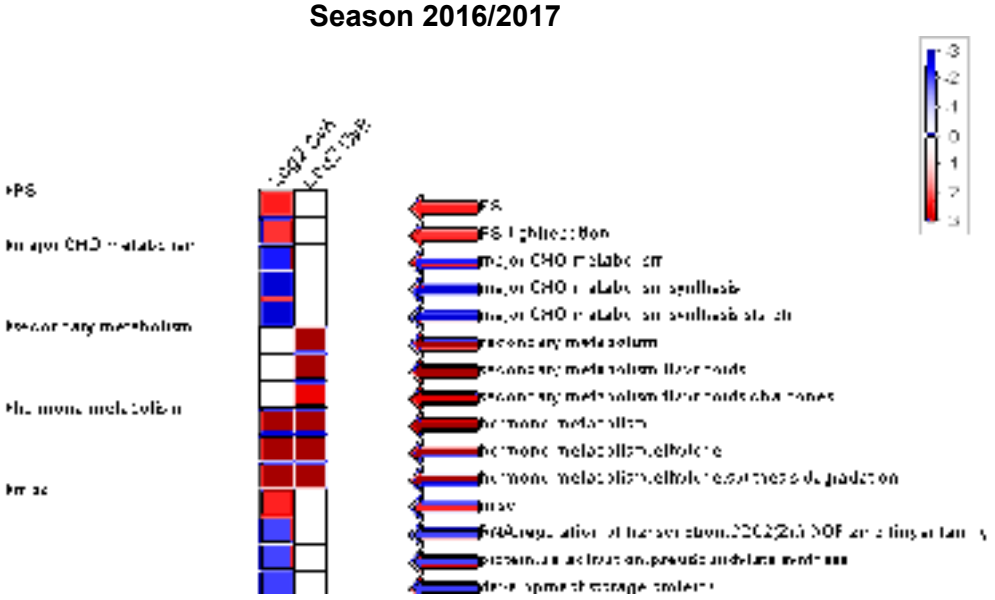

Figure 4-14. PageMan diagram representing changes in potato tubers during senescent sweetening transition for season 1 (2016/2017). Wilcoxon rank sum test (Benjamini-Hochberg corrected) was employed to identify BINs whose contents were differentially regulated. Each coloured block represents an individual BIN/sub-BIN or gene in the PageMan analysis. The colour represents the direction and strength of the regulation (Logarithm to the base 2 colour scale; Red, highly up-regulated and Blue, highly down-regulated). Abbreviations: CvA, Arsenal; and CvB, VR 808.

VR 808 exhibited up-regulation in transcripts associated with flavonoid synthesis. Chalcone synthase (CHS, EC 2.3.1.74) and flavanone 3-hydroxylase (F3H, EC 1.14.11.9) genes were over-expressed. CHS is a key enzyme of the flavonoid/isoflavonoid biosynthesis pathway (Dao *et al*., 2011). F3H plays important roles in flavonoid biosynthesis (Owens *et al*., 2008; Flachowsky *et al*., 2012).

Major carbohydrate metabolism genes down-regulated in Arsenal during the onset of SS were associated with starch synthesis, including granule-bound starch synthase 1 (EC 2.4.1.242), 1,4-α-glucan branching enzyme (EC 2.4.1.18), APL3 and ADG1. APL3 and ADG1 encode the large and the small subunits of AGPase 1, respectively. The large subunit catalyses the first, rate limiting step in starch biosynthesis whereas the small subunit is the catalytic isoform responsible for AGPase activity. The presence of the small subunit is required for large subunit stability. Therefore, these changes observed in major carbohydrate metabolism are a potential reason for the sugar accumulation reported in Arsenal.

4.4.2.1. Effect of long-term storage on carbohydrate metabolism during season 1 (2016/2017)

As significant changes were found in transcripts associated with carbohydrate metabolism during senescent sweetening transition for season 1 (2016/2017), different carbohydrate metabolism pathways were investigated. Changes associated with carbohydrate metabolism might underpin the transition to sweetening. Therefore, genes associated with sucrose-starch metabolism (involved in sugar recycling) and glycolytic pathways (related to turnover of sugars) were examined. A comparison of significant changes observed for Arsenal and VR 808 in sucrose-starch metabolism and glycolytic pathways is presented in Figure 4-15, and 4-16, respectively. In terms of sucrose-starch metabolism, Arsenal showed a strong down-regulation in AGPase (large subunit), and 1,4-α-glucan branching enzyme. Moreover, both cultivars exhibited down-regulation in α-amylase 2 (EC 3.2.1.1) as well as up-regulation in vacInv. Regarding the glycolytic pathways, similar behaviour for Arsenal and VR 808 were observed. However, Arsenal, which has a senescent sweetening susceptible profile, showed a strong down-regulation in GPT2. GPT2 is involved in the transport of glucose-6-phosphate into the plastids. The down-regulation observed in Arsenal could implicate that sugar phosphates are restricted in terms of plastid import where they are used for starch synthesis. In addition, trehalose and T6P synthesis processes were down-regulated in Arsenal while up-regulated in VR 808. T6P is a signalling metabolite that regulates carbon metabolism, developmental processes, and growth in plants.

4.4.2.2. Effect of long-term storage on SnRK genes during season 1 (2016/2017)

In addition to the differences previously observed between Arsenal (susceptible to sweetening) and VR 808 (sweetening resistant) cultivars, changes in sucrose non-fermenting-related protein kinase (SnRK) genes were observed (Figure 4-17). During the SS transition, SnRK3.15 was down-regulated while SnRK 2.6 was up-regulated. Both cultivars presented similar behaviour in SnRK genes expression. However, VR 808 had a stronger response within changes. Many studies have demonstrated that SnRK genes play various roles in the metabolism and development of plants (Halford & Hardie, 1998; Johnson *et al*., 2002; Mustilli *et al*., 2002; Boudsocq *et al*., 2004; Umezawa *et al*, 2004; Fujii & Zhu, 2009; Nakashima *et al*., 2009; Sun *et al*., 2010; Zheng *et al*., 2010; Fujii *et al*., 2011; Ghillebert *et al*., 2011).

Season 2016/2017

Arsenal

VR 808

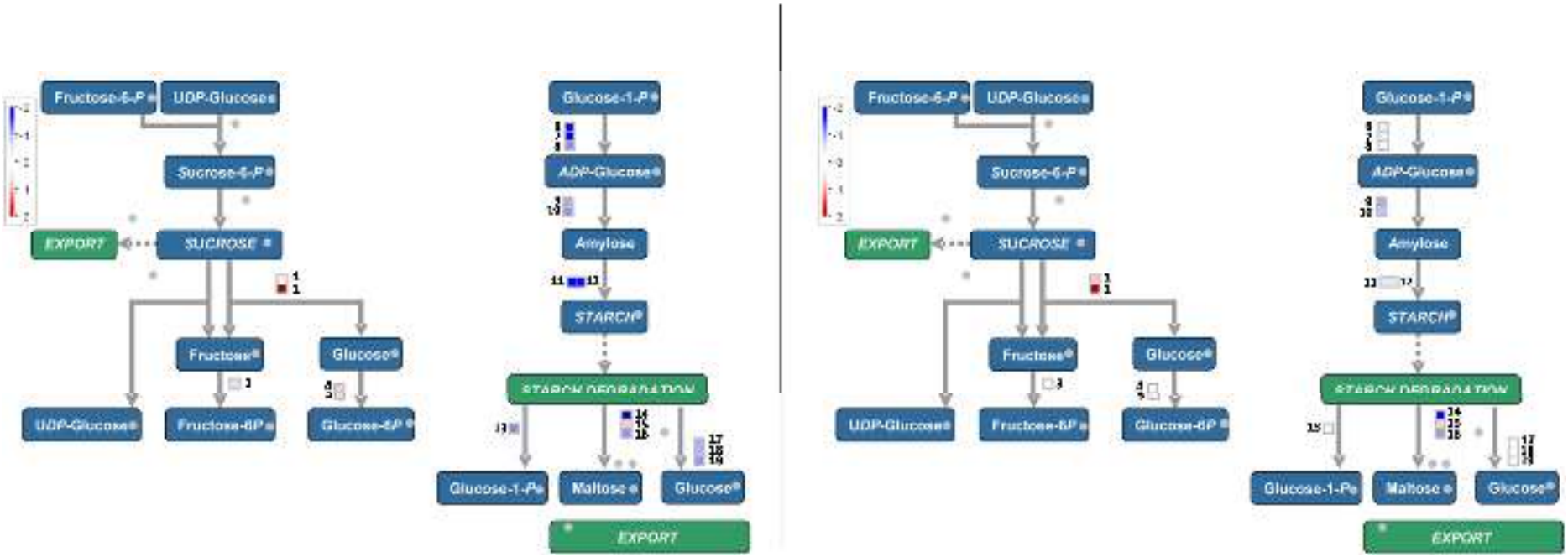

Figure 4-15. MapMan scheme representing sucrose-starch metabolism pathways gene expression for Arsenal and VR 808 tubers during senescent sweetening transition for season 1 (2016/2017). One-way ANOVA using time as factor was employed to identify significant changes during senescent sweetening transition. The colour represents the direction and strength of the regulation (Logarithm to the base 2 colour scale; Red, highly up-regulated and Blue, highly down-regulated). Abbreviations: 1, neutral invertase; 2, vacuolar invertase; 3, fructokinase; 4, hexokinase; 5, hexokinase; 6, ADP-glucose pyrophosphorylase (AGPase) (large subunit); 7, AGPase (large subunit); 8, AGPase (small subunit); 9, granule-bound starch synthase; 10, granule-bound starch synthase; 11, 1,4-α-glucan branching enzyme; 12, 1,4-α-glucan branching enzyme; 13, α-glucan phosphorylase; 14, α-amylase; 15, unknown protein; 16, α-amylase; 17, 4-α-glucanotransferase; 18, 4-α-glucanotransferase; 19, 4-α-glucanotransferase.

POTATOES

Season 2016/2017

Arsenal

VR 808

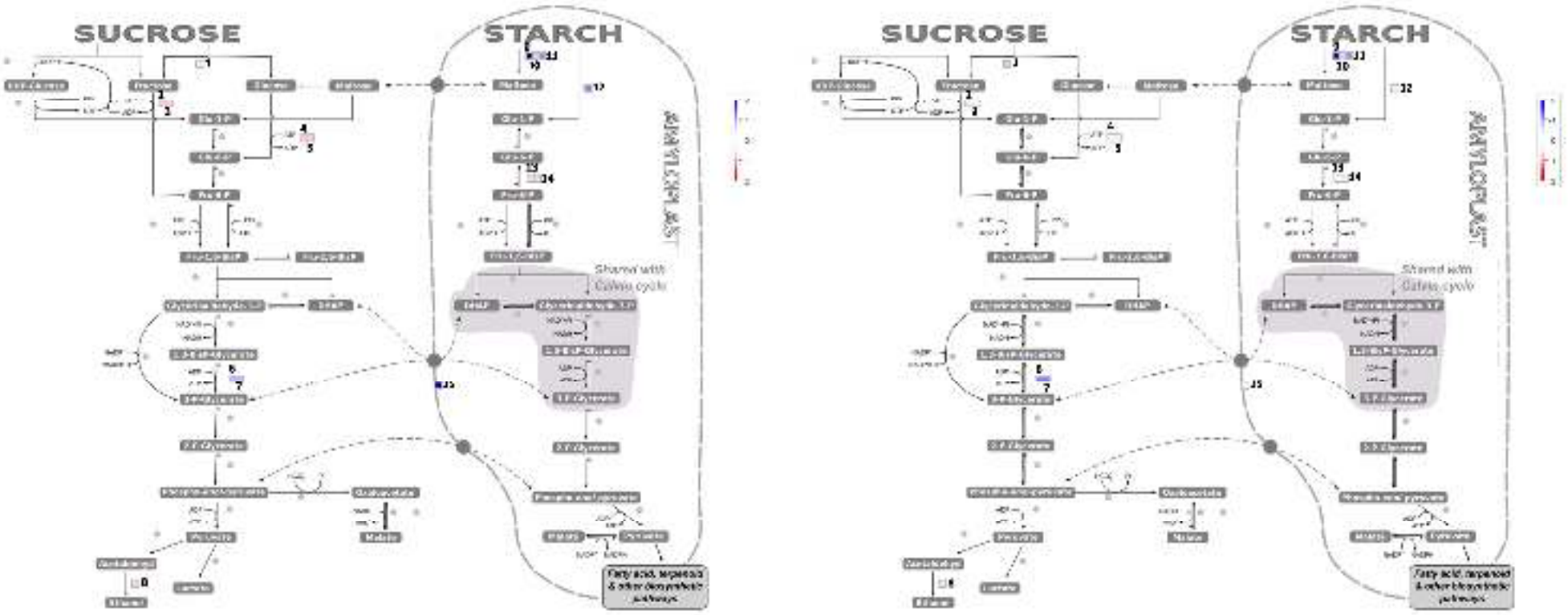

Figure 4-16. MapMan scheme representing glycolysis pathways gene expression for Arsenal and VR 808 tubers during senescent sweetening transition for season 1 (2016/2017). One-way ANOVA using time as factor was employed to identify significant changes during senescent sweetening transition. The colour represents the direction and strength of the regulation (Logarithm to the base 2 colour scale; Red, highly up-regulated and Blue, highly down-regulated). Abbreviations: 1, neutral invertase; 2, hexokinase; 3, hexokinase; 4, hexokinase; 5, hexokinase; 6, phosphatase; 7, phosphatase; 8, oxido-reductase; 9, α-amylase; 10, unknown protein; 11, α-amylase; 12, α-glucan phosphorylase; 13, phosphofructokinase; 14, phosphofructokinase; 15, glucose-6-phosphate translocator 2.

POTATOES

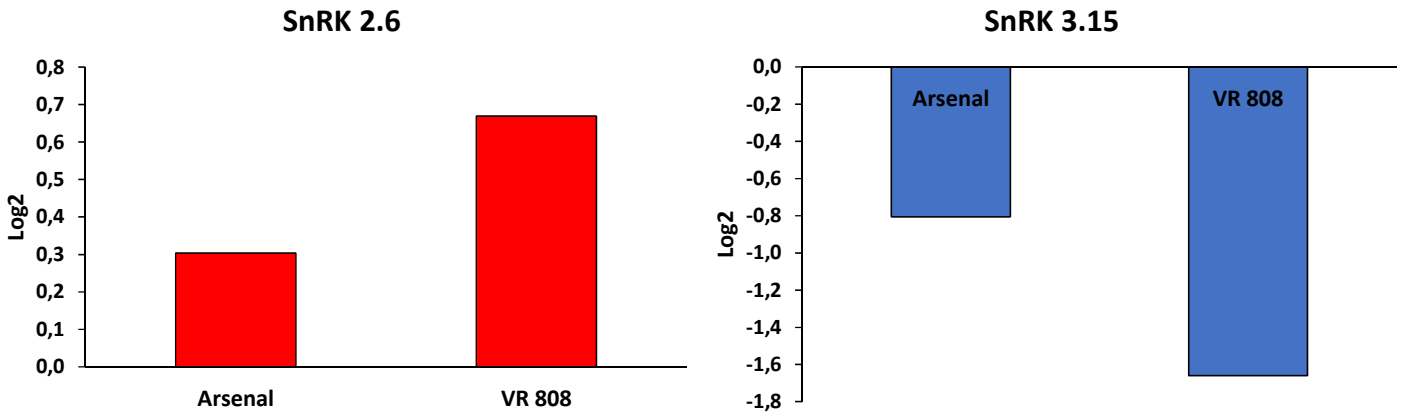

Figure 4-17. SnRK gene expression for Arsenal and VR 808 tubers during senescent sweetening transition for season 1 (2016/2017). One-way ANOVA using time as factor was employed to identify significant changes during senescent sweetening transition. The colour represents the direction of the regulation (Logarithm to the base 2 colour scale; Red, up-regulated and Blue, down-regulated).

4.4.3. Gene expression and biological processes influenced by long-term storage during season 2 (2017/2018)

During the second season, 4 different time points were under study. These time points were at 30, 37, 40 and 53 weeks after storage, corresponding to prior to sugar accumulation stage, senescent sweetening transition and late storage.

A total of 558 genes in Arsenal and 58 genes in VR 808 were differentially expressed over time. Significant changes were observed in genes associated with photosynthesis, major carbohydrate metabolism, lipid metabolism, metal handing, hormone metabolism, stress, nucleotide metabolism, regulation of transcription, DNA synthesis, signalling, development and transport (Figure 4-18).

Season 2017/2018

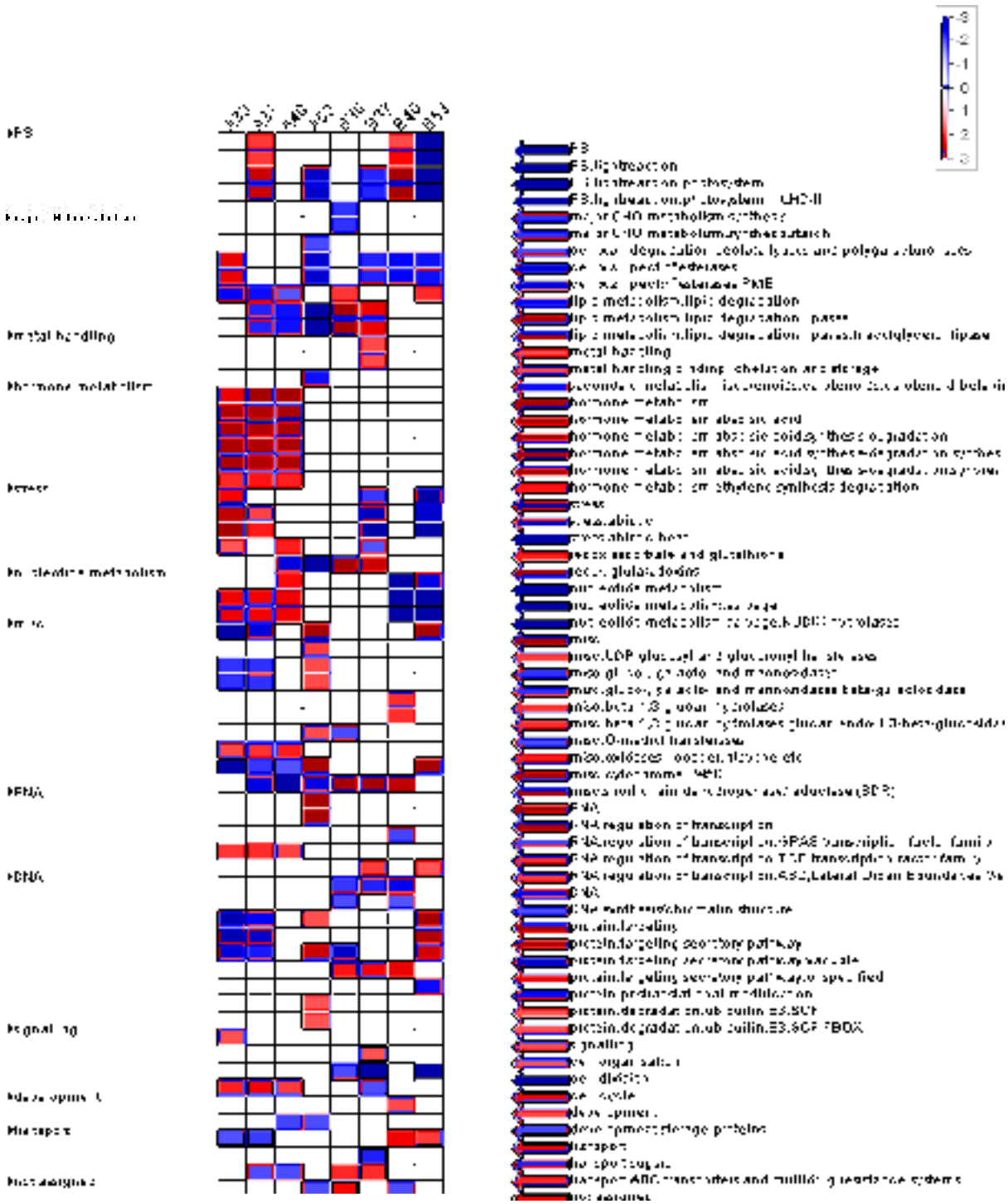

Figure 4-18. PageMan diagrams representing changes in potato tubers during long-term storage at 9°C. Differential gene expression of potato tubers during long-term storage in season 2 (2017/2018). A Wilcoxon rank sum test (Benjamini-Hochberg corrected) was employed to identify BINs whose contents were differentially regulated. Each coloured block represents an individual BIN/sub-BIN or gene in the PageMan analysis. The colour represents the direction and strength of the regulation (Red, up-regulated and Blue, down-regulated). Abbreviations: A, Arsenal; B, VR 808. 30, 37, 40, 53 represent weeks of storage.

