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Rate of Cooling Alters Chip Color, Sugar Contents, and Gene Expression Profiles in Stored Potato Tubers

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Abstract When stored at temperatures below 10 °C, potatoes accumulate sucrose and the reducing sugars glucose and fructose. This process, cold-induced sweetening, has been studied extensively because potatoes with elevated reducing sugar contents produce undesirable, dark-colored products and acrylamide, a suspected carcinogen, during high-temperature cooking. Potatoes in commercial storages are cooled slowly, but many research studies have used potatoes cooled rapidly. In this study, effects of cooling rate and variety on chip color, sugars, and gene expression were examined. Sucrose and reducing sugar contents were substantially lower in slowly cooled than in rapidly cooled tubers of 'Snowden' and "MegaChip' for the first 11 weeks after cooling to 3 °C began. Differences in gene expression for *VInv*, *β-amylase*, *SPS*, *AGPase* and *GBSS* were observed between cooling treatments and varieties. Overall, the data showed that cooling rate, time in storage, and variety influenced multiple aspects of cold-induced sweetening.

Resumen Cuando se almacena a temperaturas por debajo de los 10 °C, las papas acumulan sacarosa y los azúcares reductores glucosa y fructosa. Este proceso de endulzamiento inducido por el frío, se ha estudiado extensivamente porque las papas con contenidos elevados de azúcares reductores producen productos indeseables de color oscuro y acrilamida, sospechosa de cancerígena, durante el cocinado a alta temperatura. Las papas, en almacenamientos comerciales se enfrían lentamente, pero muchos estudios e investigaciones han usado papas enfriadas rápidamente. En este estudio se examinaron los niveles de enfriamiento y la variedad en el color de la hojuela, azucares y expresión de genes. Los contenidos de sacarosa y de azucares reductores fueron substancialmente más bajos en tubérculos enfriados lentamente que los enfriados rápidamente de "Snowden" y "MegaChip" por las primeras once semanas después de que empezó el enfriamiento a 3 °C. Se observaron diferencias en la expresión de genes para VInv, β-amylasa, SPS, AGPasa y GBSS entre tratamientos de enfriamiento y variedades. En general, los datos mostraron que el nivel de enfriamiento, el tiempo de almacenamiento y la variedad influenciaron múltiples aspectos del endulzamiento inducido por el frío.

Keywords Cold-induced sweetening · Low-temperature sweetening · Vacuolar acid invertase · ADP-glucose pyrophosphorylase · Granule-bound starch synthase · Chipping potato tuber processing quality

Introduction

The majority of the potato (*Solanum tuberosum* L.) tubers grown in the United States are processed to make fried products such as potato chips and French fries (NASS-USDA 2015). To meet with the approval of consumers, potato tubers must produce flavorful, light-colored fried products; these traits depend largely upon the reducing sugar content in raw tubers. Glucose and fructose are the major reducing sugars found in potato tubers. These sugars react with amino acids during frying in the non-enzymatic Maillard reaction (Maillard 1912) to produce a complex mixture of compounds that contributes to final product appearance and taste. When

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reducing sugar contents are high, dark-colored and bitter-tasting products are produced (Shallenberger et al. 1959). Acrylamide, a suspected carcinogen, is another product of the Maillard reaction (Mottram et al. 2002; Hogervorst et al. 2010). Avoiding high reducing sugar contents is an effective approach for avoiding high amounts of acrylamide in fried potato products (Amrein et al. 2003; Biedermann-Brem et al. 2003, Bethke and Bussan 2013).

Potato tubers that are stored at temperatures below approximately 10 °C accumulate more sucrose than those stored at higher temperatures, and some of this sucrose is converted to glucose and fructose by the action of vacuolar invertase (VInv). Sugar accumulation at temperatures below 10 °C is called cold-induced sweetening (CIS; Sowokinos 2001) or low-temperature sweetening. Tubers intended for frying are usually stored at 8-10 °C to minimize CIS and maintain acceptable color in fried products. Storage at these relatively warm temperatures, however, increases tuber sprouting and losses from disease relative to storage at 3-5 °C. As such, it would be advantageous to develop varieties that could be stored at 3-5 °C without undergoing CIS. Likewise, rapid cooling to lower temperatures would be beneficial with regard to spouting and disease development, as long as rapid cooling did not result in undesirable changes in processing quality.

Biochemical reactions that convert starch to sucrose in stored potato tubers have been identified, and the enzymes catalyzing most of them have been characterized (Chen et al. 2001; Sowokinos 2001; Bethke 2014; Schreiber et al. 2014). The responses to cold of some of these enzymes and the genes that encode them have been studied in stored potato tubers (Pressey 1970; Hill et al. 1996; Stark et al. 1996; Zrenner et al. 1996; Nielsen et al. 1997; Deiting et al. 1998; Krause et al. 1998; Matsuura-Endo et al. 2004; Bagnaresi et al. 2008; Chen et al. 2008; Bhaskar et al. 2010; Wu et al. 2011; Brummell et al. 2011; Ou et al. 2013). However, these studies were carried out using tubers that were subjected to abrupt temperature changes, usually one or two weeks after harvest. In North American commercial storages, on the other hand, potatoes that will be processed into chips and fries are usually stored using protocols that include a preconditioning period of two weeks or more (Sowokinos and Preston 1988; Forbush and Brook 1993; Brook et al. 1995; Walsh 1995; Holley 2003), application of a chemical sprout inhibitor, and gradual cooling at a rate of 0.15–0.25 °C per day to the final holding temperature (Stark and Love 2003). Therefore, previous research on tuber responses to low-temperature storage has, in many instances, used storage conditions that differ significantly from those used commercially.