4.4.3.1. Gene expression and biological processes influenced by long-term storage during season 2 (2017/2018) in Arsenal

In Arsenal, at 30, 37, and 40 weeks of storage, genes encoding the ABA biosynthetic enzymes 9-cis-epoxycarotenoid dioxygenase (NCED, EC 1.13.11.51) (Nambara & Marion-Poll, 2005) were up-regulated as well as gibberellin (GA) 20-oxidase (EC 1.14.11) genes. GA 20-oxidase activity is suggested to be one of the principal points of regulation in the GA-biosynthetic pathway (reviewed by Hedden & Kamiya, 1997). Moreover, teosinte branched1/cycloidea/proliferating cell factor (TCP) family of transcription factors genes were up-regulated. Endogenous ABA is involved in the regulation of wound-induced suberization and the processes that protect surface cells from water vapour loss and death by dehydration (Lulai *et al*., 2008). Both ABA and ethylene are required for dormancy induction, but only ABA is needed to maintain bud dormancy (reviewed by Suttle, 2004). TCP are involved in the regulation of cell growth and proliferation, performing diverse functions in plant growth and development and have been shown to be targets of pathogenic effectors and are likely to play a vital role in plant immunity (Bao *et al*., 2019). At 53 weeks after storage, protein degradation genes were up-regulated. Signalling genes encoding leucine-rich kinase family proteins were up-regulated at 30 weeks of storage. Wu *et al*. (2009) suggested these genes may participate in the responses against environmental stresses and disease resistance in potato. In addition, kip-related protein 3 (KRP3) genes, negative regulator of cell division (De Velder *et al*., 2001; Verkest *et al*., 2005; Weinl *et al*., 2005; Liu *et al*., 2008), were up-regulated at 30, 37, and 40 weeks after storage. Patatin group precursor genes involved in storage were down-regulated after the onset of senescent sweetening. Down-regulation was observed in genes encoding β-galactosidases (EC 3.2.1.23) at 30, and 37 weeks, followed by up-regulation at 53 weeks of storage. β-galactosidases are associated with fruit softening (Gross, 1984; Gross & Sams, 1984; Redgwell *et al*., 1992).

4.4.3.2. Gene expression and biological processes influenced by long-term storage during season 2 (2017/2018) in VR 808

VR 808 exhibited down-regulation in starch synthase (EC 2.4.1.21) and 1,4-α-glucan branching enzyme at 40 weeks of storage. Genes encoding pectin methyl esterase (PME, EC 3.1.1.11) were under-expressed at 37, 40, and 53 weeks of storage. Orthologous PME genes have been shown to impact on the texture of fruit from many species (reviewed in Fischer & Bennett, 1991). As pectin is a major component of the cell wall and the middle lamella, its structure is likely to be an important factor in texture in potato tubers as well as other plant tissues (Fischer & Bennett, 1991). Up-regulation in metal handing genes was observed at 37 weeks of storage. At 40 weeks of storage, GRAS family transcription factors were down-regulated. GRAS genes play diverse roles in root and shoot development, GAs signalling, and phytochrome A signal transduction (Bolle, 2004). At 30, and 40 weeks of storage, down-regulation in genes encoding WD-40 repeat family proteins, known to serve as platforms for the assembly of protein complexes or mediators of transient interplay among other proteins. In the model plant Arabidopsis thaliana, members of this superfamily are increasingly being recognized as key regulators of plant-specific developmental events (van Nocker & Ludwig, 2003).

At 30, 37 and 40 weeks after storage, genes encoding ADP-ribosylation factor (ARF) were up-regulated. ARF regulates metabolism and antioxidant capacity in transgenic potato tubers (Zuk *et al*., 2003). Transgenic plants resulted in the increase of soluble sugar-to-starch ratio parameter when compared to un-transformed plants (Zuk *et al*., 2003). Genes encoding protein phosphatase 2A (PP2A, EC 3.1.3.16) were down-regulated in VR 808 at 53 weeks of storage. PP2A may be involved in sucrose-phosphate synthesis (Reimholz *et al*., 1994). Genes encoding actin binding protein family associated with cellular organization were up-regulated at 37 weeks after storage. The actin cytoskeleton functions in the generation and maintenance of cell morphology and polarity, in endocytosis and intracellular trafficking, in contractility, motility and cell division (Winder & Ayscough, 2005). Cell division genes encoding regulator of chromosome condensation (RCC1) family protein were down-regulated at 30, 37, and 53 weeks after storage.

In plants, RCC1 molecules act mainly as regulating factors for a series of downstream genes during biological processes such as the UV-B response and cold tolerance (Heijde & Ulm, 2012; Ji *et al*., 2015). Transport sugars genes encoding major facilitator superfamily proteins were under-expressed at 37 weeks of storage. These proteins are involved in glucose transmembrane transport (Saier *et al*., 1999).

4.4.3.3. Differences between Arsenal and VR 808 in gene expression and biological processes influenced by long-term storage during season 2 (2017/2018)

Arsenal and VR 808 showed opposite behaviour in gene expression related to lipid degradation, abiotic stress, nucleotide metabolism, short chain dehydrogenase/reductase and transport. Gene expression was down-regulated for lipid metabolism, glutaredoxin, and transport in Arsenal and up-regulated in VR 808. On the contrary, stress abiotic and nucleotide metabolism were up-regulated in Arsenal and down-regulated in VR 808. The rest of significant changes in gene expression presented no clear pattern. For this year, no significant changes in SnRK genes were reported during the storage period.

4.4.3.4. Effect of long-term storage on carbohydrate metabolism during season 2 (2017/2018)

In terms of carbohydrate metabolism, differences in gene expression from the previous season were observed in the cultivars. Although no significant changes in gene expression were reported according to Wilcoxon rank sum test (Benjamini-Hochberg corrected), further details regarding sucrose-starch metabolism (Figure 4-19) and glycolysis pathways (Figure 4-20) were analysed. Arsenal showed a general down-regulation at 53 weeks of storage in AGPase (large and small subunits), starch synthase I, 1,4-α-glucan branching enzyme, and 4-alpha-glucanotransferase (EC 2.4.1.25). Furthermore, results in GPT2 expression were reproduced from season 1 (2016/2017). Arsenal exhibited a gradually and strong decrease in GPT2 transcripts whereas it had a little increase in VR 808 over the storage period (Figure 4-20). The GPT2 expression was progressively down-regulated over the storage period for the susceptible cultivar following the trend of reducing sugar accumulation. Additionally, down-regulation in triose-phosphate isomerase (TPI, EC 5.3.1.1) was observed in both cultivars after senescent sweetening transition, presenting VR 808 a higher response.
GPT2 gene showed a gradual down-regulation over time in Arsenal, which accumulates reducing sugars. The decrease in gene expression was subsequently correlated to the reducing sugar accumulation during senescent sweetening transition. Hence, we suggested *GPT2* to be a candidate gene involved in sugar accumulation during long-term storage. The initial identification of this GPT2 differential gene expression on the microarray was therefore checked and confirmed by qRT-PCR.

Season 2017/2018

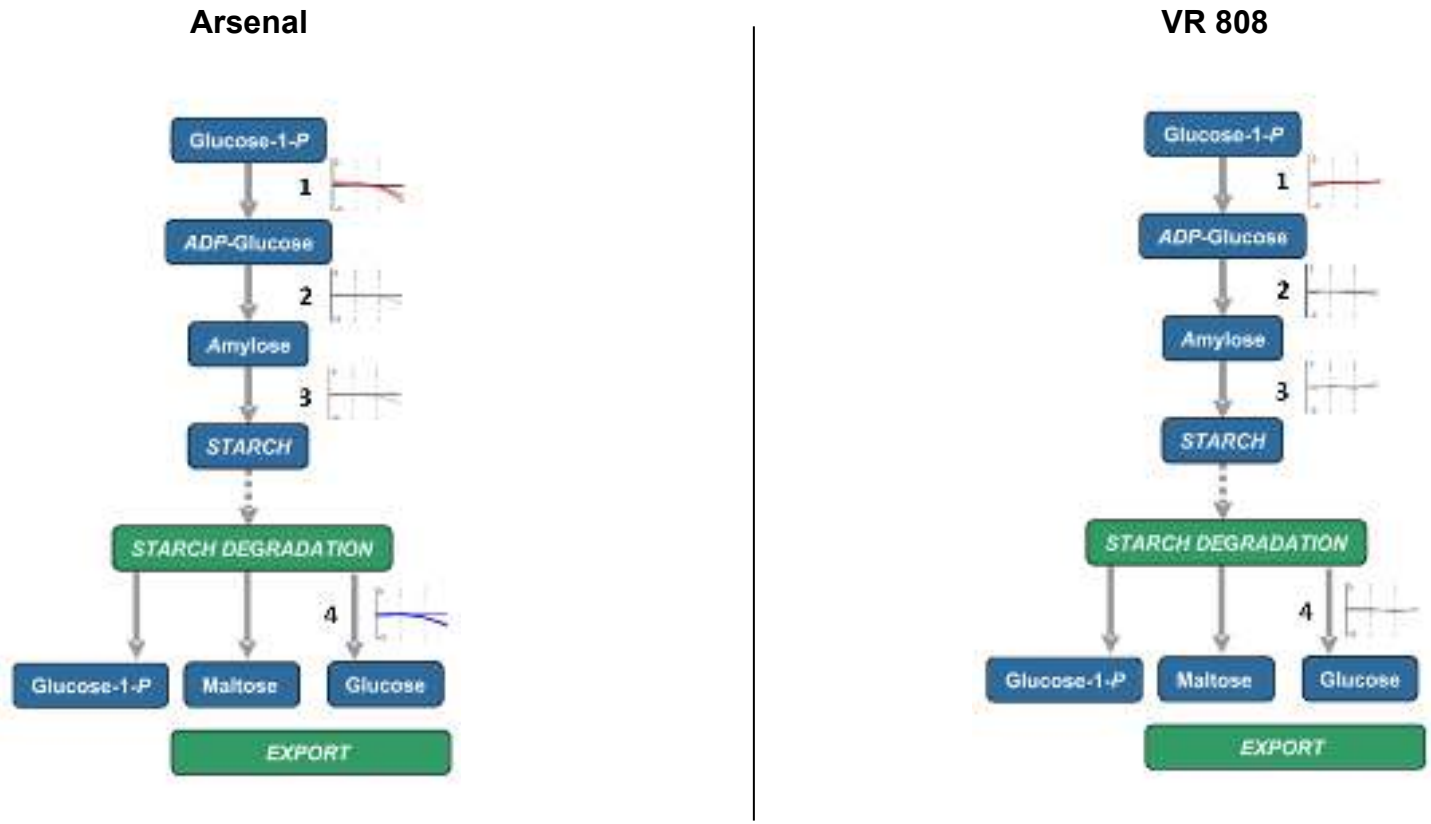

Figure 4-19. MapMan scheme representing starch metabolism pathway gene expression for Arsenal and VR 808 for season 2 (2017/2018). One-way ANOVA using time as factor (Benjamini-Hochberg corrected) was employed to identify significant changes over storage at 30, 37, 40, and 53 weeks of storage. The lines on the graphics represent the direction of the regulation. Abbreviations: 1, ADP-glucose pyrophosphorylase (AGPase, large and small subunits); 2, starch synthase; 3, 1,4-α-glucan branching enzyme; 4, 4-α-glucanotransferase.

POTATOES

Season 2017/2018

Arsenal

VR 808

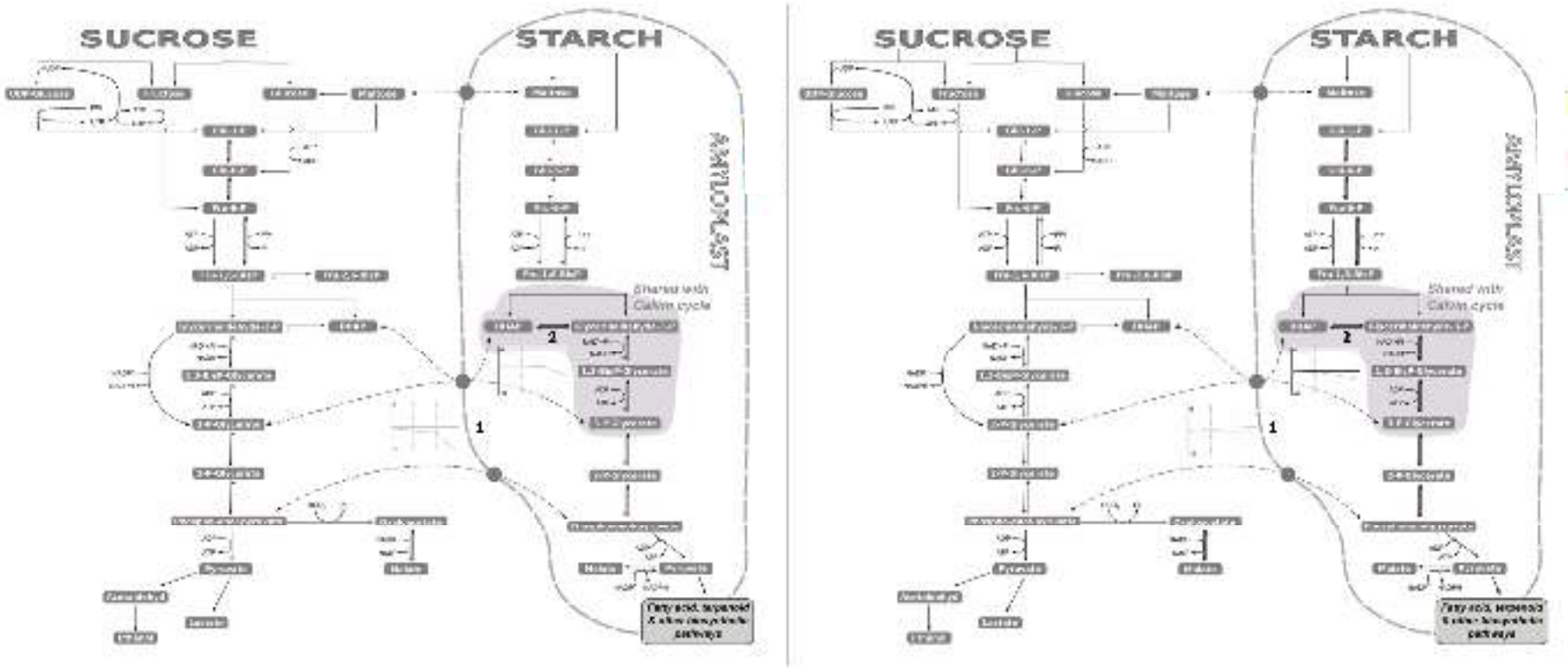

Figure 4-20. MapMan scheme representing glycolysis pathways gene expression for Arsenal and VR 808 for season 2 (2017/2018). One-way ANOVA using time as factor (Benjamini-Hochberg corrected) was employed to identify significant changes over storage at 30, 37, 40, and 53 weeks of storage. The lines on the graphics represent the direction of the regulation. Abbreviations: 1, glucose-6-phosphate translocator 2 (GPT2); 2, triose-phosphate isomerase (TPI).

4.4.4. qRT-PCR analysis of GPT2

Analysis was performed using the same samples and time points from season 2 (2017/2018) microarray experiment. qRT-PCR data confirmed the results obtained by microarray analysis. GPT2 expression was decreasing over time in Arsenal (Figure 4-21A). Moreover, a further qRT-PCR analysis was performed using season 3 (2018/2019) material in order to identify GPT2 expression as a common mechanism associated to sugar accumulation during long-term storage. In this experiment, 2 susceptible to SS, and 2 with stable sugar profile cultivars, were selected and compared during the SS transition (Figure 4-21B). Results suggested down-regulation in GPT2 gene is a common mechanism in cultivar susceptible to senescent sweetening.

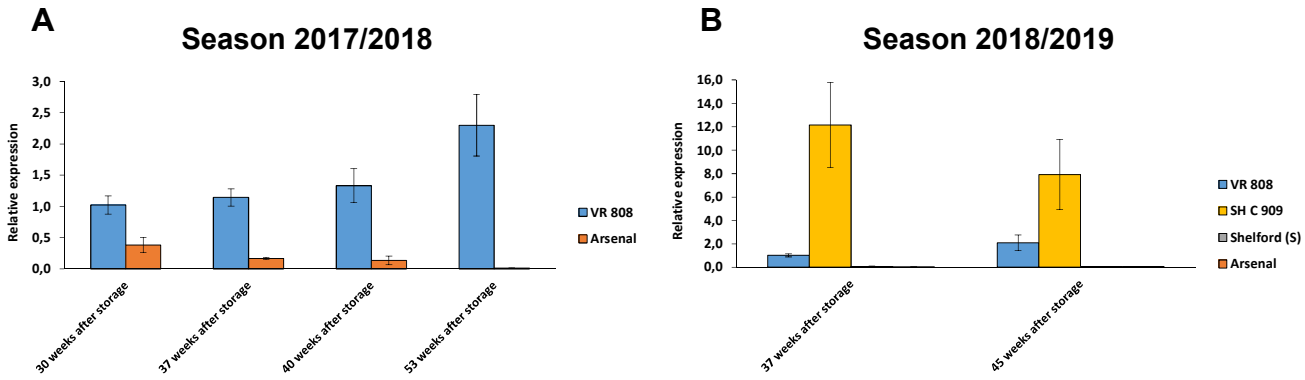

Figure 4-21. GPT2 expression in potato tubers during long-term storage at 9°C. All cultivars were from Shropshire location. **A.** Comparison between susceptible to SS and stable profile cultivars over time for season 2 (2017/2018). **B.** Comparison between 2 susceptible to SS and 2 stable profiles cultivars during SS for season 3 (2018/2019). Samples included the same cultivars studied previously. Results suggested GPT2 down-regulation is a common mechanism in susceptible to SS cultivars.

4.4.5. Assessment of transcriptome profiling results

Results from transcriptome profiling during year 1 (2016/2017) indicated differences in carbohydrate metabolism and flavonoids biosynthesis between Arsenal (susceptible to sweetening) and VR 808 (resistant to sweetening). In order to obtain further insights of whether this differential gene expression had an effect in the accumulation of reducing sugars, metabolites fluxes, carbohydrate metabolism enzymes activities, and total polyphenols content were analysed.

4.4.5.1. Metabolites flux estimation of [U-^{14}C] glucose metabolism

During season 3 (2018/2019) flux estimates from metabolism of [U-^{14}C] glucose were determined at 33, and 43 weeks of storage, both time points were prior to senescent sweetening. Potato tuber discs were incubated with [U-^{14}C] glucose for three hours. [U-^{14}C]-labelled extracts were fractionated into CO_2, starch, cell wall and protein, neutral, anionic and cationic fractions as well as glucose, fructose, and sucrose. A two-way ANOVA was carried out using factors cultivar and time of storage. Percentages of metabolised [U-^{14}C] glucose in potato tuber discs are presented in Table 4-2. [U-^{14}C]-labelled CO_2 was significant different dependent

on cultivar and time ($P < 0.05$). Percentage of metabolised [U-^{14}C] glucose into starch increased over time ($P = 0.001$) in both cultivars. However, no differences ($P > 0.05$) between cultivars were reported. No differences ($P > 0.05$) were observed for glucose, fructose, sucrose, cell wall and protein. Neutral fraction was significant different ($P < 0.01$) between cultivars at 33 weeks of storage. In addition, anionic and cationic fractions changed over time ($P < 0.05$).

Table 4-2. [U-^{14}C] in metabolic fraction in potato tuber discs at 33 and 43 weeks of storage for season 3 (2018/2019). Data presented is the percentage of [U-^{14}C] glucose metabolised per gram of fresh weight.

Metabolic fraction	14C in metabolic fraction in tuber discs (% of metabolised)			
	33 weeks of storage		43 weeks of storage	
	Arsenal	VR 808	Arsenal	VR 808
Glucose	0,96 ± 0,52	0,61 ± 0,09	2,62 ± 1,15	1,17 ± 0,10
Fructose	1,21 ± 0,60	0,66 ± 0,07	1,97 ± 0,65	1,14 ± 0,14
Sucrose	0,76 ± 0,18	0,76 ± 0,16	2,01 ± 0,76	2,63 ± 1,44
Starch	0,61 ± 0,08	1,04 ± 0,11	4,91 ± 1,40	4,72 ± 0,77
CO_2	64,77 ± 7,66	37,82 ± 3,18	28,55 ± 3,72	28,9 ± 4,55
Cell wall, Protein	1,59 ± 0,51	1,84 ± 0,04	1,49 ± 0,12	1,61 ± 0,03
Neutral fraction	7,02 ± 1,57	14,83 ± 0,65	26,39 ± 3,41	27,28 ± 3,41
Anionic fraction	11,60 ± 3,01	12,09 ± 1,27	16,12 ± 1,77	15,36 ± 0,29
Cationic fraction	2,28 ± 0,52	3,57 ± 0,18	6,05 ± 0,60	4,69 ± 0,91

4.4.5.2. Carbohydrate metabolism enzymes activity measurement

Enzymatic activities from carbohydrate metabolism were measured for season 1 (2016/2017). AGPase, UGPase, and PGM were analysed at 20, 26, and 30 weeks of storage (Figure 4-22A). In addition, HK, FK, SuSy, vacInv, and cytInv were under study at 30 weeks after storage, a post-sweetening stage (Figure 4-22B). A two-way ANOVA was performed using cultivar and time of storage as factors. HK, SuSy, vacInv and cytInv, AGPase, and UGPase activities exhibited no differences ($P > 0.05$) between cultivars or changes over time ($P > 0.05$). However, significant differences affected by cultivar for FK ($P < 0.05$) and by cultivar and time for PGM ($P < 0.005$) were observed. VR 808 exhibited a significantly lower FK specific activity compared to Arsenal at 30 weeks of storage. Besides, VR 808 showed a progressive decrease in the specific activity of PGM after 20 weeks of storage.

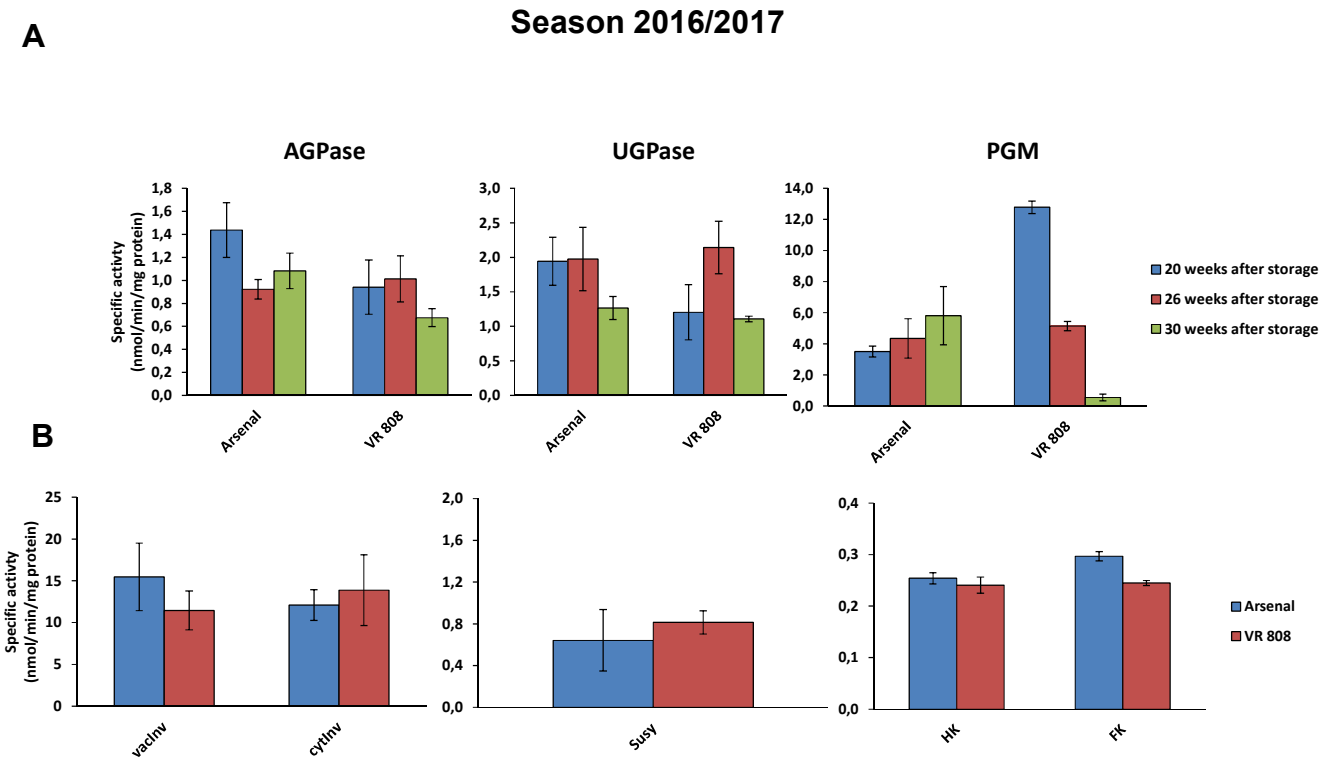

Figure 4-22. Measurement of specific activity of carbohydrate metabolism enzymes during senescent sweetening in season 1 (2016/2017). **A.** Enzyme activity of AGPase, UGPase, and PGM during senescent sweetening transition (20 and 26 weeks of storage) and post-sweetening at 30 weeks of storage. **B.** Enzyme activity of vacInv, cytInv, SuSy, HK, and FK. Values are means ± SE (three biological replicates from one experiment). Abbreviations: AGPase, ADP-glucose pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase; PGM, phosphoglucomutase; vacInv, vacuolar invertase; cytInv, cytosolic invertase; Susy, sucrose synthase; HK, hexokinase; FK, fructokinase.

4.4.6.3. Quantification of total polyphenols

Phenolic compounds are secondary metabolites produced in plants that have a common structure based on an aromatic ring with one or more hydroxyl substituents (Beckman, 2000; Parr & Bolwell, 2000; Valcarcel *et al*., 2015). These compounds can be divided according to their chemical structure into flavonoids, phenolic acids, tannins, stilbenes, coumarins, and lignans (Ignat *et al*., 2011; Lemos *et al*., 2015). Their presence affects the sensory qualities of plant-derived processed foods, including taste, colour, and texture (Kroon & Williamson, 1999; Alasalvar *et al*., 2001; Rytel *et al*., 2014). Differences in gene expression related to flavonoid biosynthesis between cultivars were reported during senescent sweetening for season 1 (2016/2017). It was hypothesized that flavonoid synthesis may be acting as a sink for reducing sugars in VR 808, resistant to sweetening cultivar, avoiding their accumulation. Therefore, total polyphenols were quantified at 20, 26 and 30 weeks after storage in year 1 (Figure 4-23). These time points were related to senescent sweetening transition and a post-sweetening stage. A two-ways ANOVA was performed using cultivar and time of storage as factors. Results showed no significant differences ($P > 0.05$) between cultivars as well as no significant changes ($P > 0.05$) influenced by time of storage.

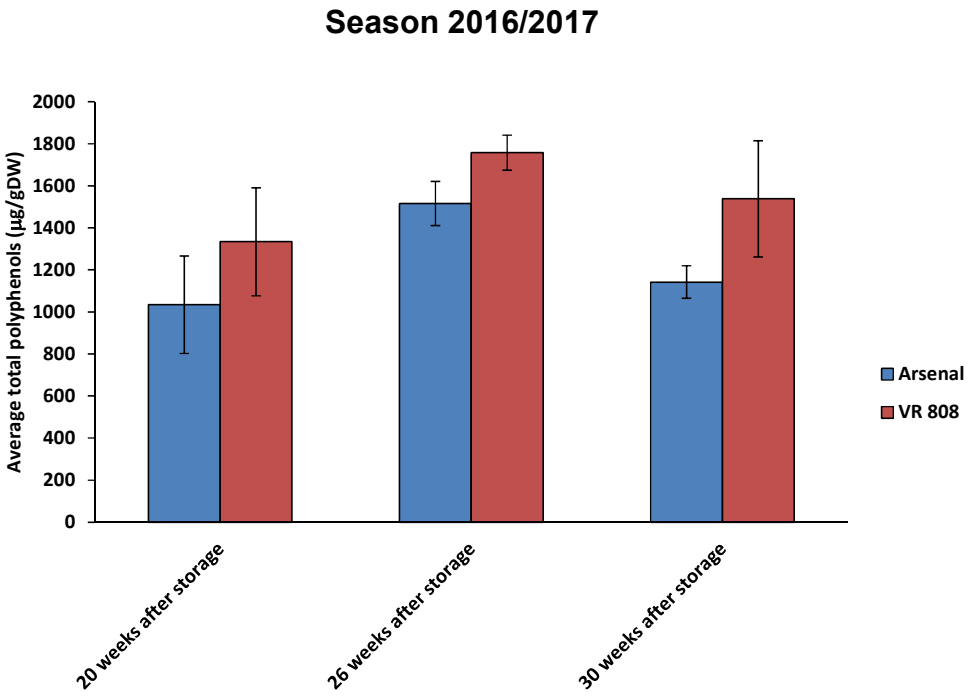

Figure 4-23. Quantification of total polyphenols content in Arsenal and VR 808 tubers during season 1 (2016/2017). Values are means ± SE (four biological replicates from one experiment).

5. DISCUSSION

5.1. Assessment of sugar accumulation and processing quality during storage

In general, Lady Rosetta, Shelford (Shropshire), and Arsenal, had the highest reducing sugars accumulation over the storage period and, SH C 909 and VR 808, showed no accumulation of glucose in any case as well as presented the lowest fructose accumulation.

The level of sugars in potato tubers is an important factor affecting quality in potatoes. The principal reason is the fact that the reducing sugars such as glucose and fructose react with free amino acids during frying to produce distasteful dark processed fries and chips via a non-enzymatic Maillard-type reaction (Shallenberger *et al*., 1959). This reaction has played an important role in the appearance and taste of foods since it is related to aroma, taste and colour. Moreover, acrylamide is present in different foods processed at high temperature and it is formed from asparagine in the presence of a carbonyl compound such a reducing sugar in the process of Maillard reactions (Mottram *et al*., 2002; Stadler *et al*., 2002). During the 3 years of this study, we reported a darkening increased over the storage period following reducing sugars trend. Susceptible cultivars exhibited darker fry colour than varieties with a stable sugar profile. Darker fry colours were reported to be more related to high glucose rather than fructose or sucrose content. Fry colour depends on the quantity of superficial reducing sugars and the temperature of frying oil as well as frying time (Pedreschi, 2009). A darker fry colour has been reported to be correlated to a higher glucose (Coleman *et al*., 1993; Pritchard & Adam, 1994) as well as higher acrylamide (Shepherd *et al*., 2010) contents.

The benefit of storing potatoes at 8-12°C is the minimum accumulation of sugars in tubers. This storage method keeps the stored potatoes suitable for table and processing purposes. However, the relatively high temperature favours sprouting and sprout growth once the natural dormancy period of potato is over. Hence, use of sprout suppressant becomes essential under these methods of potato storage. CIPC is considered as the most effective sprout suppressant for potatoes and it is usually applied as a post-harvest fogging treatment on stored potatoes (Paul *et al*., 2016). CIPC is a selective and systemic herbicide with an ability to translocate acropetally in plant system (Ashton & Crafts, 1981). CIPC acts as a mitotic inhibitor by interfering the process of spindle formation during the cell division (Vaughn & Lehnen, 1991). It is known to inhibit protein synthesis, RNA synthesis, activity of β-amylase along with suppression of transpiration and respiration and interfere with oxidative phosphorylation and photosynthesis (Vaughn & Lehnen, 1991). The CIPC treatment starts after the wound-healing period, since wound-healing requires the production of new cell layers resulting from cell division, and before dormancy break or sprout growth initiation (Kleinkopf *et al*., 2003). In the present study, CIPC treatment had an effect on the fry colour of crisps. Within the same cultivar, crisps from untreated potato tubers showed a darker fry colour than CIPC-treated tubers. Higher sugar content and darker fry colour observed in untreated tubers might be due to would-induced catabolic response and/or sink demand. With the onset of sprouting, tubers become a source organ for the growing sprout (Sonnewald, 2001). This is accompanied by structural and metabolic changes as well as by an altered level of gene expression (Ronning *et al*., 2003; Viola *et al*., 2007; Hartmann *et al*., 2011). Initial bud outgrowth does not require massive reserve mobilisation but is fed by sucrose-synthesising capacity that ensures rapid conversion of hexoses into sucrose that can be transported into growing buds to meet its energy demand. This was concluded from labelling experiments which revealed similar metabolic competence, but different metabolite pools in dormant and open tuber buds with respect to sugar metabolism (Viola *et al*., 2007). While resting buds contained only limited amounts of soluble sugars, there was a massive increase especially in the amount of sucrose at bud break indicating that sucrose unloading into the buds is a prerequisite for bud outgrowth.

Accumulation of reducing sugars must be avoided as it leads to both deterioration in processing quality and the risk of acrylamide production (Fuller & Hughes, 1984). The amount of free sugar tubers accumulate depends on the cultivar (van Vliet & Schriemer 1960; Burton 1969; Samotus *et al*., 1974; Coffin *et al*., 1987; Richardson *et al*., 1990; Zrenner *et al*., 1996). Although the

cultivars that are most susceptible to senescent sweetening tend to have short dormancy there are important exceptions to this rule such as Maris Piper and Record (Colgan *et al*., 2012). In addition to the effect of cultivar, growing conditions that affect the maturity of tubers at harvest can impact on the timing of the onset of senescent sweetening and an effect of storage temperature is also evident (Groves *et al*., 2005).

Considerable variation between different potato genotypes have been observed (Amrein *et al*., 2003; Kumar *et al*., 2004; Elmore *et al*., 2007) and this affects processing properties. Moreover, sugar content is affected by environmental factors during potato cultivation. Pre- and post-harvest environmental and management factors are important, including temperature, mineral nutrition and water availability during cultivation, crop maturity at harvest, mechanical stress and storage conditions (Kumar *et al*., 2004). Temperature during cultivation is a major factor because the processes of photosynthesis, transpiration, translocation of carbohydrates and respiration are all temperature dependent. The optimum temperature range for most varieties is quite narrow, between 15°C and 20°C (Kumar *et al*., 2004). Soil nitrogen levels also appear to be important: De Wilde *et al*. (2006) showed that the levels of tuber sugars rose in nitrogen-deprived potatoes by up to 100% compared with adequately fertilized potatoes, and Kumar *et al*. (2004) similarly reported that plants adequately fertilized with nitrogen had lower reducing-sugar concentrations at harvest. Sulphur deprivation also causes large increases in sugar concentrations (Elmore *et al*., 2007). In addition, Muttucumaru *et al*. (2015) observed that a lack of irrigation in the field-grown potatoes resulted in a lower reducing sugar concentration in four out of five varieties in the study (Lady Claire, Saturna, Ramos, and Hermes).

Senescent sweetening resistant and susceptible varieties showed similar behaviours in all years. This work allowed to identify the sweetening transition, and sampling of tubers for subsequent physiological, biochemical and molecular analysis.

5.2. Investigation of oxidative stress during long-term storage

Reactive oxygen species (ROS) are well recognized for playing a dual role as both deleterious and beneficial species depending on their concentration in plants. At high concentration ROS cause damage to biomolecules, whereas at low/moderate concentrations they act as second messengers in intracellular signalling cascades that mediate several responses in plant cells (Gechey & Hille, 2005). Among the ROS, H_2O_2 is the one which received most of the attention in the last years. H_2O_2 is the result of a two-step reduction molecular oxygen and has a relatively long lifespan in comparison to other ROS. The long half-life (1 ms) of H_2O_2 and its small size allow it to traverse cellular membranes and migrate in different compartments, which facilitates its signalling functions (Bienert *et al*., 2006). It is well known that H_2O_2 is a regulator of a multitude of physiological processes like acquiring resistance, cell wall strengthening, senescence, phytoalexin production, photosynthesis, stomatal opening and the cell cycle (Petrov & Van Breusegem, 2012) and is essential for suberization in potatoes (Razem & Bernards, 2002).The dual role played by ROS require the very strict control of H_2O_2 concentration in plant cells. The biological effect of H_2O_2 is mostly dependent on its concentration, but also on the site of production, the developmental stage of the plant and previous exposures to different kinds of stress.

H_2O_2 has a potential role in cellular and membrane damage as a consequence of an imbalance between its production and antioxidant defences. This imbalance could lead to an oxidative stress condition, and subsequent damage of the mitochondria and amyloplast membrane. Malfunction in the mitochondrial machinery would result in a reduced capacity of respiration of reducing sugars, producing their accumulation. In the same way, damage of amyloplast membrane could expose the starch to amylolytic enzymes increasing its degradation and, therefore, accumulation of reducing sugars.

H_2O_2 is the longest living ROS and is considered as the predominant ROS involved in cellular signalling (Bienert *et al*., 2006). H_2O_2 content was quantified as a marker of oxidative stress in tubers during storage. An increase of H_2O_2 content during senescent sweetening transition

would suggest oxidative stress may be linked to the sugar accumulation in tubers. Both cultivars exhibited significant differences during the storage period. However, tubers from each did not exhibit a specific increase of H_2O_2 associated with the onset on sweetening.

Changes in absolute level of H_2O_2 were not observed during long-term storage. Nevertheless, oxidative damage may have occurred due to localised concentration in specific organelles or due to controlled sudden spikes. For that reason, an additional experiment was performed in order to determine if H_2O_2 produced lipid membrane damage at cellular level over time. MDA levels were quantified as an indirect measurement of lipid peroxidation in lipid membranes (Fletcher *et al*., 1973; Konze & Elstner, 1978; Dhindsa *et al*., 1981). Results in lipid peroxidation showed similar behaviours for both cultivars. An increase at the beginning of the storage period was observed which may be related to storage-induced stress suffered by the tuber. During the long-term storage MDA levels remained stable, suggesting no increase of oxidative damage in lipid membranes. In the present work, we report no increase of markers of oxidative stress during storage, suggesting senescent sweetening may not be linked to oxidative stress.

Aging and senescence are distinctly different but overlapping developmental processes. Aging encompasses the entire lifespan of an organism, whereas senescence can be thought of as the final developmental phase that culminates in death. Aging and senescence are the result of complex changes in basic plant metabolism and, although the two are distinguishable, they do share similarities at the biochemical level. For example, a gradual disruption of membrane integrity, resulting in loss of compartmentation of cytoplasmic organelles and increased permeability of the plasma membrane, is a widely reported phenomenon common to both progressive aging and senescence of plant tissues (Thompson, 1988). Membrane integrity declines with advancing age of potato seed-tubers (Knowles & Knowles, 1989). In senescing plant tissues, lipid peroxidation plays a role in the loss of membrane integrity (Leshem, 1987; Gidrol *et al*., 1989) and evidence of extensive lipid peroxidation during prolonged storage of potato seed-tubers has been reported (Kumar & Knowles, 1993). Moreover, recent studies using diaminobenzidine tetrahydrochloride (DAB) and nitroblue tetrazolium (NBT) staining indicated a relationship between the onset of senescent sweetening and an increase in ROS, suggesting senescent sweetening resistant varieties exhibit a delayed rise in ROS accumulation (Carvalho, 2018). These findings support the hypothesis that senescent sweetening may be produced by an increase of oxidative damage. However, since DAB stain is dependent not only on ROS but also on the presence of peroxidase, this might explain the observed difference. Moreover, membrane permeability may change over time leading to a better access of the stain into the tissue.

Loss of membrane integrity in the amyloplast due to an increase of lipid peroxidation could expose the starch to amylolytic enzymes leading to an accumulation of sugars. Nonetheless, MDA measurements reported in this chapter suggest that if there is a loss of membrane integrity is not caused by oxidative damage. We showed previously there was no evidence of oxidative damage related to accumulation of sugars during long-term storage. In addition, a general decrease in fatty acids ($P < 0.001$) associated with the senescent sweetening transition was observed in this work. However, Spychalla and Desborough (1990) proposed induced or initial high levels of membrane lipid unsaturation mitigated increases in tuber membrane permeability during storage, and alterations in the levels of fatty acids had little bearing upon tuber membrane permeability. Literature has also reported mixed evidence of how long-term stored affects amyloplast membrane integrity in potato tubers. Electron micrographs of potato tubers stored at 10°C for 8 months indicated that the amyloplast membrane was still intact and continuous around starch granules in both normal and prematurely sweetened tissue (Sowokinos *et al*., 1985). Moreover, different authors reported fragmented or disintegrated starch granules membranes in potato tubers during storage (Ohad *et al*., 1971; Sowokinos *et al*., 1987).