Plant tissue often has different responses to rapidly versus gradually imposed abiotic stresses, so it is possible that molecular and biochemical responses of rapidly cooled potato tubers could differ from the responses of tubers cooled slowly. Previous work has shown that responses of some genes to slow cooling differed from those observed in studies of tubers cooled rapidly (Wiberley-Bradford et al. 2016). Slow cooling has been observed to decrease CIS in fresh market (Burton 1969) and fry processing (Pritchard and Adam 1992) varieties, but no reports have described the impact of cooling rate on chipping potatoes. In addition, extended preconditioning (Walkof and Chubey 1969; Pritchard and Adam 1992) may improve processing quality, and application of the most common sprout inhibitor, isopropyl *N*-(3-chlorophenyl) carbamate (CIPC), may have lasting impacts on tuber sugar contents and metabolism (Yang et al. 1999; Copp et al. 2000; Blenkinsop et al. 2002). The present study was carried out in order to compare directly chip colors, sugar contents, and gene expression responses of chipping potato tubers subjected to rapid and slow cooling after extensive preconditioning and application of CIPC. Rapid cooling is much more convenient for laboratory studies, but if the results of such studies do not reflect the responses of slowly cooled tubers, their applicability to commercial practice is limited.

Materials and Methods

Plant Materials

Greenhouse-grown 'MegaChip' (MC; Groza et al. 2007) seed tubers were planted at the University of Wisconsin Hancock Agricultural Research Station in Hancock, Wisconsin, during the 2012 growing season, and commercially obtained 'MegaChip' and 'Snowden' (Sno; Peloquin et al. 1994) seed tubers were planted at that site during the 2013 growing season. Standard cultivation and management practices for irrigated potatoes were employed during growth. Tubers were mechanically lifted and hand-harvested on September 17, 2012 and September 18, 2013 and stored in the University of Wisconsin Storage Research Facility at 13 °C to promote final skin set and formation of wound periderm. Tubers were treated with CIPC on October 16, 2012 and November 6, 2013 to prevent sprouting.

The storage temperature protocols used for these experiments are illustrated in Fig. 1. Cooling began after tubers reached the preferred sucrose and glucose concentrations for preconditioned chipping potatoes, 0.7 and 0.035 mg g^{-1} FW, respectively in this case. In chipping potatoes stored commercially, this process can take anywhere from a few weeks to two months or more (Sowokinos and Preston 1988; Forbush and Brook 1993; Brook et al. 1995; Bethke 2014). Tubers that are preconditioned appropriately prior to cooling often undergo smaller increases in reducing sugar contents during cooling than those that are not preconditioned appropriately (Pritchard and Adam 1992).

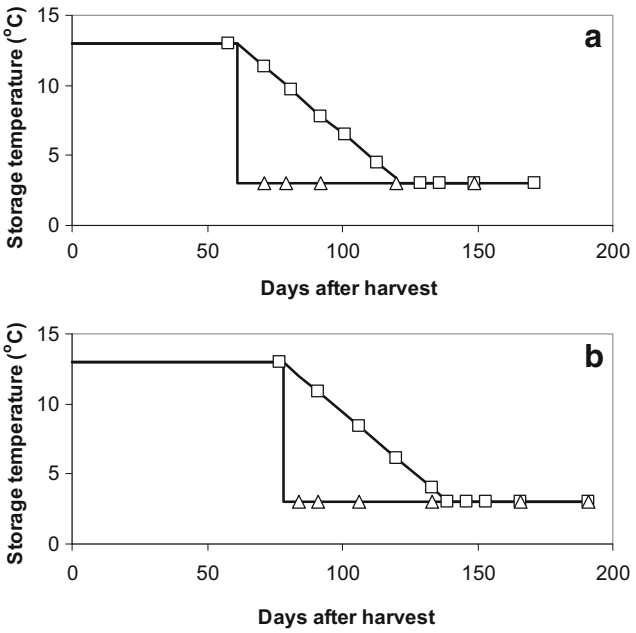

Fig. 1 Storage temperature protocols for tubers harvested in 2012 (**a**) and 2013 (**b**). Sampling time points for slowly cooled tubers are indicated with squares; those for rapidly cooled tubers, with triangles. In 2012, tubers were harvested on September 17; in 2013, tubers were harvested on September 18

For tubers that were cooled slowly, in 2012, cooling of storage lockers to their final set point temperature of 3 °C began on November 17 at a maximum rate of 0.18 °C per day; the final temperature set point was reached in the second week of January 2013, 122 days after harvest (DAH). In 2013, cooling of storage lockers to this temperature began on December 4 at the same rate as in 2012; the final temperature set point was reached in the first week of February 2014, 139 DAH. For rapid cooling, tubers were moved from 13 °C directly to a 3 °C storage locker on November 20, 2012 and December 4, 2013.

At each sampling time, six (2012) or nine (2013) tubers per variety were selected at random for chipping, molecular, and biochemical analysis. The first sampling of each year was carried out before cooling started, on November 14, 2012 and December 4, 2013 (58 and 77 DAH, respectively). Tubers that were cooled rapidly (RC) were sampled 1, 2, 4, 8, 12 (2012 and 2013), and 16 (2013 only) weeks after being moved to 3 °C (71, 79, 92, 120, and 149 DAH in 2012; 84, 91, 106, 133, 166, and 191 DAH in 2013). Tubers that were cooled slowly (SC) were sampled every 10 days (2012) or two weeks (2013) during temperature ramping and then 0 (in 2013), 1, 2, 4, and 8 weeks after reaching 3 °C (Fig. 1). In 2012, these samplings were carried out 71, 81, 93, 101, and 113 DAH during cooling and 129, 136, 149, and 171 DAH after reaching 3 °C. In 2013, they were carried out 91, 106, 120, and 133 DAH during cooling and 139, 146, 153, 166, and 191 DAH after reaching 3 °C.

Chipping Analysis

Unpeeled tubers were cut in half lengthwise through the stem scar and two or three, 1-mm-thick slices were cut from one tuber half for frying. Slices were fried in cottonseed oil at 188 °C for 2 min 10 s. Fried chips were crushed for color analysis using a D25LT colorimeter (HunterLab, Reston, VA). Each sample consisted of 18 crushed chips, three chips from each of six tubers (2012) or two chips from each of nine tubers (2013). A single chip color sample was prepared at each sampling time for each variety and treatment combination. Samples with Hunter L-values of 55 or higher may be acceptable to the potato processing industry.

Sugar, mRNA, and Protein Extraction and Analysis

At each sampling time, two tissue samples were collected from the center of each sampled tuber, frozen in liquid nitrogen, and stored at -80 °C until use. Each tissue sample was a cylinder of tuber tissue with a fresh weight of approximately 0.6 g. One sample from each tuber had sucrose, glucose, and fructose extracted for quantification using high-performance liquid chromatography as described by Bethke et al. (2009). The other tissue sample had RNA and protein extracted for analysis. For each variety at each sampling time and cooling rate, the six or nine tissue samples were separated into three pairs (2012) or trios (2013), and each pooled pair or trio of samples was ground under liquid nitrogen using a freezer mill (model 6770, SPEX SamplePrep, Metuchen, NJ) to make three pooled tissue samples for each variety at each sampling time and cooling rate. Ground samples were stored at -80 °C.

RNA was extracted from ground tissue using the Agilent Plant RNA Isolation Mini Kit (Agilent Technologies, Inc., Santa Clara, CA) according to the manufacturer's instructions. Twenty microliters of extraction solution were used per milligram of tissue. RNA integrity was checked visually using agarose gel electrophoresis, and purity was evaluated using a NanoDrop 1000 (NanoDrop Products, Wilmington, DE) with absorbance values measured at 230, 260, and 280 nm. In 2012, 600 ng of total RNA were DNase treated using a DNase Treatment Kit (Ambion, Austin, TX) according to the manufacturer's instructions. In 2013, 600 ng of total RNA were DNase treated with RQ1 RNase-free DNase (Promega Corporation, Madison, WI) according to the manufacturer's instructions. In both years, the DNase-treated RNA was reverse transcribed with M-MLV reverse transcriptase, RNase H minus (Promega Corporation), according to the manufacturer's instructions. After reactions were completed, nuclease-free water was added to each sample to bring the total volume to 80 μL. Two microliters of the reverse transcription product (containing 25 ng of total RNA) were used as template for each quantitative PCR. For each gene, only a

single product was formed, and calculated PCR efficiencies were between 90% and 110%.

Quantitative PCR was carried out using a BioRad iCycler (2012) or a BioRad MyiQ Real-Time PCR Detection System (2013) with Maxima SYBR Green/ Fluorescein qPCR Master Mix (Fermentas Inc., Glen Burnie, MD). The thermal protocol consisted of one cycle of 95 °C for 10 min, followed by 35 cycles of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. A melt curve was then collected with temperatures from 55 to 95 °C. Fluorescence data were collected during the extension phase and the melt curve. Reactions for all genes in each sample were carried out in triplicate. Primer sequences and final concentrations are given in Wiberley-Bradford et al. (2016).

Protein extraction and desalting were carried out as described by Bhaskar et al. (2010); about 0.5 g of pooled, ground tissue was homogenized in 2 mL of extraction buffer. Protein concentrations in the extracts were determined using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA). Extracts were assayed for acid invertase activity as described previously (Bhaskar et al. 2010). Samples were assayed in triplicate, and activities were expressed as nmol glucose min^{-1} mg^{-1} protein. Assays were carried out both on extracts that had been vortexed to inactivate endogenous invertase inhibitors and on non-vortexed extracts.