Scavenging or detoxification of excess ROS is achieved by an efficient antioxidant defence system comprising of the non-enzymatic as well as enzymatic antioxidants (Schreck & Baeuerle, 1991; Noctor & Foyer, 1998; Møller, 2001). The enzymatic antioxidants include SOD, catalase (CAT), glutathione peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH)

cycle such as APX, MDHAR, DHAR, and GR (Noctor & Foyer, 1998, Möller *et al*., 2001). AsA, GSH, carotenoids, tocopherols, and phenolics serve as potent non-enzymatic antioxidants within the cell. Maintenance of a high antioxidant capacity to scavenge the toxic ROS has been linked to increased tolerance of plants to these environmental stresses (Zaefyzadeh *et al*., 2009; Chen *et al*., 2010). Considerable progress has been made in improving stress-induced oxidative stress tolerance in crop plants by developing transgenic lines with altered levels of antioxidants (Allen *et al*., 1997; Faize *et al*., 2011). Simultaneous expression of multiple antioxidant enzymes has been shown to be more effective than single or double expression for developing transgenic plants with enhanced tolerance to multiple environmental stresses (Lee *et al*., 2007). Plants have developed antioxidant defence systems to minimize the concentrations of ROS and to protect plant cells from oxidative damage (Noctor & Foyer, 1998). Given the lack of any evidence for oxidative stress in stored tubers, the hypothesis that antioxidant systems were upregulated to deal with an increased production of oxidants after prolonged storage was tested. An important role in the antioxidant defence system has been attributed to the ascorbate-glutathione pathway, which is catalysed by a set of four enzymes (Noctor & Foyer, 1998; Asada, 2006). The specific activity of this set of enzymes from the ascorbate-glutathione cycle was quantified to monitor any change during storage that could be related to senescent sweetening. Results showed fluctuations in the specific activity of antioxidant enzymes during storage. However, there was no consistent change in antioxidant enzyme activity associated with the onset of senescent sweetening. These data indicate a lack of support for the hypothesis that senescent sweetening is associated with oxidative stress in stored potato tubers.

5.3. Metabolome profiles of potato tubers during long-term storage

In season 1 (2016/2017) and during the senescent sweetening transition, a general increase in amino acids was observed for both cultivars. During year 3 (2018/2019), differences in amino acids were observed between metabolite profiles of the cultivars. Results suggested that sugars profile may be related to the amino acids content since PCA separate resistant cultivars from the rest of the cultivars mainly by the amino acids content at 43 weeks after storage, during the senescent sweetening period. Variable trends in free amino acids contents in potato tubers during the storage have been previously reported (Talley *et al*., 1984). Černá and Kracmar (2010) reported that the storage duration and cultivar have a significant effect on the total amino acids content. An increase in free amino acids content that occurred during the latter part of long-term storage (for up to 40 weeks) has been related to an upturn of proteinase activity on the break of dormancy (Brierley *et al*., 1996).

As previously described, reducing sugars, such as glucose and fructose, react with free amino acids during high-temperature cooking and processing in the Maillard reaction (Nursten, 2005; Mottram, 2007; Halford *et al*., 2011). The relationship between reducing sugars, asparagine, and acrylamide formation in potato products during cooking and processing is complex. Asparagine is present approximately at one-third of the total free amino acid pool (Eppendorfer & Bille 1996; Oruna-Concha *et al*., 2001; Amrein *et al*., 2003; Elmore *et al*., 2007; Carillo *et al*., 2012; Halford *et al*., 2012; Muttucumaru *et al*., 2013). Due to this fact, sugar concentrations might be expected to be the limiting factor for acrylamide formation as asparagine is found in such a high concentration. However, the evidence is mixed. Some studies have reported sugar concentrations as the limiting factor (Amrein *et al*., 2003; Becalski *et al*., 2004; de Wilde *et al*., 2006), while others authors have observed asparagine concentration or asparagine concentration as a proportion of the total free amino acid pool to be also important (Elmore *et al*., 2007; Shepherd *et al*., 2010; Halford *et al*., 2012; Muttucumaru *et al*., 2014).

In addition, both cultivars exhibited an increase in organic acids after the onset of senescent sweetening. Wichrowska *et al*. (2009) described that the content of organic acids in potato tubers depends on cultivar and storage conditions. An increase in organic acids might be the result of reduced respiration as a consequence of mitochondrial damage produced by oxidative stress (Salvato *et al*., 2014).

On the contrary, total fatty acids decreased after 20 weeks of storage for both cultivars.

Spychalla and Desborough (1990) revealed that potato cultivars with higher levels of fatty acid unsaturation had lower rates of membrane electrolyte leakage and lower sugar contents. However, at the present work both Arsenal and VR 808 exhibited similar unsaturated fatty acids while differences in reducing sugars content.

A progressive decrease in PGM activity was observed in VR 808 during storage in season 1 (2016/2017). In potato tubers, the reduction in the activity of plastidial PGM leads to both a reduction in starch accumulation and an increased sucrose accumulation (Fernie *et al*., 2001). The accumulation of sucrose observed in VR 808 could be related to a reduction in the PGM activity, suggesting the accumulation of reducing sugars and sucrose observed in Arsenal might be due to different mechanisms.

5.4. Changes in transcript levels associated with long-term storage in potato tubers

The aim of this work was to utilize transcriptome profiling to understand how long-term storage affects potato gene expression to promote senescent sweetening. A microarray experiment was carried out using two different time points representing senescent sweetening transition during the first year. For the second year, the experiment was conducted using 4 time points, including prior to sweetening stage, senescent sweetening transition and late storage stage. qRT-PCR was performed to verify the transcriptome results.

Carbohydrates provide energy and building blocks for plant growth and development. Furthermore, soluble sugars including glucose, fructose and sucrose are known to act as signal molecules to regulate the expression of many key genes involved in plant metabolic processes and defence responses, consequently regulating plant growth and development (Rolland *et al*., 2006; Mishra *et al*., 2009; Ruan *et al*., 2010; Cho & Yoo, 2011; Li *et al*., 2011). Carbohydrates are also central to quality and yield of crops. In fleshy fruits, the accumulation of soluble sugars during fruit development largely determines their sweetness at harvest. Plants have evolved an elaborate system for sugar metabolism and accumulation in sink cells (Li *et al*., 2012).

The results of both transcriptome analyses suggested that carbohydrate metabolism was altered during the storage period. In the major carbohydrate metabolism, genes associated with starch synthesis were down-regulated in both analyses for the susceptible cultivar, presenting these genes up-regulation for the stable profile cultivar. In addition, Arsenal exhibited up-regulation of FK genes as well as higher specific activity of this enzyme compared to VR 808. FK efficiently catalyses the phosphorylation of fructose to fructose 6-phosphate. However, it has been suggested that FK has little impact on glycolysis and starch synthesis (Davies *et al*., 2005).

For season 1 (2016/2017), genes encoding AGPase were down-regulated in Arsenal during the senescent sweetening transition. In season 2 (2017/2018), Arsenal also exhibited a general down-regulation in starch synthesis, including AGPase genes, at 53 weeks of storage. In heterotrophic storage organs such as potato tubers, most of the incoming sucrose is converted to starch as a long-term carbon store for reproductive growth. AGPase catalyses the first committed step of starch synthesis in the plastid, converting glucose 1-phosphate and ATP to ADP-Glc and PPi. ADP-Glc is subsequently used by starch synthases and branching enzymes to elongate the glucan chains of the starch granule. AGPase is a heterotetramer that contains two large (AGPS, 51 kDa) and two slightly smaller subunits (AGPB, 50 kDa) (Morell *et al*., 1987, Okita *et al*., 1990). Work with Arabidopsis mutants (Neuhaus & Stitt, 1990) and potato tubers (Geigenberger *et al*., 2004) showed that the enzyme catalyses a near rate-limiting step in the pathway of starch synthesis. There is evidence for the in vivo role of posttranslational redox modulation of AGPase in regulating starch synthesis in heterotrophic potato tubers (Tiessen *et al*., 2002) and photosynthetic leaves of potato, pea, and Arabidopsis plants (Hendriks *et al*., 2003). Posttranslational redox activation of AGPase allows the rate of starch synthesis to be increased in response to external inputs and independently of any increase in the levels of glycolytic intermediates (Tiessen *et al*., 2002). More recent studies in potato tubers revealed that sucrose and glucose lead to redox activation of AGPase via two different signaling pathways involving SnRK1 and hexokinase, respectively (Tiessen *et al*., 2003). Hexokinase and

SnRK1 are both implicated in a regulatory network that controls the expression and phosphorylation of cytosolic enzymes in response to sugars (Smeekens, 2000). How they are linked to reductive activation of AGPase and starch synthesis in the plastid remains unresolved. Trehalose metabolism has been implicated in the regulation of sugar utilization in yeast and plants (Thevelein & Hohmann, 1995; Eastmond & Graham, 2003; Gancedo & Flores, 2004).

Genes included in the minor carbohydrate biosynthesis suggested down-regulated production of T6P in Arsenal potato tubers. Trehalose and the metabolism associated with its synthesis have been proposed to be a component of the plant's sugar signalling system (Paul, 2007; Paul *et al*., 2008). T6P is an intermediate product of trehalose biosynthesis. T6P is a product of the reaction between UDP-Glc and G6P (Cabib & Leloir, 1958), which is catalysed by TPS. T6P is further metabolised to trehalose by TPP (Cabib & Leloir, 1958; O'Hara *et al*., 2013), which is eventually hydrolysed by trehalase into glucose (Elbein *et al*., 2003). In potato, T6P overproduction has been shown to cause the down-regulation of cell proliferation and delayed growth and sprouting (Debast *et al*., 2011). It has been reported that the addition of T6P to isolated chloroplasts leads to redox activation of AGPase (Kolbe *et al*., 2005). Lunn *et al*. (2006) reported that rising sugar levels in plants are accompanied by increases in the level of T6P, redox activation of AGPase and the stimulation of starch synthesis in vivo. These results indicate that T6P acts as a signalling metabolite of sugar status in plants and support the proposal that T6P mediates sucrose-induced changes in the rate of starch synthesis (Lunn *et al*., 2006).

Debast *et al*., (2011) reported that transgenic potato plants with elevated T6P levels displayed reduced starch content, decreased ATP contents, and increased respiration rate diagnostic for high metabolic activity. On the contrary, lines with significantly reduced T6P showed accumulation of soluble carbohydrates, hexose phosphates, and ATP, no change in starch when calculated on a fresh weight basis, and a strongly reduced tuber yield. T6P-accumulating tubers were strongly delayed in sprouting, while those with reduced T6P sprouted earlier than the wild type (Debast *et al*., 2011). This observation may be related to the fact that potato cultivars that are most susceptible to SS tend to have short dormancy (Colgan *et al*., 2012).

T6P is considered a signal regulating plant sugar metabolism, growth and development, possibly due to its interaction with sucrose non-fermenting (SNF) kinases (Lunn *et al*., 2014). T6P acts as an intermediary, increasing the rate of starch synthesis via the redox activation of AGPase (Kolbe *et al*., 2005). T6P has no significant inhibitory effects on the hexokinase activities of spinach (Wiese *et al*., 1999), Arabidopsis (Eastmond *et al*., 2002), or tomato (Kandel-Kfir *et al*., 2006). However, T6P indirectly responds to glucose or fructose but is directly influenced by sucrose (Yadav *et al*., 2014).

In Arabidopsis, T6P signalling is partially mediated through inhibition of the SnRK1 (Debast *et al*., 2011). Protein phosphorylation is involved in regulation of various cellular activities in plants and one of the main signals mediating the responses to environmental stresses (Laurie & Halford, 2001; Yoshida *et al*., 2006; Fujii *et al*., 2007; Movahed *et al*., 2012; Hong *et al*.,2013). The SnRKs are a gene family coding for Ser/Thr protein kinases and play important roles in linking abiotic stress tolerance and the metabolic responses of plants (Qin *et al*., 2011; Bing *et al*., 2013; Tao & Lu, 2013). Based on sequence similarity, domain structure and metabolic roles, the plant SnRK family is divided into three subfamilies: SnRK1, SnRK2 and SnRK3. Several studies have demonstrated that these three subfamilies play various roles in the metabolism and development of plants. SnRK1 plays an important role in regulating carbon metabolism and energy conversion in plants (Halford & Hardie, 1998; Ghillebert *et al*., 2011), SnRK2 members are the major players in plant responses to osmotic stresses (Boudsocq *et al*., 2004; Umezawa *et al*, 2004; Fujii & Zhu, 2009; Fujii *et al*., 2011), ABA dependent and independent stomatal closure-opening (Mustilli *et al*., 2002), fruit development (Sun *et al*., 2010), seed dormancy (Zheng *et al*., 2010) and germination (Johnson *et al*., 2002, Nakashima *et al*., 2009), while SnRK3 is involved in plant development, calcium-responsive regulatory loop and ABA sensitivity.

In addition, we reported a GPT2 gene was progressively down-regulated over time in the susceptible cultivar. This decrease in expression during the senescent sweetening transition was related to the increase of reducing sugars previously observed. A number of genes have been identified as being up-regulated by exogenous increases in sugar, including At1g61800, encoding a GPT2 (Knappe *et al*., 2003). GPT2 is involved in the transport of G6P across plastid membranes in return for inorganic phosphate (Niewiadomski *et al*., 2005). Microarray analyses have shown that GPT2 expression has been associated with impaired carbon metabolism (Kunz *et al*., 2010), senescence (Pourtau *et al*., 2006), and increases in carbon fixation due to increased light (Athanasiou *et al*., 2010). Moreover, GPT2 has been suggested to be associated with sugar sensing by affecting the balance of metabolites between cellular compartments (Dyson *et al*., 2015).

Kunz *et al*. (2010) showed that GPT2 expression is up-regulated in mutants impaired in starch synthesis (Kunz *et al*., 2010). In Arabidopsis, whereas the glucose-6-phosphate translocator 1 (GPT1) is constitutively present in particular cells such as stomatal guard cells of leaves or cells of the root tip, GPT2 is induced when carbohydrate metabolism is impaired, e.g. at higher concentrations of soluble sugars (Kunz *et al*., 2010). In potato tubers, the under-expression of GPT2 gene could lead to a decrease in transport of G6P into the amyloplasts for the synthesis of ADP-Glc, which is the substrate for starch synthases and represents the first committed precursor for starch synthesis.

The key enzyme in starch biosynthesis, the stroma-localised AGPase, catalyses the ATP-dependent conversion of G1P to ADP-Glc, the substrate for starch synthases. A knockout mutation in the catalytic subunit of AGPase in Arabidopsis results in a lack of starch in all parts of the plants (Lin *et al*., 1988), as is the case for a mutant plant with a defect in the plastid-localised PGM, catalysing the reversible conversion of G6P to G1P as substrate for AGPase (Caspar *et al*., 1985; Kofler *et al*., 2000; Periappuram *et al*., 2000). During the season 1 (2016/2017) of this project, VR 808 exhibited a progressive decrease in PGM activity at 26 weeks of storage. In plastids of heterotrophic tissues, G6P can be imported from the cytosol via a glucose-6-phosphate/phosphate translocator (GPT) and converted to starch via PGM, AGPase and starch synthases. The proposed role of the GPT is delivery of G6P to non-green plastids as carbon skeletons for starch biosynthesis and/or to the oxidative pentose phosphate pathway (Kammerer *et al*., 1998; Rolletschek *et al*., 2007; Zhang *et al*., 2008).

Microarray analyses revealed that GPT2 was substantially up-regulated in a pgm mutant or in a wild type fed with glucose (Thimm *et al*., 2004; Bläsing *et al*., 2005; Pourtau *et al*., 2006). In Arabidopsis leaves, GPT2 is strongly induced by light and contributes significantly to the measurable G6P transport activity of mutants impaired in starch biosynthesis (Kunz *et al*., 2010, Weise *et al*., 2019). GPT2 has been suggested to be a safety valve under situations when carbohydrate metabolism is impaired or in the presence of increased soluble sugar concentrations (Kunz *et al*., 2010). Moreover, inverse correlation of GPT2 and cwInv gene expression has been reported (Ferreira *et al*., 2010).

Weise *et al*. (2019) reported that both redox responsive transcription factor 1 (RRTF1) and high amounts of cytosolic triose phosphate are required for induction of the expression of GPT2 in Arabidopsis leaves. In the present study, VR 808 exhibited increasing transcript levels of GPT2 as well as a gradual decrease of PGM activity at 26 weeks of storage during year 1. However, potato tubers from both cultivars showed similar expression in RRTF1 genes. In mutants of Arabidopsis that are unable to synthesize starch due to a mutation in the gene encoding the plastid PGM, GPT2 transcripts amounts were more than two-fold higher than in the wild type (Weise *et al*., 2019). Potato lines with decreased activities of plastidial PGM exhibited a remarkable (up to 40%) decrease in the accumulation of starch, and significant increases in the levels of sucrose and hexose phosphates (Tauberger *et al*., 2000).

Cytosolic expression of yeast invertase in potato tubers leads to reduced starch content and increased respiration. Moreover, UDP-Glc production is associated with a reduced expression of cell wall biosynthetic genes (Ferreira & Sonnewald, 2012). In addition, Ferreira and

Sonnewald (2012) observed the transgenic tubers are characterized by elevated expression of senescence-associated genes, coupled to reduced expression of genes related to photosynthesis and the cytoskeleton. Increased respiration, observed in Arsenal tubers, might be due to sugar signalling via released T6P inhibition of the SnRK1 complex. In Arsenal, expression of the GPT2 was significantly down-regulated during the storage for year 1 and 2. This could lead to a shift in the cytosolic to plastidic G6P ratio and hence might limit starch synthesis, but also the oxidative pentose phosphate pathway.

In Arabidopsis, GPT2 is rapidly induced by both glucose and sucrose, and thus is essential for leaf growth and acclimation of metabolism to daily environmental changes (Gonzali *et al*., 2006; Athanasiou *et al*., 2010; Dyson *et al*., 2014; Dyson *et al*., 2015; Van Dingenen *et al*., 2016). The induction of GPT2 by glucose is dependent on its concentration, and does not occur in response to light, ABA, or other indirect signalling pathways (Chen *et al*., 2019). Chen *et al*. (2019) suggested that when sugars are increased in the cytosol, the expression levels of sugar-responsive genes such as GPT2 increase by the coordinate actions of WRKY18, WRKY53, and HAC1. The increased cytosolic sugar content could then be lowered by more active sugar import into cellular compartments (e.g. amyloplast in potato tubers).

In the mature leaves of most plants, photosynthates formed during C3 photosynthesis are used in the formation of sucrose, which is allocated via the phloem to the heterotrophic plant organs, such as young leaves, roots, seeds, fruits, or tubers. In these sink tissues, sucrose serves as a source of carbon and energy and is cleaved by the action of invertases or sucrose synthase. Finally, the products of these reactions are converted into hexose phosphates.

Plastids of non-photosynthetic plant tissues depend metabolically on the supply of ATP and carbon compounds. In general, plastids are not able to generate hexose phosphates from C3 compounds due to the absence of fructose-1,6-bisphosphatase activity (Entwistle & ap Rees, 1988). Non-green plastids of heterotrophic tissues import carbon as a source of biosynthetic pathways and energy and, in the case of amyloplasts of storage tissues, the site of starch synthesis. Within plastids, carbon can be used in the biosynthesis of starch or as a substrate for the oxidative pentose phosphate pathway. Several studies have reported that this transport in different plant tissues is mediated by a phosphate translocator that imports hexose phosphates in exchange with inorganic phosphate or C3 sugar phosphates (Borchert *et al*., 1989, 1993; Hill & Smith, 1991, 1995; Neuhaus *et al*., 1993; Flügge & Weber, 1994; Schünemann & Borchert, 1994; Flügge, 1995; Schott *et al*., 1995; Quick & Neuhaus, 1996). Although G6P has been reported to be the preferred hexose phosphate taken up by non-green plastids (Kammerer *et al*., 1998), in amyloplasts from wheat endosperm, G1P rather than G6P is the precursor of starch biosynthesis (Tyson & ap Rees, 1988; Tetlow *et al*., 1994). Amyloplasts from potato tubers showed to use G1P rather than G6P to support starch synthesis (Naeem *et al*., 1997), although previous studies reported that these plastids were able to transport G6P but not G1P (Schott *et al*., 1995).