Statistics

Statistical analyses were carried out with Tukey's HSD test or Student's t-test, as appropriate, using JMP® Pro version 11.0.0 (SAS Institute, Cary, NC). All significant differences are $p < 0.05$.

Results

Chip Color Assessment

Chips made from SC tubers of MC (2012 and 2013) and Sno (2013) were lighter in color than those from RC tubers for at least 11 weeks after cooling began (Figs. 2, 3). Sixteen weeks after cooling began, however, chips from RC and SC tubers were similar in color (Figs. 2, 3). The color of chips from SC tubers of both varieties did not darken appreciably until after tubers reached 3 °C. For SC MC, chip darkening was first observed approximately one week after tubers reached 3 °C (Fig. 2, 3a). Dark chips were first observed four weeks after reaching 3 °C for SC Sno tubers (Fig. 2b, 3b).

Sugar Contents of Stored Tubers

In 2013–14, sucrose contents of RC MC tubers increased significantly within two weeks of reaching 3 °C (Fig. 4a). A significant increase in RC Sno tuber sucrose content was observed a week earlier (Fig. 4b). In SC MC and Sno tubers, sucrose contents increased after eight weeks of cooling and were significantly greater than early amounts one week after reaching 3 °C. maximum sucrose content was higher in RC than in SC tubers, and the sucrose content of RC tubers was higher than that of SC tubers until 13 weeks after cooling began (Fig. 4a-b). The same trends were observed in SC MC tubers in 2012–13 (data not shown). In RC Sno and MC tubers, glucose and fructose contents increased two or four weeks, respectively, after tubers were moved to 3 °C (Fig. 4c-f). Glucose and fructose contents of SC MC and Sno tubers remained constant during cooling until 11 (MC) or 13 (Sno) weeks after cooling began, which corresponded to two or four weeks after reaching 3 °C. Reducing sugar contents in SC tubers were less than those in RC tubers throughout the storage period, although the magnitude of this difference decreased late in storage. Sugar contents of MC tubers in 2012–13 showed the same behavior as MC tubers in 2013–14 with regard to sugar contents (data not shown).

Expression of Sucrose and Starch Metabolism Genes

In RC MC and Sno tubers, expression of *VInv* increased within one week of tubers being placed at 3 °C and decreased later in storage (Fig. 5a, b). Peak transcript accumulation was observed four weeks after tubers reached 3 °C for RC MC and one to two weeks after tubers reached 3 °C for RC Sno. In SC tubers of both varieties, peak *VInv* transcript accumulation was observed after eight weeks of cooling, one week before tubers reached 3 °C. Expression of a VInv inhibitor gene (*INH*) decreased in both varieties when tubers began cooling (Fig. 5c, d). *INH* accumulation decreased until eight to nine weeks after the start of cooling and then increased slightly by the last sampling in all variety and treatment combinations except SC Sno. These trends in *VInv* and *INH* expression were also observed in 2012–13 MC tubers (data not shown). Extractable VInv activity increased gradually in MC and Sno tubers after cooing began (Fig. 5e, f). Invertase activity tended to be lower in SC Sno and MC, respectively, than in RC Sno and MC. Expression of sucrose synthase (*SuSy*), the other enzyme that catabolizes sucrose in stored potato tubers, decreased early in storage of RC MC and Sno tubers but increased dramatically by 12 weeks after tubers were moved to 3 °C (Fig. 5g, h). In SC MC and Sno tubers, *SuSy* decreased slightly early in storage and remained low through approximately eight weeks after the start of cooling. A large increase in *SuSy* expression was observed 13 weeks after cooling began in RC MC and Sno and 16 weeks after cooling began in SC Sno. *SuSy* expression in 2012–13 MC tubers also decreased early in storage and increased later (data not shown).

Expression of *β-amylase*, which functions in starch degradation, increased in both varieties and both treatments until it reached a peak eight weeks after the start of