In conclusion, long-term storage caused changes in carbohydrate metabolism and a progressive decrease in sugar transporters gene expression in susceptible cultivars. GPT2 was consistently down-regulated in sweetening tubers. This fact might limit sugar supply to the plastids, which could lead to a down-regulation of starch biosynthesis genes. These results suggest that down-regulation of sugar phosphate transport is a crucial factor that promotes senescent sweetening during long-term storage. Hence, we have identified GPT2 as a possible candidate gene involved in the mechanisms of senescent sweetening. Insight into the underlying mechanism that causes accumulation of sugars in stored potato tubers is needed to fully understand the senescent sweetening process.

6. CONCLUSIONS

Control of potato quality during storage represents a significant problem for the potato processing industry and little is known regarding the mechanisms of senescent sweetening. The Ph.D. research project adopts physiological, biochemical, and molecular approaches to elucidate downstream biochemical and molecular responses to long-term storage that may influence carbohydrate metabolism resulting in senescent sweetening. Potential mechanisms include enhanced starch degradation, reduced starch resynthesis, and reduced catabolism of sugars.

6.1 Assessment of physiological changes of potato tubers during long-term storage

Long-term storage had a significant impact on sugar accumulation in potato tubers. Varieties showed differences in senescent sweetening susceptibility. Accumulation of sugars was higher in Arsenal, Lady Rosetta and Shelford than in VR 808 and SH C 909. Sugar content was higher in untreated tubers compared to CIPC-treated tubers in year 1. During this year, Arsenal showed senescent sweetening after 26 weeks of storage. However, for the second year Arsenal exhibited a later onset of senescent sweetening, after 37 weeks of storage. In year 3, susceptible cultivars were observed to accumulate sugars after 43 of storage. Furthermore, the Shelford variety showed a difference in sugar accumulation depending on growing location.

Despite changes in the timing of accumulation, the relative timing was consistent. In conclusion, senescent sweetening has a strong genetic component that is overlaid by environmental factors, as demonstrated by impact of year and growing location. This fact suggests that breeding for senescent sweetening resistance is a feasible objective.

6.2 Senescent sweetening and its relationship with oxidative stress

A relationship between the onset of senescent sweetening and oxidative stress was not observed during the storage period. Arsenal and VR 808 exhibited differences in H_2O_2 content, however, no consistent increase was observed related to the onset of senescent sweetening. Similarly, although the content of MDA (a marker of oxidative stress) exhibited a transient increase in both cultivars early in storage, MDA levels rapidly returned to a basal level and a second increase associated with senescent sweetening was not observed. Antioxidant enzyme activities were observed to change over the course of the storage period, however there was again no consistent change associated with the transition to senescent sweetening.

The data presented in this work therefore provides no evidence for an association between oxidative stress and the induction of senescent sweetening. Some previous reports have suggested a greater oxidative burden in aged tubers (Kumar and Knowles 1996; Kumar et al. 1997) although the relationship between increased oxidative burden and senescent sweetening was not established. On the contrary, Sowokinos et al. (1987) clearly demonstrated that damage to the amyloplast membrane was associated with sugar accumulation and although it was assumed that loss of membrane integrity was associated with oxidative stress this was not demonstrated. However, Carvalho (2018) did show a loss of membrane integrity and also demonstrated an increase in the reactive oxygen species superoxide and hydrogen peroxide associated with senescent sweetening.

Taken together, it appears that long-term storage of potato tubers can induce oxidative stress however, the relationship between oxidative damage and sugar accumulation is less clear. Other work presented in this report indicates that genetically programmed changes in tuber metabolism may be a more significant factor in senescent sweetening.

6.3 Potato tuber metabolome during long-term storage

Cultivars showed similar behaviours in terms of metabolome during the storage. The main difference observed was in the amino acids content, which it has been postulated to be cultivar-

dependent. The amino acids content separate the cultivars into different groups based on their senescent sweetening susceptibility, suggesting a relationship between amino acids and sugar content.

Despite the observed differences in some amino acids, metabolic profiles are remarkably similar between cultivars. Moreover, and as evidenced from the data in this work, the metabolic adjustments leading to sweetening are minor. It might therefore be expected that only a few key genes are significant in producing the sweetening effect. Therefore, a small number of QTL might be expected to have a large effect which means that marker assisted breeding may be a powerful tool in the creation of sweetening resistant cultivars.

6.4 Changes in transcript levels associated with long-term storage in potato tubers

The key finding of this work is the observation that transcripts encoding the plastid hexose-phosphate transporter GPT2 are consistently reduced in abundance across all cultivars tested during the transition to senescent sweetening. During storage, potato tubers undergo futile rounds of starch degradation in the amyloplast, export of sugars to the cytosol, phosphorylation of sugars in the cytosol, import of phosphorylated sugars to the amyloplast and starch resynthesis in the amyloplast. GPT2 plays a crucial role in this cycle where it is responsible for the import of phosphorylated sugars back into the amyloplast. A reduction of GPT2 is likely to result in reduced import of sugar phosphates back into the amyloplast thereby reducing substrate availability for starch resynthesis and increasing the levels of free sugars and sugar-phosphates in the cytosol. This is further compounded by the observation that transcripts associated with starch synthesis are also depleted during the senescent sweetening transition. It is reasonable to hypothesise that starch biosynthesis transcripts are downregulated as a result of substrate limitation suggesting that the primary cause of senescent sweetening is the reduction in GPT2 capacity. These data indicate that GPT2 is a potential target for marker assisted breeding.

6.5 Mechanisms of senescent sweetening

In the present work, results suggest that senescent sweetening may be the consequence of altered carbohydrate metabolism. During the three years of study, all cultivars accumulated sucrose at similar levels. The accumulation of sucrose might result from a general water-deficit stress induced by long-term storage. As previously described, drought stress may affect vacuolar transporters. In this context, all cultivars under study might exhibit vacuolar accumulation of sucrose due to drought stress following long-term storage.

GPT2 expression was significantly lower in cultivars susceptible to senescent sweetening compared to resistant cultivars. GPT2 is involved in the transport of glucose-6-phosphate into the plastids. In potato tubers, the down-regulation observed in susceptible cultivars could implicate that sugar phosphates are unable to be transported into the amyloplasts, where they are used for starch synthesis. As a consequence, the decreased content in starch substrates in the amyloplasts might lead to the down-regulation in starch synthesis genes observed in Arsenal. Since reducing sugars could not been used for starch resynthesis, they would start to accumulate during long-term storage resulting in senescent sweetening.

Although differences related to senescent sweetening were observed between cultivars, the reason for these differences and what are the molecular triggers of onset remain to be elucidated.

6.6 Implications of this work

The main contributions of the present research project are a better understanding of the physiological, biochemical, and molecular changes in potato tubers during long-term storage. A further understanding of the processes underlying senescent sweetening will enable strategies

for control by optimising storage regimes and will underpin breeding programmes for the development of senescent sweetening-resistant varieties.

7. REFERENCES

Abdel-Ghany, S. E. (2009). Contribution of plastocyanin isoforms to photosynthesis and copper homeostasis in *Arabidopsis thaliana* grown at different copper regimes. *Planta*, *229*(4), 767–779. https://doi.org/10.1007/s00425-008-0869-z

Alasalvar, C., Grigor, J. M., Zhang, D., Quantick, P. C., & Shahidi, F. (2001). Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. *Journal of Agricultural and Food Chemistry*, *49*, 1410–1416. https://doi.org/10.1021/jf000595h

Allen, R. D., Webb, R. P., & Schake, S. A. (1997). Use of transgenic plants to study antioxidant defences. *Free Radical Biology and Medicine*, *23*(3), 473–479.

Amrein, T. M., Bachmann, S., Noti, A., Biedermann, M., Barbosa, M. F., Biedermann-Brem, S., Grob, K., Keiser, A., Realini, P., Escher, F., & Amadó, R. (2003). Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. *Journal of Agricultural and Food Chemistry*, *51*(18), 5556–5560. https://doi.org/10.1021/jf034344v

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*, *141*, 391–396.

Ashton, F. A., & Crafts, A. S. (1981). Mode of action of herbicides. 2. New York: Wiley.

Athanasiou, K., Dyson, B. C., Webster, R. E., & Johnson, G. N. (2010). Dynamic acclimation of photosynthesis increases plant fitness in changing environments. *Plant Physiology*, *152*, 366–373. https://doi.org/10.1104/pp.109.149351

Bao, S., Zhang, Z., Lian, Q., Sun, Q., & Zhang, R. (2019). Evolution and expression of genes encoding TCP transcription factors in *Solanum tuberosum* reveal the involvement of StTCP23 in plant defence. *BMC Genetics*, *20*(91). https://doi.org/10.1186/s12863-019-0793-1

Bartoli, C. G., Pastori, G. M., & Foyer, C. H. (2000). Ascorbate Biosynthesis in Mitochondria Is Linked to the Electron Transport Chain between Complexes III and IV. *Plant Physiology*, *123*, 335-344.

Baxter, C. J., Redestig, H., Schauer, N., Repsilber, D., Patil, K. R., Nielsen, J., Selbig, J., Liu, J., Fernie, A. R., & Sweetlove, L. J. (2007). The metabolic response of heterotrophic Arabidopsis cells to oxidative stress. *Plant Physiology*, *143*, 312–325.

Becalski, A., Lau, B. P. Y., Lewis, D., Seaman, S. W., Hayward, S., Sahagian, M., Ramesh, M., & Leclerc, Y. (2004). Acrylamide in French fries: Influence of free amino acids and sugars. *Journal of Agricultural and Food Chemistry*, *52*, 3801–3806. https://doi.org/10.1021/jf0349376

Beck, E., & Ziegler, P. (1989). Biosynthesis and degradation of starch in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *40*, 95– 117.

Beckman, C. H. (2000). Phenolic-storing cells: Keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiological and Molecular Plant Pathology*, *57*, 101–110. https://doi.org/10.1006/pmpp.2000.0287

Bell-Lelong, D. A., Cusumano, J. C., Meyer, K., & Chapple, C. (1997). Cinnamate-4-hydroxylase expression in Arabidopsis. Regulation in response to development and the environment. *Plant Physiology*, *113*(3), 729–738. https://doi.org/10.1104/pp.113.3.729

Bhaskar, P. B., Wu, L., Busse, J. S., Whitty, B. R., Hamernik, A. J., Jansky, S. H., Buell, C. R., Bethke, P. C., & Jiang, J. (2010). Suppression of the vacuolar invertase gene prevents cold-induced sweetening in potato. *Plant Physiology*, *154*, 939-948.

Bienert, G. P., Møller, A. L. B., Kristiansen, K. A., Schulz, A., Moller, I. M., Schjoerring, J. K., & Jahn, T. P. (2007). Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *Journal of Biological Chemistry*, *282*, 1183–1192.

Bienert, G. P., Schjoerring, J. K., & Jahn, T. P. (2006). Membrane transport of hydrogen peroxide. *Biochimica et Biophysica Acta*, *1758*(8), 994–1003. Doi: 10.1104/pp.106.090431

Bing, L., Feng, C. C., Li, J. L., Li, X. X., Zhao, B. C., Shen, Y. Z., Huang, Z. J., & Ge, R. C. (2013). Overexpression of the AtSTK gene increases salt, PEG and ABA tolerance in Arabidopsis. *Journal of Plant Biology*, *56*, 375–382. https://doi.org/10.1007/s12374-013-0154-y

Bläsing, O. E., Gibon, Y., Günther, M., Höhne, M., Morcuende, R., Osuna, D., Thimm, O., Usadel, B., Scheible, W. R., & Stitt, M. (2005). Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. *Plant Cell*, *176*(1), 3257–3281. https://doi.org/10.1105/tpc.105.035261

Bolle, C. (2004). The role of GRAS proteins in plant signal transduction and development. *Planta*, *218*, 683–692. Doi: 10.1007/s00425-004-1203-z

Bolwell, G. P., Bindschedler, L. V., Blee, K. A., Butt, V. S., Davies, D. R., Gardner, S. L., Gerrish, C., & Minibayeva, F. (2002). The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. *Journal of Experimental Botany*, *53*, 1367–1376.

Borchert, S., Große, H., & Heldt, H. W. (1989). Specific transport of inorganic phosphate, glucose 6-phosphate, dihydroxyacetone phosphate and 3-phosphoglycerate into amyloplasts from pea roots. *FEBS Letters*, *253*, 183–186. https://doi.org/10.1016/0014-5793(89)80955-X

Borchert, Sieglinde, Harborth, J., Schünemann, D., Hoferichter, P., & Heldt, H. W. (1993). Studies of the enzymic capacities and transport properties of pea root plastids. *Plant Physiology*, *101*, 303–312. https://doi.org/10.1104/pp.101.1.303

Boudsocq, M., Barbier-Brygoo, H., & Laurière, C. (2004). Identification of nine sucrose nonfermenting 1-related protein kinases 2 activated by hyperosmotic and saline stresses in *Arabidopsis thaliana*. *Journal of Biological Chemistry*, *279*, 41758–41766. https://doi.org/10.1074/jbc.M405259200

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brierley, E. R., Bonner, P. L. R., & Cobb, A. H. (1996). Factors influencing the free amino acid content of potato (*Solanum tuberosum* L.) tubers during prolonged storage. *Journal of the Science of Food and Agriculture*, *70*, 515–525. ISSN 0022-5142

Burton, W. G. (1969). The sugar balance in some British potato varieties during storage. II. The effects of tuber age, previous storage temperature, and intermittent refrigeration upon low-temperature sweetening. *Europe Potato Journal*, *12*, 81-95.

Burton, W. G. (1989). Post-harvest physiology. In Burton, W.G. (Ed.), *The Potato*, 3rd ed. Longman Scientific and Technical, Harlow, pp. 423–522.

Burton, W. G., van Es, A., & Hartmans, K. J. (1992). The physics and physiology of storage. In: Paul, H., editor. *The potato crop*. London: Chapman and Hall, pp. 608–727.

Cabib, E., & Leloir, L. F. (1958). The biosynthesis of trehalose phosphate. *The Journal of Biological Chemistry*, *231*, 259–275.

Carillo, P., Cacace, D., De Pascale, S., Rapacciuolo, M., & Fuggi, A. (2012). Organic vs. traditional potato powder. *Food Chemistry*, *133*, 1264–1273.

Carvalho, C. (2018). Understanding mechanisms and identifying markers for the onset of senescent sweetening. *AHDB Potatoes Report No. 2018/1.* https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/11140014%20Senescent%20Markers%20FINAL.pdf

Caspar, T., Huber, S. C., & Somerville, C. (1985). Alterations in Growth, Photosynthesis, and Respiration in a Starchless Mutant of *Arabidopsis thaliana* (L.) Deficient in Chloroplast Phosphoglucomutase Activity. *Plant Physiology*, *79*, 1–7. https://doi.org/10.1104/pp.79.1.11

Černá, M., & Kráčmar, S. (2010). The effect of storage on the amino acids composition in potato tubers. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *58*(5), 49–56. https://doi.org/10.11118/actaun201058050049

Chen, Q., Xu, X., Xu, D., Zhang, H., Zhang, C., & Li, G. (2019). WRKY18 and WRKY53 coordinate with HISTONE ACETYLTRANSFERASE1 to regulate rapid responses to sugar. *Plant Physiology*, *180*, 2212–2226. https://doi.org/10.1104/pp.19.00511

Chen, Q., Zhang, M., & Shen, S. (2010). Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (*Helianthus tuberosus* L.). *Acta Physiologiae Plantarum*, *33*(2), 273–278.

Cho, Y. H., & Yoo, S. D. (2011). Signaling role of fructose mediated by FINS1/FBP in *Arabidopsis thaliana*. *PLoS Genetics*, *7*(1), 1001263. https://doi.org/10.1371/journal.pgen.1001263

Coffin, R. H., Yada, R. Y., Parkin, K. L., Grodzinski, B., & Stanley, D. W. (1987). Effect of low temperature storage on sugar concentrations and chip color of certain processing potato cultivars and selections. *Journal of Food Science*, *52*, 639-645.

Coleman, W. K., Tai, G. C. C., Clayton, S., Howie, M., & Pereira, A. (1993). A portable monitor for the rapid assessment of processing quality of stored potato tubers. *American Potato Journal*, *70*(12), 909-923.

Colgan, R., Rees, D., & Briddon, A. (2012). Senescent sweetening. *Potato Council Research Review 2012/6*. https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/20126%20Senescent%20Sweetening%20R442.pdf

Considine, M. J., Goodman, M., Echtay, K. S., Laloi, M., Whelan, J., Brand, M. D., & Sweetlove, L. J. (2003). Superoxide stimulates a proton leak in potato mitochondria that is related to the activity of uncoupling protein. *Journal of Biological Chemistry*, *278*, 22298–22302.

Couée, I., Sulmon, C., Gouesbet, G., & El Amrani, A. (2006). An involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *Journal of Experimental Botany*, *57*, 449–459.

Cunnington, A. C. (2008). Developments in potato storage in Great Britain. *Potato Research*, *51***,** 403.
Doi: 10.1016/j.foodchem.2011.08.088

Dao, T. T., Linthorst, H. J., & Verpoorte, R. (2011). Chalcone synthase and its functions in plant resistance. *Phytochemistry Reviews*, *10*, 397–412.
Doi: 10.1007/s11101-011-9211-7

Davies, H. V., Shepherd, L. V. T., Burrell, M. M., Carrari, F., Urbanczyk-Wochniak, E., Leisse, A., Hancock, R. D., Taylor, M., Viola, R., Ross, H., McRae, D., Willmitzer, L., & Fernie, A. R. (2005). Modulation of fructokinase activity of potato (*Solanum tuberosum*) results in substantial shifts in tuber metabolism. *Plant and Cell Physiology*, *46*, 1103–1115. https://doi.org/10.1093/pcp/pci123

de Pinto, M. C., Paradiso, A., Leonetti, P., & De Gara, L. (2006). Hydrogen peroxide, nitric oxide and cytosolic ascorbate peroxidase at the crossroad between defence and cell death. *The Plant Journal*, *48*, 784–795.

De Veylder, L., Beeckman, T., Beemster, G. T. S., Krols, L., Terras, F., Landrieu, I., Van Der Schueren, E., Maes, S., Naudts, M., & Inzé D. (2001). Functional analysis of cyclin-dependent kinase inhibitors of Arabidopsis. *Plant Cell*, *13*, 1653–1667.

De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroodt, J. M., & Verhé, R. (2006). Influence of fertilization on acrylamide formation during frying of potatoes harvested in 2003. *Journal of Agricultural and Food Chemistry*, *54*, 404–408. https://doi.org/10.1021/jf0521810

Debast, S., Nunes-Nesi, A., Hajirezaei, M. R., Hofmann, J., Sonnewald, U., Fernie, A. R., & Börnke, F. (2011). Altering trehalose-6-phosphate content in transgenic potato tubers affects tuber growth and alters responsiveness to hormones during sprouting. *Plant Physiology*, *156*(4), 1754–1771. https://doi.org/10.1104/pp.111.179903

Desikan, R., Mackerness, S. A. H., Hancock, J. T., & Neill, S. J. (2001). Regulation of the Arabidopsis transcriptome by oxidative stress. *Plant Physiology*, *127*(1), 159– 172.

Dethloff, F., Orf, I., & Kopka, J. (2017). Rapid in situ ^{13}C tracing of sucrose utilization in Arabidopsis sink and source leaves. *Plant methods*, *13*, 87. https://doi.org/10.1186/s13007-017-0239-6

Dhindsa, R. S., Plumb-Dhlndsa, P., & Thorpe, T. A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation; and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, *32*, 93-101.

Driskill, E. P., Knowles, L. O., & Knowles, N. R. (2007). Temperature-induced changes in potato processing quality during storage are modulated by tuber maturity. *American Journal of Potato Research, 84*, 367-383.

Ducreux, L. J. M., Morris, W. L., Prosser, I. M., Morris, J. A., Beale, M. H., Wright, F., Shepherd, T., Bryan, G. J., Hedley, P. E., & Taylor, M. A. (2008). Expression profiling of potato germplasm differentiated in quality traits leads to the identification of candidate flavour and texture genes. *Journal of Experimental Botany*, *59*, 4219–4231. Doi: 10.1093/jxb/ern264

Dynowski, M., Schaaf, G., Loque, D., Moran, O., & Ludewig, U. (2008). Plant plasma membrane water channels conduct the signalling molecule H_2O_2. *Biochemical Journal*, *414*, 53–61.