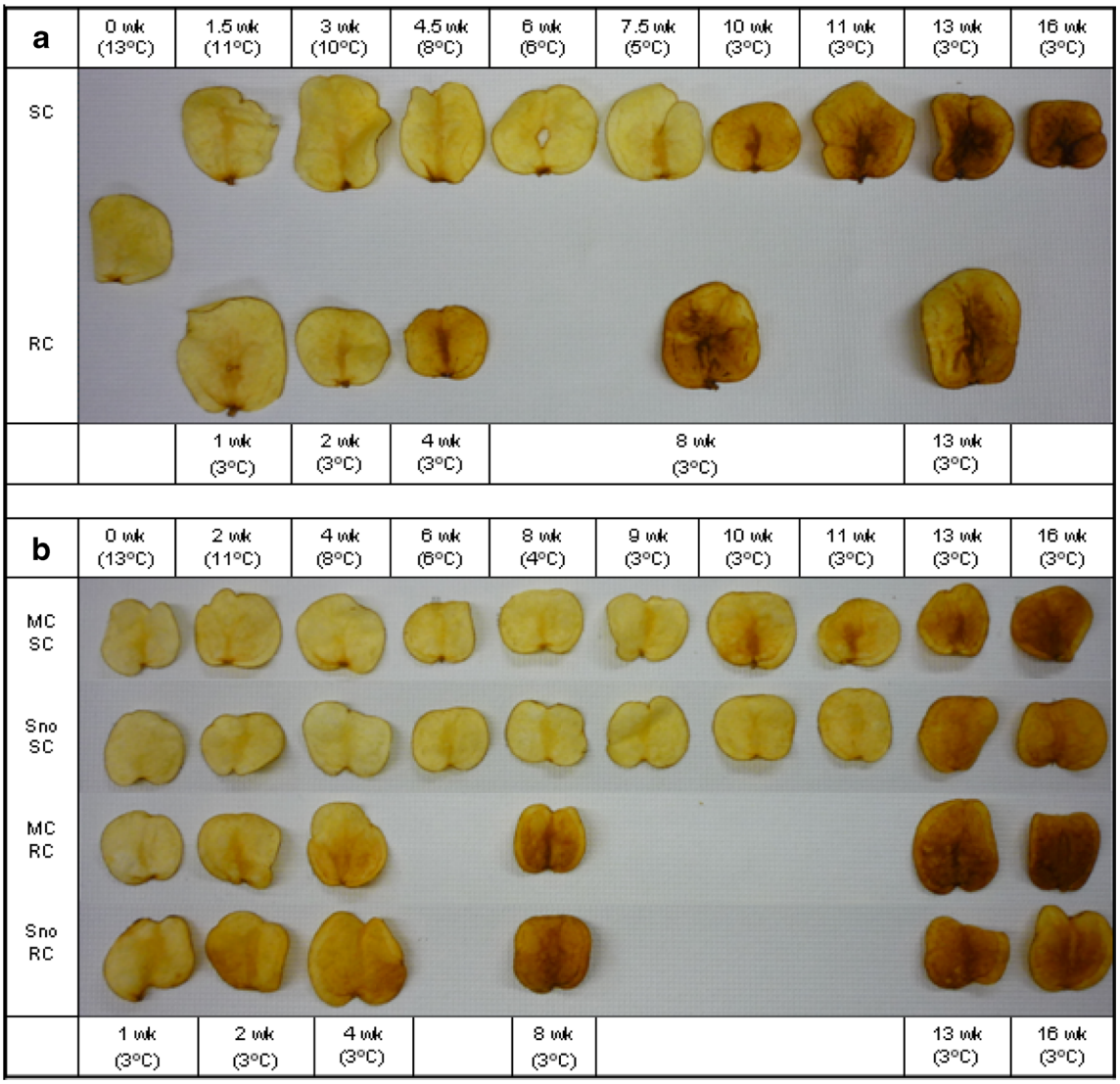

Fig. 2 Representative chips from slowly cooled (*SC*) and rapidly cooled (*RC*) MegaChip (*MC*) tubers from all sampling time points in 2012–13 (**a**) and MC and Snowden (*Sno*) tubers in 2013–14 (**b**). The time since the beginning of cooling in weeks (*wk*) is indicated above the storage temperature of the tubers at each sampling time

cooling (Fig. 6a, b). *β-amylase* expression was less in SC than in RC tubers of both varieties through eleven weeks after the start of cooling, but *β-amylase* expression was similar in SC and RC tubers of both varieties at the end of storage. Expression of UDP-glucose pyrophosphorylase (*UGPase*), which also functions in conversion of starch to sucrose, decreased slightly in both varieties and treatments until approximately eight weeks after the start of cooling and then increased slightly through the last sampling (Fig. 6c, d). Few differences in *UGPase* expression were observed between the two temperature treatments, although expression in SC tubers tended to be less than that in RC tubers. Expression of sucrose phosphate synthase (*SPS*), which combines UDP-glucose produced by UGPase with fructose 6-phosphate to produce sucrose 6-phosphate, remained constant throughout storage in SC tubers of both varieties (Fig. 6e, f). In RC tubers, *SPS* expression was highest two weeks after tubers were placed at 3 °C but decreased and remained constant at later times. Similar patterns of expression for these genes were observed in 2012–13 MC tubers (data not shown).

Expression of ADP-glucose pyrophosphorylase (*AGPase*) and granule-bound starch synthase (*GBSS*), both of which are involved in starch resynthesis, decreased dramatically in RC tubers, and to a lesser extent in SC tubers, one to two weeks after tubers reached 3 °C (Fig. 7). At later times in storage, *AGPase* expression increased slowly in RC and SC tubers, but *GBSS* expression did not. At the end of storage, *AGPase* and *GBSS* expression were similar in RC and SC tubers of Sno but not of MC. Similar patterns of expression for *AGPase* and *GBSS* were observed in 2012–13 MC tubers (data not shown).