Dyson, B. C., Allwood, J. W., Feil, R., Xu, Y., Miller, M., Bowsher, C. G., Goodacre, R., Lunn, J. E., & Johnson, G. N. (2015). Acclimation of metabolism to light in *Arabidopsis thaliana*: The glucose 6-phosphate/phosphate translocator GPT2 directs metabolic acclimation. *Plant, Cell and Environment*, *38*, 1404–1417. https://doi.org/10.1111/pce.12495

Dyson, B. C., Webster, R. E., & Johnson, G. N. (2014). GPT2: A glucose 6-phosphate/phosphate translocator with a novel role in the regulation of sugar signalling during seedling development. *Annals of Botany*, *113*, 643–652. https://doi.org/10.1093/aob/mct298

Eastmond, P. J., & Graham, I. A. (2003). Trehalose metabolism: A regulatory role for trehalose-6-phosphate? *Current Opinion in Plant Biology*, *6*(3), 231–235. https://doi.org/10.1016/S1369-5266(03)00037-2

Eastmond, P. J., Van Dijken, A. J. H., Spielman, M., Kerr, A., Tissier, A. F., Dickinson, H. G., Jones, J. D. G., Smeekens, S. C., & Graham, I. A. (2002). Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. *Plant Journal*, *29*, 225–235. https://doi.org/10.1046/j.1365-313x.2002.01220.x

Elbein, A. D., Pan, Y. T., Pastuszak, I., & Carroll, D. (2003). New insights on trehalose: A multifunctional molecule. *Glycobiology*, *13*(4), 17–27. https://doi.org/10.1093/glycob/cwg047

Elmore, J. S., Mottram, D. S., Muttucumaru, N., Dodson, A. T., Parry, M. A. J., & Halford, N. G. (2007). Changes in free amino acids and sugars in potatoes due to sulfate fertilization and the effect on acrylamide formation. *Journal of Agricultural and Food Chemistry*, *55*(13), 5363–5366. https://doi.org/10.1021/jf070447s

Entwistle, G., & Rees, T. A. (1988). Enzymic capacities of amyloplasts from wheat (*Triticum aestivum*) endosperm. *The Biochemical Journal*, *255*, 391–396. https://doi.org/10.1042/bj2550391

Eppendorfer, W. H., & Bille, S. W. (1996). Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilisation and phosphorus and potassium deficiency. *Journal of the Science of Food and Agriculture*, *71*, 449–458.
Doi: 10.1002/(SICI)1097-0010(199608)71:4<449::AID-JSFA601>3.0.CO;2-N

Faize, M., Burgos, L., Faize, L., Piqueras, A., Nicolas, E., Barba-Espin, G., Clemente-Moreno, M. J., Alcobendas, R., Artlip, T., & Hernandez, J. A. (2011). Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. *Journal of Experimental Botany*, *62*(8), 2599–2613.

Fauconnier, M. L., Rojas-Beltran, J., Delcarte, J., Dejaeghere, F., Marlier, M., & du Jardin, P. (2002). Lipoxygenase pathway and membrane permeability and composition during storage of potato tubers (*Solanum tuberosum* L. cv Bintje and Desiree) in different conditions. *Plant Biology*, *4*(1), 77-85.

Fernie, A. R., Roessner, U., Trethewey, R. N., & Willmitzer, L. (2001). The contribution of plastidial phosphoglucomutase to the control of starch synthesis within the potato tuber. *Planta*, *213*, 418–426.

Ferreira, S. J., & Sonnewald, U. (2012). The mode of sucrose degradation in potato tubers determines the fate of assimilate utilization. *Frontiers in Plant Science*, *3*(23), 1–18. https://doi.org/10.3389/fpls.2012.00023

Ferreira, S. J., Senning, M., Sonnewald, S., Keßling, P. M., Goldstein, R., & Sonnewald, U. (2010). Comparative transcriptome analysis coupled to X-ray CT reveals sucrose

supply and growth velocity as major determinants of potato tuber starch biosynthesis. *BMC Genomics*, *11*, 93. https://doi.org/10.1186/1471-2164- 11-93

Fischer, R. L., & Bennett, A. B. (1991). Role of cell wall hydrolases in fruit ripening. *Annual Review of Plant Physiology and Plant Molecular Biology*, *42*, 675–703.

Flachowsky, H., Halbwirth, H., Treutter, D., Richter, K., Hanke, M. V., Szankowski, I., Gosch, C., Stich, K., & Fischer, T. C. (2012). Silencing of flavanone-3-hydroxylase in apple (*Malus domestica* Borkh.) leads to accumulation of flavanones, but not to reduce fire blight susceptibility. *Plant Physiology and Biochemistry*, *51*, 18-25. Doi: 10.1016/j.plaphy.2011.10.004

Fletcher, B. L., Dillard, C. J., & Tappel A. L. (1973). Measurement of fluorescent lipid peroxidation products in biological systems. *Analytical Biochemistry*, *52*, 1-9.

Flügge, U. I., & Weber, A. (1994). A rapid method for measuring organelle-specific substrate transport in homogenates from plant tissues. *Planta*, *194*, 181–185. https://doi.org/10.1007/BF00196386

Flugge, U.L. (1995). Phosphate translocation in the regulation of photosynthesis. *Journal of Experimental Botany*, *46*, 1317–1323. https://doi.org/10.1093/jxb/46.special_issue.1317

Foito, A., Byrne, S. L., Hackett, C. A., Hancock, R. D., Stewart, D., & Barth, S. (2013). Short-term response in leaf metabolism of perennial ryegrass (*Lolium perenne*) to alterations in nitrogen supply. *Metabolomics*, *9*, 145–156. Doi: 10.1007/s11306-012-0435-3

Fujii, H., & Zhu, J. K. (2009). Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 8380–8385. https://doi.org/10.1073/pnas.0903144106

Fujii, H., Verslues, P. E., & Zhu, J. K. (2007). Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in Arabidopsis. *Plant Cell*, *19*, 485–494. https://doi.org/10.1105/tpc.106.048538

Fujii, H., Verslues, P. E., & Zhu, J. K. (2011). Arabidopsis decuple mutant reveals the importance of SnRK2 kinases in osmotic stress responses in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 1717–1722. https://doi.org/10.1073/pnas.1018367108

Fuller, T. J., & Hughes, J. C. (1984). Factors influencing the relationships between reducing sugars and fry colour of potato tubers of cv. Record. *International Journal of Food Science & Technology*, *19*(4), 455-467.

Gancedo, C., & Flores, C. L. (2004). The importance of a functional trehalose biosynthetic pathway for the life of yeasts and fungi. *FEMS Yeast Research*, *4*(4-5), 351–359. https://doi.org/10.1016/S1567-1356(03)00222-8

Gechev, T. S., & Hille, J. (2005). Hydrogen peroxide as a signal controlling plant programmed cell death. *Journal of Cell Biology*, *168*, 17–20.

Gechev, T. S., Van Breusegem, F., Stone, J. M., Denev, I., & Laloi, C. (2006). Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *Bioessays*, *28*, 1091–1101.

Geigenberger, P., Stitt, M., & Fernie, A. R. (2004). Metabolic control analysis and regulation of the conversion of sucrose to starch in growing potato tubers. *Plant, Cell and Environment*, *27*(6), 655–673. https://doi.org/10.1111/j.1365-3040.2004.01183.x

Ghillebert, R., Swinnen, E., Wen, J., Vandesteene, L., Ramon, M., Norga, K., Rolland, F., & Winderickx, J. (2011). The AMPK/SNF1/SnRK1 fuel gauge and energy regulator: Structure, function and regulation. *FEBS Journal*, *278*(21), 3978–3990. https://doi.org/10.1111/j.1742-4658.2011.08315.x

Gidrol, X., Serghini, H., Noubhani, A., Mocquot, B., & Mazalik, P. (1989). Biochemical changes induced by accelerated aging in sunflower seeds. I. Lipid peroxidation and membrane damage. *Physiologia Plantarum*, *76*, 591-597.

Gong, X., Liu, M., Zhang, L., Ruan, Y., Ding, R., Ji Y., Zhang, N., Zhang, S., Farmer, J., & Wang, C. (2015). Arabidopsis AtSUC2 and AtSUC4 encoding sucrose transporters, are required for abiotic stress tolerance in an ABA-dependent pathway. *Physiologia Plantarum*, *153*, 119–136. Doi: 10.1111/ppl.12225

Gonzali, S., Loreti, E., Solfanelli, C., Novi, G., Alpi, A., & Perata, P. (2006). Identification of sugar-modulated genes and evidence for in vivo sugar sensing in Arabidopsis. *Journal of Plant Research*, *119*, 115–123. https://doi.org/10.1007/s10265-005-0251-1

Gross, K. C. (1984). Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. *Physiologia Plantarum*, *62*, 25–32.

Gross, K. C., & Sams, C. E. (1984). Changes in cell wall neutral sugar composition during fruit ripening: a species survey. *Phytochemistry*, *23*, 2457–2461.

Groves, S., Wiltshire, J., Briddon, A. & Cunnington, A. (2005). Managing maturity to improve crop processing quality and storage. *AHDB Potato Council report* 807/236, Kenilworth, Warwickshire, CV8 2TL, GB.

Hajirezaei, M. R., Bornke, F., Peisker, M., Takahata, Y., Lerchl, J., Kirakosyan, A., & Sonnewald, U. (2003). Decreased sucrose content triggers starch breakdown and respiration in stored potato tubers (*Solanum tuberosum*). *Journal of Experimental Botany*, *54*, 477–488.

Halford, N. G., & Hardie, D. G. (1998). SNF1-related protein kinases: global regulators of carbon metabolism in plants? *Plant Molecular Biology*, *37*, 735–748. Doi: 10.1023/A:1006024231305

Halford, N. G., Curtis, T. Y., Muttucumaru, N., Postles, J., & Mottram, D. S. (2011). Sugars in crop plants. *Annals of Applied Biology*, *158*, 1–25. https://doi.org/10.1111/j.1744-7348.2010.00443.x

Halford, N. G., Muttucumaru, N., Powers, S. J., Gillatt, P. N., Hartley, L., Elmore, J. S., & Mottram, D. S. (2012). Concentrations of free amino acids and sugars in nine potato varieties: effects of storage and relationship with acrylamide formation. *Journal of Agricultural and Food Chemistry*, *60*, 12044–12055. Doi: 10.1021/jf3037566

Hancock, R. D. (2017). Oxidative Stress. *In*: Thomas B., Murray B.G. & Murphy D.J. (eds.) *Encyclopedia of Applied Plant Sciences*, Vol 1, Waltham, MA: Academic Press, pp 27-35.

Hancock, R. D., Morris, W. L., Ducreux, L. J. M., Morris, J. A., Usman, M., Verrall, S. R., Fuller, J., Simpson, C. G., Zhang, R., Hedley, P. E., & Taylor, M. A. (2014). Physiological, biochemical and molecular responses of the potato (*Solanum tuberosum* L.) plant to moderately elevated temperature. *Plant, Cell and Environment*, *37*(2), 439–450. https://doi.org/10.1111/pce.12168

Hartmann, A., Senning, M., Hedden, P., Sonnewald, U., & Sonnewald, S. (2011). Reactivation of meristem activity and sprout growth in potato tubers require both cytokinin and gibberellin. *Plant Physiology*, *155*(2), 776–796. https://doi.org/10.1104/pp.110.168252

Hasegawa, M., Bressan, R., & Pardo, J. M. (2000). The dawn of plant salt tolerance genetics. *Trends in Plant Science*, *5*, 317–319. Doi: 10.1016/S1360-1385(00)01692-7

Hedrich, R., Sauer, N., & Neuhaus, H. E. (2015). Sugar transport across the plant vacuolar membrane: nature and regulation of carrier proteins. *Current Opinion in Plant Biology*, *25*, 63–70. Doi: 10.1016/j.pbi.2015.04.008

Heijde, M., & Ulm, R. (2012). UV-B photoreceptor-mediated signalling in plants. *Trends in Plant Science*, *17*(4) 230–237. https://doi.org/10.1016/j.tplants.2012.01.007

Hendriks, J. H. M., Kolbe, A., Gibon, Y., Stitt, M., & Geigenberger, P. (2003). ADP-Glucose Pyrophosphorylase Is Activated by Posttranslational Redox-Modification in Response to Light and to Sugars in Leaves of Arabidopsis and Other Plant Species. *Plant Physiology*, *133*(2), 838–849. https://doi.org/10.1104/pp.103.024513

Hertog, M., Tijskens, L. M. M., & Hak, P. S. (1997). The effects of temperature and senescence on the accumulation of reducing sugars during storage of potato (*Solanum tuberosum* L) tubers: A mathematical model. *Postharvest Biology and Technology*, *10*(1), 67-79.

Hill, L. M., & Smith, A. M. (1991). Evidence that glucose 6-phosphate is imported as the substrate for starch synthesis by the plastids of developing pea embryos. *Planta*, *185*, 91–96. https://doi.org/10.1007/BF00194519

Hill, L. M., & Smith, A. M. (1995). Coupled Movements of Glucose 6-Phosphate and Triose Phosphate through the Envelopes of Plastids from Developing Embryos of Pea *(Pisum sativum* L.). *Journal of Plant Physiology*, *146*, 411–417. https://doi.org/10.1016/S0176-1617(11)82001-8

Hodges, D., DeLong, J., Forney, C., & Prange, R. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, 207, 604–611. https://doi.org/10.1007/s004250050524

Hong, L., Hu, B., Liu, X., He, C. Y., Yao, Y., Li, X. L., & Li, L. (2013). Molecular cloning and expression analysis of a new stress-related AREB gene from *Arachis hypogaea*. *Biologia Plantarum*, *57*, 56–62. https://doi.org/10.1007/s10535-012-0236-6

Huang, B., Hu, J., & Rohrer, J. (2016). Determination of Carbohydrates in Kombucha Using HPAE-PAD. Thermo Fisher Scientific, Sunnyvale, CA, USA.

Huege, J., Sulpice, R., Gibon, Y., Lisec, J., Koehl, K., & Kopka, J. (2007). GC-EI-TOF-MS analysis of in vivo carbon-partitioning into soluble metabolite pools of higher plants by monitoring isotope dilution after $^{13}CO_2$ labelling. *Phytochemistry*, *68*(16), 2258–2272. https://doi.org/10.1016/j.phytochem.2007.03.026

Ignat, I., Volf, I., & Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, *126*(4), 1821–1835. https://doi.org/10.1016/j.foodchem.2010.12.026

Jammer, A., Gasperl, A., Luschin-Ebengreuth, N., Heyneke, E., Chu, H., Cantero-Navarro, E., Großkinsky, D. K., Albacete, A. A., Stabentheiner, E., Franzaring, J., Fangmeier, A., Graaff, E. Van Der, & Roitsch, T. (2015). Simple and robust determination of the activity signature of key carbohydrate metabolism enzymes for physiological phenotyping in model and crop plants. *Journal of Experimental Botany*, *66*(18), 5531–5542. https://doi.org/10.1093/jxb/erv228

Ji, H., Wang, Y., Cloix, C., Li, K., Jenkins, G. I., Wang, S., Shang, Z., Shi, Y., Yang, S., & Li, X. (2015). The Arabidopsis RCC1 Family Protein TCF1 Regulates Freezing Tolerance and Cold Acclimation through Modulating Lignin Biosynthesis. *PLoS Genetics*, *11*, e1005471. https://doi.org/10.1371/journal.pgen.1005471

Johnson, R. R., Wagner, R. L., Verhey, S. D., & Walker-Simmons, M. K. (2002). The abscisic acid-responsive kinase PKABA1 interacts with a seed-specific abscisic acid response element-binding factor, TaABF, and phosphorylates TaABF peptide sequences. *Plant Physiology*, *130*, 837–846. https://doi.org/10.1104/pp.001354

Kammerer, B., Fischer, K., Hilpert, B., Schubert, S., Gutensohn, M., Weber, A., & Flügge, U. L. (1998). Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: The glucose 6-phosphate/phosphate antiporter. *Plant Cell*, *10*, 105–117. https://doi.org/10.1105/tpc.10.1.105

Kandel-Kfir, M., Damari-Weissler, H., German, M. A., Gidoni, D., Mett, A., Belausov, E., Petreikov, M., Adir, N., & Granot, D. (2006). Two newly identified membrane-associated and plastidic tomato HXKs: Characteristics, predicted structure and intracellular localization. *Planta*, *224*, 1341–1352. https://doi.org/10.1007/s00425-006-0318-9

Kirsch, M., An, Z., Viereck, R., Low, R., & Rausch, T. (1996). Salt stress induces an increased expression of V-type H(+)-ATPase in mature sugar beet leaves. *Plant Molecular Biology*, *32*, 543–547. Doi: 10.1007/BF00019107

Kiyosue, T., Abe, H., Yamaguchi-Shinozaki, K., & Shinozaki, K. (1998). ERD6 a cDNA clone for an early dehydration-induced gene of Arabidopsis, encodes a putative sugar transporter. *Biochimica et Biophysica Acta*, *1370*, 187–191. Doi: 10.1016/S0005-2736(98)00007-8

Kleinkopf, G. E., Oberg, N. A., & Olsen, N. (2003). Sprout inhibition in storage: current status, new chemistries and natural compounds. *American Journal of Potato Research*, *80*, 317–327. Doi: 10.1007/BF02854316

Knappe, S., Flügge, U. I., & Fischer, K. (2003). Analysis of the plastidic phosphate translocator gene family in Arabidopsis and identification of new phosphate translocator-homologous transporters, classified by their putative substrate-binding site. *Plant Physiology*, *131*, 1178–1190. https://doi.org/10.1104/pp.016519

Knowles, N. R, & Knowles, L. O. (1989). Correlations between electrolyte leakage and degree of saturation of polar lipids from aged potato (*Solanum tuberosum* L) tuber tissue. *Annals of Botany*, *63*, 331-338.

Kofler, H., Häusler, R. E., Schulz, B., Gröner, F., Flügge, U. I., & Weber, A. (2000). Molecular characterisation of a new mutant allele of the plastid phosphoglucomutase in Arabidopsis, and complementation of the mutant with the wild-type cDNA. *Molecular and General Genetics*, *263*, 978–986. https://doi.org/10.1007/PL00008698

Kolbe, A., Tiessen, A., Schluepmann, H., Paul, M., Ulrich, S., & Geigenberger, P. (2005). Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 11118–11123. https://doi.org/10.1073/pnas.0503410102

Konze, J. R., & Elstner, E. F. (1978). Ethane and ethylene formation by mitochondria as indication of aerobic lipid degradation in response to wounding of plant tissue. *Biochimica et Biophysica Acta*, *528*, 213-221.

Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., & Scharf, K. D. (2007). Complexity of the heat stress response in plants. *Current Opinion in Plant Biology*, *10*, 310–316.

Kroon, P. A., & Williamson, G. (1999). Hydroxycinnamates in plants and food: Current and future perspectives. *Journal of the Science of Food and Agriculture*, *79*(3), 355–361. https://doi.org/10.1002/(SICI)1097-0010(19990301)79:3<355::AID-JSFA255>3.0.CO;2-G

Kuhlemeier, C., Green, P. J., & Chua, N. H. (1987). Regulation of gene expression in higher plants. *Annual Review of Plant Physiology*, *38*, 221-257.

Kumar, D., Singh, B. P., & Kumar, P. (2004). An overview of the factors affecting sugar content of potatoes. *Annals of Applied Biology*, *145*(3), 247–256. https://doi.org/10.1111/j.1744-7348.2004.tb00380.x

Kumar, G. N. M., & Knowles, N. R. (1993). Changes in lipid-peroxidation and lipolytic and free-radical scavenging enzyme-activities during aging and sprouting of potato (*Solanum tuberosum*) seed-tubers. *Plant Physiology*, *102*(1), 115-124.

Kumar, G. N. M., & Knowles, N. R. (1996). Oxidative stress results in increased sinks for metabolic energy during aging and sprouting of potato seed-tubers. *Plant Physiology, 112*, 1301-1313.

Kumar, G. N. M., Houtz, R. L., & Knowles, N. R. (1999). Age-induced protein modifications and increased proteolysis in potato seed-tubers. *Plant Physiology*, *119*, 89-99.

Kunz, H. H., Häusler, R. E., Fettke, J., Herbst, K., Niewiadomski, P., Gierth, M., Bell, K., Steup, M., Flügge, U. I., & Schneider, A. (2010). The role of plastidial glucose-6-phosphate/phosphate translocators in vegetative tissues of *Arabidopsis thaliana* mutants impaired in starch biosynthesis. *Plant Biology*, *12*, 115–128. https://doi.org/10.1111/j.1438-8677.2010.00349.x

Laurie, S., & Halford, N. G. (2001). The role of protein kinases in the regulation of plant growth and development. *Plant Growth Regulation*, *34*, 253–265. https://doi.org/10.1023/A:1013311807626

Lee, Y. P., Kim, S. H., Bang, J. W., Lee, H. S., Kwak, S. S., & Kwon, S. Y. (2007). Enhanced tolerance to oxidative stress in transgenic tobacco plants expressing three antioxidant enzymes in chloroplasts. *Plant Cell Reports*, *26*(5), 591–598.

Lemos, M. A., Aliyu, M. M., & Hungerford, G. (2015). Influence of cooking on the levels of bioactive compounds in Purple Majesty potato observed via chemical and spectroscopic means. *Food Chemistry*, *173*, 462–467. https://doi.org/10.1016/j.foodchem.2014.10.064

Leshem, Y. Y. (1987). Membrane phospholipid catabolism and Ca^{+2} activity in control of senescence. *Physiologia Plantarum*, *69*, 551-559.

Li, M., Feng, F., & Cheng, L. (2012). Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. *PLoS ONE*, *7*(3), 33055. https://doi.org/10.1371/journal.pone.0033055

Li, P., Wind, J. J., Shi, X., Zhang, H., Hanson, J., Smeekens, S. C., & Teng, S. (2011). Fructose sensitivity is suppressed in Arabidopsis by the transcription factor ANAC089 lacking the membrane-bound domain. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(8), 3436–3441. https://doi.org/10.1073/pnas.1018665108

Lin, T. P., Caspar, T., Somerville, C., & Preiss, J. (1988). Isolation and Characterization of a Starchless Mutant of *Arabidopsis thaliana* (L.) Heynh Lacking ADPglucose Pyrophosphorylase Activity. *Plant Physiology*, *86*, 1131–1135. https://doi.org/10.1104/pp.86.4.1131

Liu, J., Zhang, Y., Qin, G., Tsuge, T., Sakaguchi, N., Luo, G., Sun, K., Shi, D., Aki, S., Zheng, N., Aoyama, T., Oka, A., Yang, W., Umeda, M., Xie, Q., Gu, H., & Qu, L. J. (2008). Targeted degradation of the cyclin-dependent kinase inhibitor ICK4/KRP6 by RING-type E3 ligases is essential for mitotic cell cycle progression during Arabidopsis gametogenesis. *Plant Cell*, *20*, 1538–1554.

Liu, Y., Ren, D., Pike, S., Pallardy, S., Gassmann, W., & Zhang, S. (2007). Chloroplast-generated reactive oxygen species are involved in hypersensitive response-like cell death mediated by a mitogen-activated protein kinase cascade. *The Plant Journal*, *51*, 941–954.

Lulai, E. C., Suttle, J. C., & Pederson, S. M. (2008). Regulatory involvement of abscisic acid in potato tuber wound-healing. *Journal of Experimental Botany*, *59*, 1175–1186. Doi: 10.1093/jxb/ers296

Lundin, B., Hansson, M., Schoefs, B., Vener, A. V., & Spetea, C. (2007). The Arabidopsis PsbO2 protein regulates dephosphorylation and turnover of the photosystem II reaction centre D1 protein. *Plant Journal*, *49*(3), 528–539. https://doi.org/10.1111/j.1365-313X.2006.02976.x

Lunn, J. E., Delorge, I., Figueroa, C. M., Van Dijck, P., & Stitt, M. (2014). Trehalose metabolism in plants. *Plant Journal*, *79*, 544–567. https://doi.org/10.1111/tpj.12509

Lunn, John E., Feil, R., Hendriks, J. H. M., Gibon, Y., Morcuende, R., Osuna, D., Scheible, W. R., Carillo, P., Hajirezaei, M. R., & Stitt, M. (2006). Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. *Biochemical Journal*, *397*, 139–148. https://doi.org/10.1042/BJ20060083

Matsuura-Endo, C., Kobayashi, A., Noda, T., Takigawa, S., Yamauchi, H., & Mori, M. (2004). Changes in sugar content and activity of vacuolar acid invertase during low-temperature storage of potato tubers from six Japanese cultivars. *Journal of Plant Research*, *117*, 131–137.

Medici, A., Laloi, M., & Atanassova, R. (2014). Profiling of sugar transporter genes in grapevine coping with water deficit. *FEBS Letters*, *588*, 3989–3997. Doi: 10.1016/j.febslet.2014.09.016

Miller, G., & Mittler, R. (2006). Could heat shock transcription factors function as hydrogen peroxide sensors in plants? *Annals of Botany*, *98*, 279–288.

Miller, G., Shulaev, V., & Mittler, R. (2008). Reactive oxygen signalling and abiotic stress. *Physiologia Plantarum*, *133*, 481–489.

Mishra, B. S., Singh, M., Aggrawal, P., & Laxmi, A. (2009). Glucose and auxin signaling interaction in controlling *Arabidopsis thaliana* seedlings root growth and development. *PLoS ONE*, *4*(2), 4502. https://doi.org/10.1371/journal.pone.0004502

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Sciences*, *7*, 405–410.

Møller, I. M. (2001). Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annual Review of Plant Physiology and Plant Molecular Biology*, *52*, 561–591.

Morell, M. K., Bloom, M., Knowles, V., & Preiss, J. (1987). Subunit Structure of Spinach Leaf ADPglucose Pyrophosphorylase. *Plant Physiology*, *85*, 182–187. https://doi.org/10.1104/pp.85.1.182

Mottram, D. S. (2007). The Maillard reaction: Source of flavour in thermally processed foods. In *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*, (pp. 269–284). https://doi.org/10.1007/978-3-540-49339-6_12

Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature*, *419*(6906), 448-449.

Movahedi, S., Tabatabaei, B. E. S., Alizade, H., Ghobadi, C., Yamchi, A., & Khaksar, G. (2012). Constitutive expression of Arabidopsis DREB1B in transgenic potato

enhances drought and freezing tolerance. *Biologia Plantarum*, *56*, 37–42. https://doi.org/10.1007/s10535-012-0013-6

Mubarakshina, M. M., Ivanov, B. N., Naydov, I. A., Hillier, W., Badger, M. R., & Krieger-Liszkay, A. (2010). Production and diffusion of chloroplastic H_2O_2 and its implication to signalling. *Journal of Experimental Botany*, *61*, 3577–3587.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell and Environment*, *25*, 239–250.

Murshed, R., Lopez-Lauri, F., & Sallanon, H. (2008). Microplate quantification of enzymes of the plant ascorbate-glutathione cycle. *Analytical Biochemistry*, *383*(2), 320–322. https://doi.org/10.1016/j.ab.2008.07.020

Mustilli, A. C., Merlot, S., Vavasseur, A., Fenzi, F., & Giraudat, J. (2002). Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell*, *14*, 3089–3099. https://doi.org/10.1105/tpc.007906

Muttucumaru, N., Powers, S. J., Briddon, A., Elmore, J. S., Mottram, D. S., & Halford, N. G. (2014). Evidence for the complex relationship between the concentrations of free amino acids, sugars and acrylamide-forming potential in potato. *Annals of Applied Biology*, *164*, 286-300.

Muttucumaru, N., Powers, S. J., Elmore, J. S., Mottram, D. S., & Halford, N. G. (2015). Effects of water availability on free amino acids, sugars, and acrylamide-forming potential in potato. *Journal of Agricultural and Food Chemistry*, *63*(9), 2566–2575. https://doi.org/10.1021/jf506031w

Muttucumaru, N., Powers, S. J., Elmore, J. S., Mottram, D. S., & Halford, N. G. (2013). Effects of nitrogen and sulfur fertilization on free amino acids, sugars and acrylamide-forming potential in potato. *Journal of Agricultural and Food Chemistry*, *61*, 6734–6742. Doi: 10.1021/jf401570x

Naeem, M., Tetlow, I. J., & Emes, M. J. (1997). Starch synthesis in amyloplasts purified from developing potato tubers. *Plant Journal*, *11*(5), 1095–1103. https://doi.org/10.1046/j.1365-313X.1997.11051095.x

Nakashima, K., Fujita, Y., Kanamori, N., Katagiri, T., Umezawa, T., Kidokoro, S., Maruyama, K., Yoshida, T., Ishiyama, K., Kobayashi, M., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2009). Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. *Plant and Cell Physiology*, *50*, 1345–1363. https://doi.org/10.1093/pcp/pcp083

Nambara, E., & Marion-Poll, A. (2005). Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology*, *56*(1), 165–185. Doi: 10.1146/annurev.arplant.56.032604.144046

Neill, S. J., Desikan, R., Clarke, A., Hurst, R. D., & Hancock, J. T. (2002). Hydrogen peroxide and nitric oxide as signaling molecules in plants. *Journal of Experimental Botany*, *53*, 1237–1247. Doi: 10.1093/jexbot/53.372.1237

Neuhaus, H. E., & Stitt, M. (1990). Control analysis of photosynthate partitioning - Impact of reduced activity of ADP-glucose pyrophosphorylase or plastid phosphoglucomutase on the fluxes to starch and sucrose in *Arabidopsis thaliana* (L.) *Heynh. Planta*, *182*, 445–454. https://doi.org/10.1007/BF02411398

Neuhaus, H. E., Batz, O., Thom, E., & Scheibe, R. (1993). Purification of highly intact plastids from various heterotrophic plant tissues: Analysis of enzymic equipment and precursor dependency for starch biosynthesis. *Biochemical Journal*, *296*, 395–401. https://doi.org/10.1042/bj2960395

Nicot, N., Hausman, J. F., Hoffmann, L., & Evers, D. (2005). Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *Journal of Experimental Botany*, *56*(421), 2907–2914.

Niewiadomski, P., Knappe, S., Geimer, S., Fischer, K., Schulz, B., Unte, U. S., Rosso, M. G., Ache, P., Flügge, U. I., & Schneider, A. (2005). The Arabidopsis plastidic glucose 6-phosphate/phosphate translocator GPT1 is essential for pollen maturation and embryo sac development. *Plant Cell*, *17*, 760–775. https://doi.org/10.1105/tpc.104.029124

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Biology*, *49*, 249–279.
Nursten, H. (2005). The Maillard Reaction: Chemistry, Biochemistry and Implications. In *Advances in Carbohydrate Chemistry*.
Nyvall Collén, P., Camitz, A., Hancock, R.D., Viola, R., & Pedersén, M. (2004). Effect of nutrient deprivation and resupply on metabolites and enzymes related to carbon allocation in *Gracilaria tenuistipatata* (Rhodophyta). *Journal of Phycology*, *40*, 305–314.
O'Hara, L. E., Paul, M. J., & Wingler, A. (2013). How do sugars regulate plant growth and development? New insight into the role of trehalose-6-phosphate. *Molecular Plant*, *6*(2), 261–274. https://doi.org/10.1093/mp/sss120
Ogawa, D., Abe, K., Miyao, A., Kojima, M., Sakakibara, H., Mizutani, M., Morita, H., Toda, Y., Hobo, T., Sato, Y., Hattori, T., Hirochika, H., & Takeda, S. (2011). RSS1 regulates the cell cycle and maintains meristematic activity under stress conditions in rice. *Nature Communications*, *2*, 278. https://doi.org/10.1038/ncomms1279
Ohad, I., Friedberg, I., Ne'eman, Z., & Schramm, M. (1971). Biogenesis and degradation of starch. I. The fate of the amyloplast membranes during maturation and storage of potato tubers. *Plant Physiology*, *47*, 465–477.
Okita, T. W., Nakata, P. A., Anderson, J. M., Sowokinos, J., Morell, M., & Preiss, J. (1990). The subunit structure of potato tuber ADPglucose pyrophosphorylase. *Plant Physiology*, *93*(2), 785–790. https://doi.org/10.1104/pp.93.2.785
Oruna-Concha, M. J., Duckham, S. C., & Ames, J. M. (2001). Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking. *Journal of Agricultural and Food Chemistry*, *49*, 2414–2421. Doi: 10.1021/jf0012345
Owens, D. K., Crosby, K. C., Runac, J., Howard, B. A., & Winkel, B. S. J. (2008). Biochemical and genetic characterization of Arabidopsis flavanone 3β-hydroxylase. *Plant Physiology and Biochemistry*, *46*, 833-843.
Parr, A. J., & Bolwell, G. P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, *80*(7), 985–1012. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<985::AID-JSFA572>3.0.CO;2-7
Paul, M. (2007). Trehalose 6-phosphate. *Current Opinion in Plant Biology*, *10*(3), 303– 309. https://doi.org/10.1016/j.pbi.2007.04.001
Paul, M. J., Primavesi, L. F., Jhurreea, D., & Zhang, Y. (2008). Trehalose Metabolism and Signaling. *Annual Review of Plant Biology*, *59*, 417–441. https://doi.org/10.1146/annurev.arplant.59.032607.092945
Paul, V., Ezekiel, R., & Pandey, R. (2016). Sprout suppression on potato: need to look beyond CIPC for more effective and safer alternatives. *Journal of Food Science and Technology*, *53*(1), 1–18. https://doi.org/10.1007/s13197-015-1980-3
Pedreschi, F. (2009). Fried and Dehydrated Potato Products. In: Singh, J. & Kaur, L. (eds.) *Advances in Potato Chemistry and Technology*.
Periappuram, C., Steinhauer, L., Barton, D. L., Taylor, D. C., Chatson, B., & Zou, J. (2000). The plastidic phosphoglucomutase from Arabidopsis. A reversible enzyme reaction with an important role in metabolic control. *Plant Physiology*, *122*(4), 1193–1199. https://doi.org/10.1104/pp.122.4.1193
Pesaresi, P., Scharfenberg, M., Weigel, M., Granlund, I., Schröder, W. P., Finazzi, G., Rappaport, F., Masiero, S., Furini, A., Jahns, P., & Leister, D. (2009). Mutants, overexpressors, and interactors of Arabidopsis plastocyanin isoforms: Revised roles of plastocyanin in photosynthetic electron flow and thylakoid redox state. *Molecular Plant*, *2*(2), 236–248. https://doi.org/10.1093/mp/ssn041
Petrov, V. D., & Van Breusegem, F. (2012). Hydrogen peroxide-a central hub for information flow in plant cells. *AoB PLANTS*, pls014. https://doi.org/10.1093/aobpla/pls014
Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, *29*(9), e45.
Pourtau, N., Jennings, R., Pelzer, E., Pallas, J., & Wingler, A. (2006). Effect of sugar-induced senescence on gene expression and implications for the regulation of

senescence in Arabidopsis. *Planta*, *224*(3), 556–568. https://doi.org/10.1007/s00425-006-0243-y

Pritchard, M. K., & Adam, L. R. (1994). Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody potatoes. *American Journal of Potato Research*, *71*, 59-68.

Qin, F., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2011). Achievements and challenges in understanding plant abiotic stress responses and tolerance. *Plant and Cell Physiology*, *52*(9), 1569–1582. https://doi.org/10.1093/pcp/pcr106

Queval, G., Hager, J., Gakière, B., & Noctor, G. (2008). Why are literature data for H_2O_2 contents so variable? A discussion of potential difficulties in quantitative assays of leaf extracts. *Journal of Experimental Botany*, *59*, 135–146.

Queval, G., & Noctor, G. (2007). A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: Application to redox profiling during Arabidopsis rosette development. *Analytical Biochemistry*, *363*(1), 58-69.

Quick, W. P., & Neuhaus, H. E. (1996). Evidence for two types of phosphate translocators in sweet-pepper (*Capsicum annum* L.) fruit chromoplasts. *The Biochemical Journal*, *320*, 7–10.

Razem, F. A., & Bernards, M. A. (2002). Hydrogen peroxide is required for poly(phenolic) domain formation during wound-induced suberization. *Journal of Agricultural and Food Chemistry*, *50*, 1009-1015.

Redgwell, R. J., Melton, L. D., & Brasch, D. J. (1992). Cell wall dissolution in ripening kiwifruit (*Actinidia deliciosa*). Solubilization of the pectic polymers. *Plant Physiology*, *98*, 71–81.

Reimholz, R., Geigenberger, P., & Stitt, M. (1994). Sucrose-phosphate synthase is regulated via metabolites and protein phosphorylation in potato tubers, in a manner analogous to the enzyme in leaves. *Planta*, *192*, 480–488. https://doi.org/10.1007/BF00203585

Rhoads, D. M., & Subbaiah, C. C. (2007). Mitochondrial retrograde regulation in plants. *Mitochondrion*, *7*, 177–194.

Richardson, D. L., Davies, H. V., Ross, H. A., & Mackay, G. R. (1990). Invertase activity and its relation to hexose accumulation in potato tubers. *Journal of Experimental Botany*, *41*, 95-99.

Ritte, G., & Raschke, K. (2003). Metabolite export of isolated guard cell chloroplasts of *Vicia faba*. *New Phytologist*, *159*, 195–202.

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., & Mittler, R. (2004). When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant Physiology*, *134*(4), 1683–1696. https://doi.org/10.1104/pp.103.033431

Rolland, F., Baena-Gonzalez, E., & Sheen, J. (2006). SUGAR SENSING AND SIGNALING IN PLANTS: Conserved and Novel Mechanisms. *Annual Review of Plant Biology*, *57*, 675–709. https://doi.org/10.1146/annurev.arplant.57.032905.105441

Rolletschek, H., Nguyen, T. H., Häusler, R. E., Rutten, T., Göbel, C., Feussner, I., Radchuk, R., Tewes, A., Claus, B., Klukas, C., Linemann, U., Weber, H., Wobus, U., & Borisjuk, L. (2007). Antisense inhibition of the plastidial glucose-6-phosphate/phosphate translocator in Vicia seeds shifts cellular differentiation and promotes protein storage. *Plant Journal*, *51*(3), 468–484. https://doi.org/10.1111/j.1365-313X.2007.03155.x

Ronning, C. M., Stegalkina, S. S., Ascenzi, R. A., Bougri, O., Hart, A. L., Utterbach, T. R., Vanaken, S. E., Riedmuller, S. B., White, J. A., Cho, J., Pertea, G. M., Lee, Y., Karamycheva, S., Sultana, R., Tsai, J., Quackenbush, J., Griffiths, H. M., Restrepo, S., Smart, C. D., Fry, W. E., van der Hoeven, R., Tanksley, S., Zhang, P., Jin, H., Yamamoto, M. L., Baker, B. J., & Buell, C. R. (2003). Comparative analyses of potato expressed sequence tag libraries. *Plant Physiology*, *131*, 419–429.

Rosa, M., Hilal, M., González, J. A., & Prado, F. E. (2004). Changes in soluble carbohydrates and related enzymes induced by low temperature during early developmental stages of quinoa (*Chenopodium quinoa*) seedlings. *Journal of Plant Physiology*, *161*, 683–689.