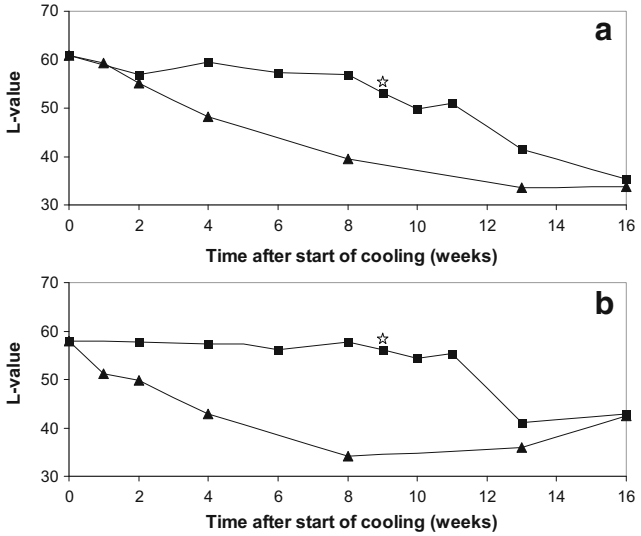

Fig. 3 Hunter L-values of chips from rapidly (triangles) or slowly (squares) cooled tubers of MegaChip (**a**) and Snowden (**b**) during and after cooling to 3 °C, 2013–14. The star indicates the date on which slowly cooled tubers reached 3 °C. Each sample was comprised of two chips from each of nine tubers. Higher L-values correspond to lighter chip color

Discussion

Changes in chip color, tuber sugar contents, and gene expression varied considerably with both cooling rate and variety. For both varieties and cooling rates, chip color darkened rapidly when storage temperature reached 3 °C. This was expected and is similar to previous observations in 'Atlantic' and 'Dakota Pearl' (Wiberley-Bradford et al. 2016) and in numerous other studies. However, acceptable (L ≥ 55) chip color was maintained six and 11 weeks longer in SC MC and SC Sno tubers, respectively, than in RC tubers of these varieties. This is in agreement with previous observations of MC and Sno tubers cooled slowly, which found Sno to be more CIS-resistant than MC (Groza et al. 2007). In view of the substantial difference in chip color between SC and RC tubers, changes in the sugars primarily responsible for chip color were investigated, as were expression levels of numerous genes involved in carbohydrate metabolism.

Tuber sucrose contents changed throughout storage in both RC and SC tubers, but the patterns of change differed between the two treatments. When tubers were cooled slowly, sucrose contents increased slowly and to only 70% of peak concentrations in RC tubers. This observation generally agrees with a previous study that compared RC and SC tubers of fresh market variety 'Majestic'. SC 'Majestic' tubers had lower sucrose contents than RC tubers through 20 weeks of cold storage (Burton 1969). When MC and Sno tubers were cooled rapidly, sugar contents increased rapidly and peaked two to four weeks after cooling began (Fig. 4a, b). This prompt increase has been seen in tubers of a number of other varieties cooled rapidly to 3-5 °C (e.g. Hill et al. 1996; Krause et al. 1998; Matsuura-Endo et al. 2004; Bagnaresi et al. 2008; He et al. 2012; Ou

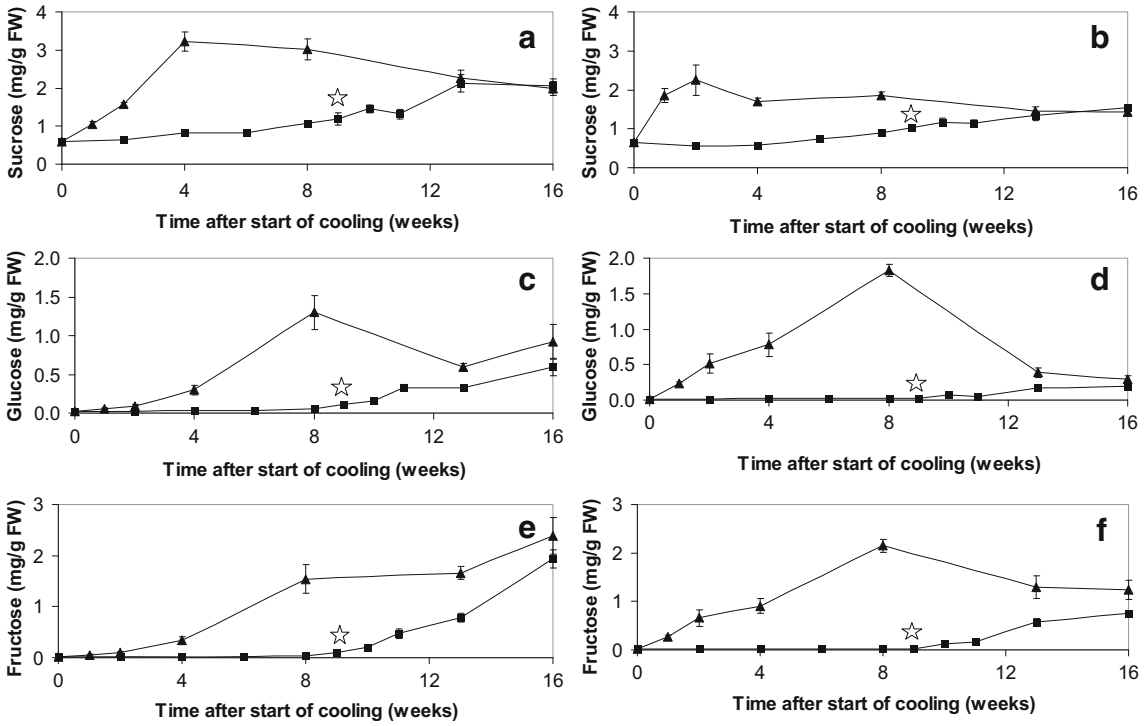

Fig. 4 Sucrose (**a**, **b**), glucose (**c**, **d**), and fructose (**e**, **f**) concentrations in MegaChip (**a**, **c**, **e**) and Snowden (**b**, **d**, **f**) tubers during and after rapid (triangles) or slow (squares) cooling to 3 °C, 2013–14. The star indicates the date on which slowly cooled tubers reached 3 °C. Bars represent mean concentrations in nine independent samples ± standard error

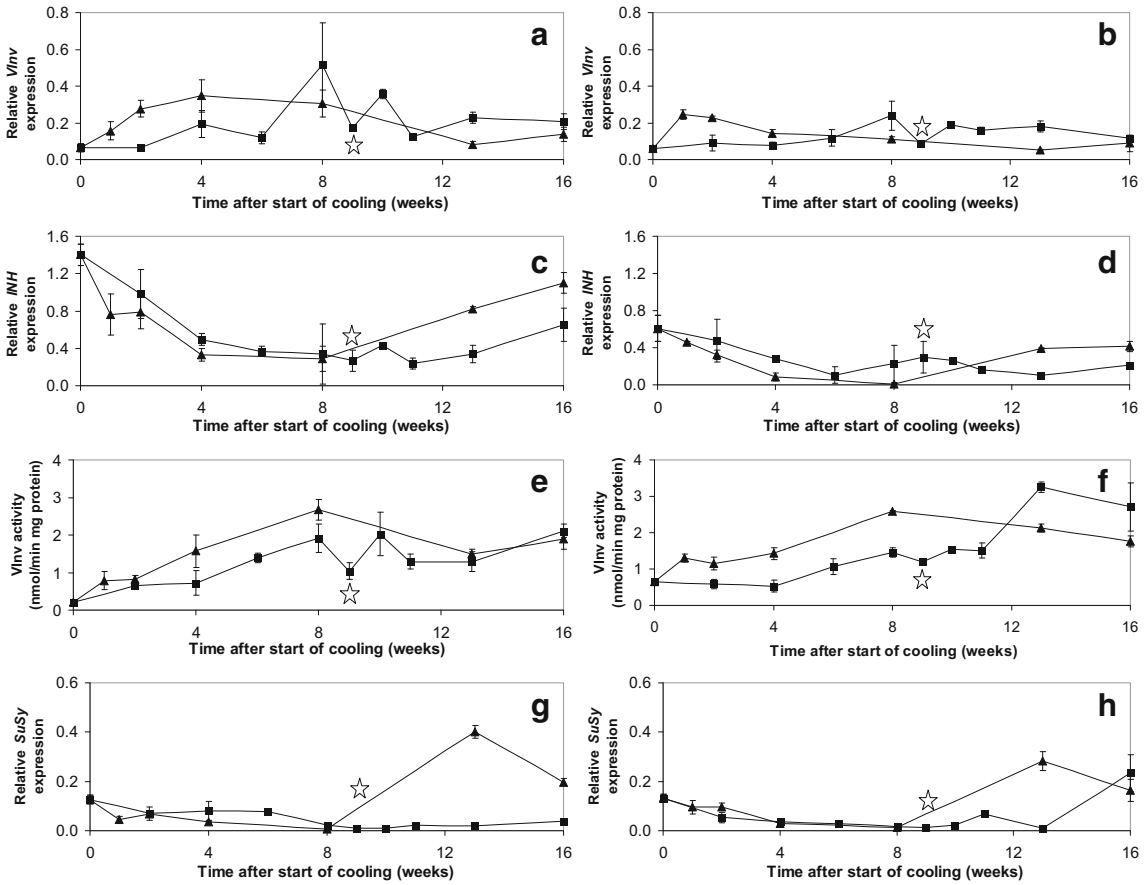

Fig. 5 *VInv* (**a**, **b**), *INH* (**c**, **d**), and *SuSy* (**g**, **h**) expression relative to *actin* expression and VInv activity (**e**, **f**) in MegaChip (**a**, **c**, **e**, **g**) and Snowden (**b**, **d**, **f**, **h**) tubers during and after rapid (triangles) or slow (squares) cooling to 3 °C, 2013–14. The star indicates the date on which slowly cooled tubers reached 3 °C. Bars represent mean expression in three independent samples ± standard error

et al. 2013). Tubers subjected to the two cooling regimes therefore exhibited significant differences in both the timing and magnitude of changes in sucrose contents.