Ruan, Y. L., Jin, Y., Yang, Y. J., Li, G. J., & Boyer, J. S. (2010). Sugar input, metabolism, and signaling mediated by invertase: Roles in development, yield potential, and

response to drought and heat. *Molecular Plant*, *3*(6), 942–955. https://doi.org/10.1093/mp/ssq044
Runquist, M., & Kruger, N. J. (1999). Control of gluconeogenesis by isocitrate lyase in endosperm of germinating castor bean seedlings. *The Plant Journal*, *19*, 423–431.
Rytel, E., Tajner-Czopek, A., Kita, A., Aniołowska, M., Kucharska, A. Z., Sokół- ŁĘtowska, A., & Hamouz, K. (2014). Content of polyphenols in coloured and yellow fleshed potatoes during dices processing. *Food Chemistry*, *161*, 224–229. https://doi.org/10.1016/j.foodchem.2014.04.002
Saier, M. H., Jr., Beatty, J. T., Goffeau, A., Harley, K. T., Heijne, W. H., Huang, S. C., Jack, D. L., Jahn, P. S., Lew, K., Liu, J., Pao, S. S., Paulsen, I. T., Tseng, T. T., & Virk, P. S. (1999). The major facilitator superfamily. *Journal of Molecular Microbiology and Biotechnology*, *1*, 257–279.
Salvato, F., Havelund, J. F., Chen, M., Rao, R. S. P., Rogowska-Wrzesinska, A., Jensen, O., Gang, D. R., Thelen, J. J., N., & Møller, I. M. (2014). The Potato Tuber Mitochondrial Proteome. *Plant Physiology*, *164*(2), 637–653.
Samotus, B., Niediwiedz, M., Kolodziej, Z., Leja, M., & Czajkowska, B. (1974). Storage and reconditioning of tubers of Polish potato varieties and strains. 2. Changes in sugar level in potato tubers of different varieties and strains during reconditioning of cold-stored potatoes. *Potato Research*, *17*, 82-96.
Schneider, C. A., Rasband, W. S., & Eliceiri. K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671 – 675.
Schott, K., Borchert, S., Müller-Röber, B., & Walter Heldt, H. (1995). Transport of inorganic phosphate and C3- and C6-sugar phosphates across the envelope membranes of potato tuber amyloplasts. *Planta: An International Journal of Plant Biology*, *196*, 647–652. https://doi.org/10.1007/BF00197327
Schreck, R., & Baeuerle, P. A. (1991). A role for oxygen radicals as second messengers. *Trends in Cell Biology*, *1*, 39–42.
Schulze, W. X., Schneider, T., Starck, S., Martinoia, E., & Trentmann, O. (2012). Cold acclimation induces changes in Arabidopsis tonoplast protein abundance and activity and alters phosphorylation of tonoplast monosaccharide transporters. *The Plant Journal*, *69*, 529–541. Doi: 10.1111/j.1365-313X.2011.04812.x
Schünemann, D., & Borchert, S. (1994). Specific Transport of Inorganic Phosphate and C3- and C6-Sugar-Phosphates across the Envelope Membranes of Tomato (*Lycopersicon esculentum*) Leaf-Chloroplasts, Tomato Fruit-Chloroplasts and Fruit-Chromoplasts. *Botanica Acta*, *107*, 461–467. https://doi.org/10.1111/j.1438-8677.1994.tb00821.x
Servaites, J. C., & Geiger, D. R. (2002). Kinetic characteristics of chloroplast glucose transport. *Journal of Experimental Botany*, *53*, 1581–1591.
Shallenberger, R. S., Smith, O., & Treadway, R. H. (1959). Role of sugars in the browning reaction in potato chips. *Journal of Agriculture and Food Chemistry*, *7*, 274-277.
Shepherd, L. V. T., Bradshaw, J. E., Dale, M. F. B., McNicol, J. W., Pont, S. D. A., Mottram, D. S., & Davies, H. V. (2010). Variation in acrylamide producing potential in potato: segregation of the trait in a breeding population. *Food Chemistry*, *123*, 568–573. Doi: 10.1016/j.foodchem.2010.04.070
Smeekens, S. (2000). Sugar-induced signal transduction in plants. *Annual Review of Plant Biology*, *51*, 49–81.
Smirnova, E., Marquis, V., Poirier, L., Aubert, Y., Zumsteg, J., Ménard, R., Miesch, L., & Heitz, T. (2017). Jasmonic Acid Oxidase 2 hydroxylates jasmonic acid and represses basal defense and resistance responses against *Botrytis cinerea* infection. *Molecular Plant*, *10*, 1159–1173.
Smith, O. (1987). Transport and storage of potatoes. In: Talburt, W.F, Smith, O. (eds) *Potato Processing Van Nostrand Reinhold*, New York, USA, pp 203–286.
Sonnewald, S., & Sonnewald, U. (2014). Regulation of potato tuber sprouting. *Planta*, *239*, 27–38. Doi: 10.1007/s00425-013-1968-z
Sonnewald, U. (2001). Control of potato tuber sprouting. *Trends in Plant Sciences*, *6*, 333–335. Doi: 10.1016/S1360-1385(01)02020-9
Souleyre, E. J. F., Iannetta, P. P. M., Ross, H. A., Hancock, R. D., Shepherd, L. V. T., Viola, R., Taylor, M. A., & Davies, H. V. (2004). Starch metabolism in developing

strawberry (Fragaria x ananassa) fruits. *Physiologia Plantarum*, *121*(3), 369–376. https://doi.org/10.1111/j.0031-9317.2004.0338.x
Sowokinos, J. R., Lulai, E. C., & Knoper, J. A. (1985). Translucent tissue defects in *Solanum tuberosum* L. I. Alterations in amyloplast membrane integrity, enzyme activities, sugars and starch content. *Plant Physiology*, *78*, 489–494.
Sowokinos, J. R., Orr, P. H., Knoper, J. A., & Varns, J. L. (1987). Influence of potato storage and handling stress on sugars, chip quality and integrity of the starch (amyloplast) membrane. *American Journal of Potato Research*, *64*, 213-226.
Spychalla, J. P., & Desborough, S. L. (1990). Fatty-acids, membrane-permeability, and sugars of stored potato-tubers. *Plant Physiology*, *94*(3), 1207-1213.
Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., Robert, M. C., & Riediker, S. (2002). Acrylamide from Maillard reaction products. *Nature*, *419*(6906), 449-450.
Stevanato, R., Fabris, S., & Momo, F. (2004). New enzymatic method for the determination of total phenolic content in tea and wine. *Journal of Agricultural and Food Chemistry*, *52*(20), 6287–6293. https://doi.org/10.1021/jf049898s
Stitt, M., & Ap Rees, T. (1980). Carbohydrate breakdown by chloroplasts of *Pisum sativum*. *Biochimica et Biophysica Acta*, *627*, 131– 143.
Stitt, M., & Heldt, H. W. (1981). Physiological rates of starch breakdown in isolated intact spinach chloroplasts. *Plant Physiology*, *68*, 755– 761.
Stürzenbaum, S. R., & Kille, P. (2001). Control genes in quantitative molecular biological techniques: the variability of invariance. *Comparative Biochemistry and Physiology B*, *130*, 281–289. Doi: 10.1016/S1096-4959(01)00440-7
Sugita, M., & Gruissem, W. (1987). Developmental, organ-specific, and light-dependent expression of the tomato ribulose-1,5-bisphosphate carboxylase small subunit gene family. *Proceedings of the National Academy of Sciences of the United States of America*, *84*, 7104–7108.
Sun, L., Zhang, M., Ren, J., Qi, J., Zhang, G., & Leng, P. (2010). Reciprocity between abscisic acid and ethylene at the onset of berry ripening and after harvest. *BMC Plant Biology*, *10*, 257–267. https://doi.org/10.1186/1471-2229-10-257
Suttipanta, N., Pattanaik, S., Kulshrestha, M., Patra, B., Singh, S. K., & Yuan, L. (2011). The transcription factor CrWRKY1 positively regulates the terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*. *Plant Physiology*, *157*(4), 2081–2093. https://doi.org/10.1104/pp.111.181834
Suttle, J. C. (2004). Physiological regulation of potato tuber dormancy. *American Journal of Potato Research*, *81*, 253–262. https://doi.org/10.1007/BF02871767
Talley, E. A., Toma, R. B., & Orr, P. H. (1984). Amino acid composition of freshly harvested and stored potatoes. *American potato journal*, *61*, 247–29. ISSN 0003-0589
Tao, X. C., & Lu, Y. T. (2013). Loss of AtCRK1 gene function in *Arabidopsis thaliana* decreases tolerance to salt. *Journal of Plant Biology*, *56*, 306–314. https://doi.org/10.1007/s12374-012-0352-z
Tauberger, E., Fernie, A. R., Emmermann, M., Renz, A., Kossmann, J., Willmitzer, L., & Trethewey, R. N. (2000). Antisense inhibition of plastidial phosphoglucomutase provides compelling evidence that potato tuber amyloplasts import carbon from the cytosol in the form of glucose-6-phosphate. *Plant Journal*, *23*(1), 43–53. https://doi.org/10.1046/j.1365-313X.2000.00783.x
Tetlow, I. J., Blissett, K. J., & Emes, M. J. (1994). Starch synthesis and carbohydrate oxidation in amyloplasts from developing wheat endosperm. *Planta*, *194*, 454– 460. https://doi.org/10.1007/BF00714456
Thevelein, J. M., & Hohmann, S. (1995). Trehalose synthase: guard to the gate of glycolysis in yeast? *Trends in Biochemical Sciences*, *20*, 3–10. https://doi.org/10.1016/S0968- 0004(00)88938-0
Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., Krüger, P., Selbig, J., Müller, L. A., Rhee, S. Y., & Stitt, M. (2004). MAPMAN: A user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant Journal*, *37*, 14–39. https://doi.org/10.1111/j.1365313X.2004.02016.x

Thompson, J. E. (1988). The molecular basis for membrane deterioration during senescence. In LD Nooden, AC Leopold, eds, *Senescence and Aging in Plants*. Academic Press, New York, pp. 51-83.

Tiessen, A., Hendriks, J. H. M., Stitt, M., Branscheid, A., Gibon, Y., Farré, E. M., & Geigenberger, P. (2002). Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: A novel regulatory mechanism linking starch synthesis to the sucrose supply. *Plant Cell*, *14*, 2191–2213. https://doi.org/10.1105/tpc.003640

Tiessen, A., Prescha, K., Branscheid, A., Palacios, N., McKibbin, R., Halford, N. G., & Geigenberger, P. (2003). Evidence that SNF1-related kinase and hexokinase are involved in separate sugar-signalling pathways modulating post-translational redox activation of ADP-glucose pyrophosphorylase in potato tubers. *Plant Journal*, *35*, 490–500. https://doi.org/10.1046/j.1365-313X.2003.01823.x

Tobin, E. M., & Silvetthorne, J. (1985). Light regulation of gene expression in higher plants. *Annual Review of Plant Physiology*, *36*, 569-593.

Tyson, R. H., & ap Rees, T. (1988). Starch synthesis by isolated amyloplasts from wheat endosperm. *Planta*, *175*, 33–38. https://doi.org/10.1007/BF00402879

Umezawa, T., Yoshida, R., Maruyama, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2004). SRK2C, a SNF1-related protein kinase 2, improves drought tolerance by controlling stress-responsive gene expression in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 17306–17311. https://doi.org/10.1073/pnas.0407758101

Usadel, B., Nagel, A., Steinhauser, D., Gibon, Y., Bläsing, O. E., Redestig, H., Sreenivasulu, N., Krall, L., Hannah, M. A., Poree, F., Fernie, A. R., & Stitt, M. (2006). PageMan: an interactive ontology tool to generate, display, and annotate overview graphs for profiling experiments. *BMC Bioinformatics*, *7*, 535.

Usadel, B., Obayashi, T., Mutwil, M., Giorgi, F. M., Bassel, G. W., Tanimoto, M., Chow, A., Steinhauser, D., Persson, S., & Provart, N. J. (2009). Co-expression tools for plant biology: Opportunities for hypothesis generation and caveats. *Plant, Cell and Environment*, *32*, 1633–1651.

Valcarcel, J., Reilly, K., Gaffney, M., & O'Brien, N. M. (2015). Antioxidant Activity, Total Phenolic and Total Flavonoid Content in Sixty Varieties of Potato (*Solanum tuberosum* L.) Grown in Ireland. *Potato Research*, *58*, 221–244. https://doi.org/10.1007/s11540-015-9299-z

Van Breusegem, F., & Dat, J. F. (2006). Reactive oxygen species in plant cell death. *Plant Physiology*, *141*, 384–390.

van Dingenen, J., de Milde, L., Vermeersch, M., Maleux, K., de Rycke, R., de Bruyne, M., Storme, V., Gonzalez, N., Dhondt, S., & Inzé, D. (2016). Chloroplasts are central players in sugar-induced leaf growth. *Plant Physiology*, *171*, 590–605. https://doi.org/10.1104/pp.15.01669

van Nocker, S., & Ludwig, P. (2003). The WD-repeat protein superfamily in Arabidopsis: conservation and divergence in structure and function. *BMC Genomics*, *4*, 50. Doi: 10.1186/1471-2164-4-50

van Vliet, W. F., & Schriemer, W. H. (1960). The sugar accumulation in potatoes kept at low temperatures studied in a small selection of samples from Dutch varieties. *European Potato Journal*, *3*, 263–271.

Vaughn, K. C., & Lehnen, L. P. (1991). Mitotic disrupter herbicides. *Weed Sciences*, *39*, 450–457.

Verkest, A., Manes, C. L., Vercruysse, S., Maes, S., Van Der Schueren, E., Beeckman, T., Genschik, P., Kuiper, M., Inze, D., & De Veylder, L. (2005). The cyclin-dependent kinase inhibitor KRP2 controls the onset of the endoreduplication cycle during Arabidopsis leaf development through inhibition of mitotic CDKA;1 kinase complexes. *Plant Cell*, *17*, 1723–1736.

Viola, R., Pelloux, J., van der Ploeg, A., Gillespie, T., Marquis, N., Roberts, A. G., & Hancock, R. D. (2007). Symplastic connection is required for bud outgrowth following dormancy in potato (*Solanum tuberosum* L.) tubers. *Plant, Cell and Environment*, *30*, 973–983. Doi: 10.1111/j.1365-3040.2007.01692.x

Wang, C. C., Meng, L. H., Gao, Y., Grierson, D., & Fu, D. Q. (2018). Manipulation of light signal transduction factors as a means of modifying steroidal glycoalkaloids accumulation in tomato leaves. *Frontiers in Plant Science*, *9*, 437. https://doi.org/10.3389/fpls.2018.00437

Wehr, N. B., & Levine, R. L. (2013). Quantification of protein carbonylation. *Methods in Molecular Biology*, *965*, 265-281.

Weinl, C., Marquardt, S., Kuijt, S., Nowack, M., Jakoby, M., Hülskamp, M., & Schnittgera, A. (2005). Novel functions of plant cyclin-dependent kinase inhibitors, ICK1/KRP1, can act non-cell-autonomously and inhibit entry into mitosis. *Plant Cell*, *17*, 1704–1722.

Weise, S. E., Liu, T., Childs, K. L., Preiser, A. L., Katulski, H. M., Perrin-Porzondek, C., & Sharkey, T. D. (2019). Transcriptional regulation of the glucose-6-phosphate/phosphate translocator 2 is related to carbon exchange across the chloroplast envelope. *Frontiers in Plant Science*, *10*, 827. https://doi.org/10.3389/fpls.2019.00827

Weise, S. E., Weber, A. P. M., & Sharkey, T. D. (2004). Maltose is the major form of carbon exported from the chloroplast at night. *Planta*, *218*, 474–482.

Wiberley-Bradford, A. E., & Bethke, P. C. (2017). Suppression of the vacuolar invertase gene delays senescent sweetening in chipping potatoes. *Journal of the Science of Food and Agriculture*, *98*, 354-360.

Wichrowska, D., Rogozińska, I., & Pawelzik E. (2009). Concentrations of some organic acids in potato tubers depending on weed control method, cultivar and storage conditions. *Polish Journal of Environmental Studies*, *18*, 487-491.

Wiese, A., Gröner, F., Sonnewald, U., Deppner, H., Lerchl, J., Hebbeker, U., Flügge, U. I., & Weber, A. (1999). Spinach hexokinase I is located in the outer envelope membrane of plastids. *FEBS Letters*, *461*, 13–18. https://doi.org/10.1016/S0014-5793(99)01417-9

Winder, S. J., & Ayscough, K. R. (2005). Actin-binding proteins. *Journal of Cell Science*, *118*, 651–654. https://doi.org/10.1242/jcs.01670

Wormit, A., Trentmann, O., Feifer, I., Lohr, C., Tjaden, J., Meyer S., Schmidt, U., Martinoia, E., & Neuhaus, H. E. (2006). Molecular identification and physiological characterization of a novel monosaccharide transporter from Arabidopsis involved in vacuolar sugar transport. *Plant Cell*, *18*, 3476–3490. Doi: 10.1105/tpc.106.047290

Wu, L., Zhang, Q., Zhou, H., Ni, F., Wu, X., & Qi, Y. (2009). Rice MicroRNA effector complexes and targets. *Plant Cell*, *21*, 3421–3435.

Yadav, U. P., Ivakov, A., Feil, R., Duan, G. Y., Walther, D., Giavalisco, P., Piques, M., Carillo, P., Hubberten, H. M., Stitt, M., & Lunn, J. E. (2014). The sucrose- trehalose 6-phosphate (Tre6P) nexus: Specificity and mechanisms of sucrose signalling by Tre6P. *Journal of Experimental Botany*, *65*, 1051–1068. https://doi.org/10.1093/jxb/ert457

Yamada K., Osakabe Y., Mizoi J., Nakashima K., Fujita Y., Shinozaki K., & Yamaguchi-Shinozaki, K. (2010). Functional analysis of an *Arabidopsis thaliana* abiotic stress-inducible facilitated diffusion transporter for monosaccharides. *Journal of Biological Chemistry*, *285*, 1138–1146. Doi: 10.1074/jbc.M109.054288

Yamada, T., Tanaka, Y., Sriprasertsak, P., Kato, H., Hashimoto, T., Kawamata, S., Ichinose, Y., Kato, H., Shiraishi, T., & Oku, H. (1992). Phenylalanine ammonia-lyase genes from *Pisum sativum*: Structure, organ-specific expression and regulation by fungal elicitor and suppressor. *Plant and Cell Physiology*, *33*, 715-725.

Yan, J., Tsuichihara, N., Etoh, T., & Iwai, S. (2007). Reactive oxygen species and nitric oxide are involved in ABA inhibition of stomatal opening. *Plant, Cell and Environment*, *30*(10), 1320–1325.

Yoshida, T., Nishimura, N., Kitahata, N., Kuromori, T., Ito, T., Asami, T., Shinozaki, K., & Hirayama, T. (2006). ABA-hypersensitive germination3 encodes a protein phosphatase 2C (AtPP2CA) that strongly regulates abscisic acid signaling during germination among Arabidopsis protein phosphatase 2Cs. *Plant Physiology*, *140*, 115–126. https://doi.org/10.1104/pp.105.070128

Zaefyzadeh, M., Quliyev, R. A., Babayeva, S. M., & Abbasov, M. A. (2009). The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. *Turkish Journal of Biology*, *33*(1), 1–7.

Zhang, L., Häusler, R. E., Greiten, C., Hajirezaei, M. R., Haferkamp, I., Neuhaus, H. E., Flügge, U. I., & Ludewig, F. (2008). Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. *Plant Biotechnology Journal*, *6*, 453–464. https://doi.org/10.1111/j.1467-7652.2008.00332.x

Zhang, Z., Yang, J., & Wu, Y. (2015). Transcriptional Regulation of Zein Gene Expression in Maize through the Additive and Synergistic Action of opaque2, Prolamine-Box Binding Factor, and O2 Heterodimerizing Proteins. *Plant Cell*, *27*(4), 1162–1172. https://doi.org/10.1105/tpc.15.00035

Zheng, Z., Xu, X., Crosley, R. A., Greenwalt, S. A., Sun, Y., Blakeslee, B., Wang, L., Ni, W., Sopko, M. S., Yao, C., Yau, K., Burton, S., Zhuang, M., McCaskill, D. G., Gachotte, D., Thompson, M., & Greene, T. W. (2010). The protein kinase SnRK2.6 mediates the regulation of sucrose metabolism and plant growth in Arabidopsis. *Plant Physiology*, *153*, 99–113. https://doi.org/10.1104/pp.109.150789

Zrenner, R., Schüler, K., & Sonnewald, U. (1996). Soluble acid invertase determines the hexose to sucrose ratio in cold-stored tubers. *Planta*, *198*, 246–252.

Zuk, M., Prescha, A., Kępczyński, J., & Szopa, J. (2003). ADP ribosylation factor regulates metabolism and antioxidant capacity of transgenic potato tubers. *Journal of Agricultural and Food Chemistry*, *51*(1), 288–294. https://doi.org/10.1021/jf020779r

8. APPENDICES

9. KNOWLEDGE EXCHANGE ACTIVITIES

AHDB Crops PhD Studentship Conference, in Warwick (2016) - 1 slide presentation
The James Hutton Institute PhD Annual Event, in Birnam (2017) - Poster
AHDB Crops PhD Studentship Conference, in Warwick (2017) - Poster
Hutton Annual Research Symposium, in Dundee (2017) - Poster
The James Hutton Institute PhD Annual Event, in Birnam (2018) - Presentation
Cell and Molecular Sciences seminar, in Dundee (2018) - Presentation
Cell and Molecular Sciences review, in Dundee (2018) - Poster
AHDB Crops PhD Studentship Conference, in Warwick (2018) - Presentation
The James Hutton Institute PhD Annual Event, in Birnam (2019) - Presentation
EAPR Post-harvest section meeting, in Norwich (2019) - Presentation
SEFARI Showcase of Postgraduate Research (2019) - Presentation

10. ACKNOWLEDGEMENTS

This study was partially funded by PepsiCo. The views expressed are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.