Changes in glucose and fructose contents also varied between RC and SC tubers. In RC tubers, changes in glucose and fructose followed a pattern similar to that of sucrose in RC tubers, but peak concentrations of glucose and fructose were observed four to six weeks after peak sucrose concentrations. This finding was similar to previous observations (Pressey 1969, 1970; Hill et al. 1996; Krause et al. 1998; Matsuura-Endo et al. 2004; Bagnaresi et al. 2008; He et al. 2012; Ou et al. 2013). Glucose and fructose contents in SC tubers also increased during storage, but this occurred several months after increases in RC tubers were observed. At all sampling times, glucose and fructose contents were lower in SC than in RC tubers. Differences were large and statistically significant four to 13 weeks after the start of cooling (Fig. 4c-f).

Expression of *VInv* increased soon after RC tubers of both varieties were moved to 3 °C, and VInv activity increased shortly thereafter. This, combined with the prompt increase in sucrose contents in RC tubers, was likely responsible for the rapid darkening of chip color observed in these tubers. Similar abrupt increases in *VInv* expression and VInv activity have been observed in tubers of other varieties cooled rapidly to low temperatures (Pressey 1969; Zrenner et al. 1996; Matsuura-Endo et al. 2004; Chen et al. 2008; Bagnaresi et al. 2008; Ou et al. 2013). In SC tubers, however, increases in *VInv* expression and invertase activity lagged far behind those observed in RC tubers, although levels of both were similar to or greater than those in RC tubers by the final sampling. Tubers subjected to the two cooling regimes exhibited significant differences in the timing and magnitude of changes in *VInv* expression and activity.

Responses of genes involved in starch resynthesis from sucrose differed in RC and SC MC and Sno tubers. In both cooling treatments, expression of *AGPase* and *GBSS* decreased early in the sampling period, but expression of both genes decreased much more rapidly in RC than in SC tubers. The rate of cooling therefore affected expression of these genes, possibly contributing to observed differences in sugar accumulation and chip color.

Expression of the VInv inhibitor *INH* decreased at the same rate in both RC and SC tubers of both varieties, indicating that changes in *INH* expression did not respond strongly to cooling

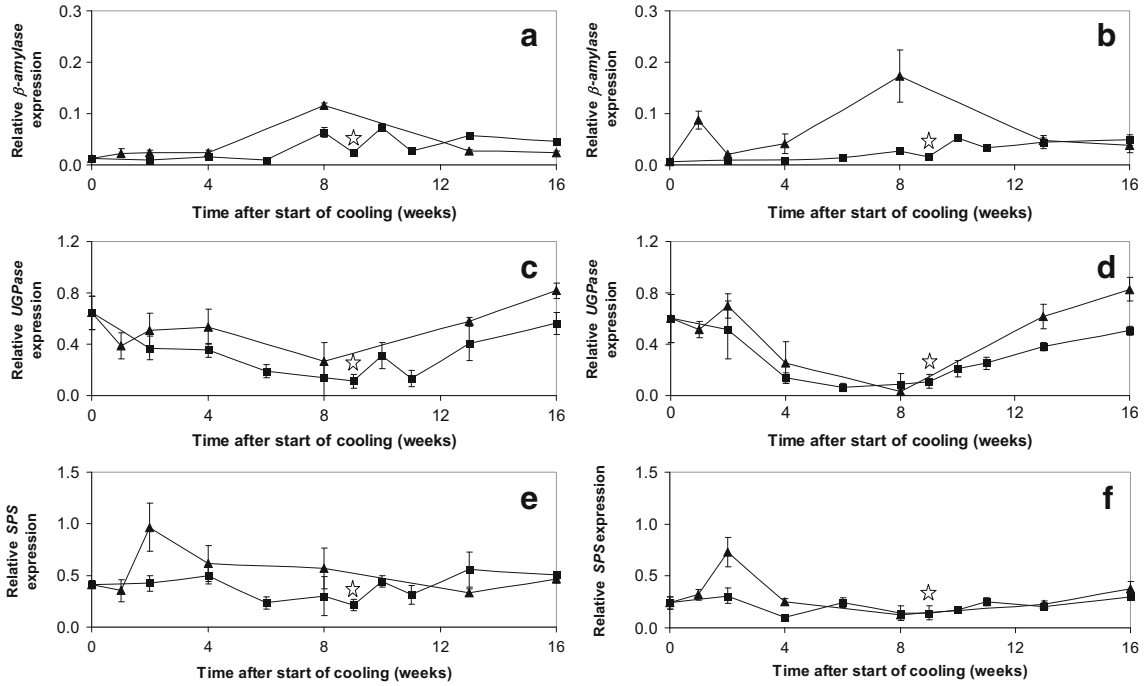

Fig. 6 *β-amylase* (**a**, **b**), *UGPase* (**c**, **d**), and *SPS* (**e**, **f**) expression relative to actin in MegaChip (**a**, **c**, **e**) and Snowden (**b**, **d**, **f**) tubers during and after rapid (triangles) or slow (squares) cooling to 3 °C, 2013–14. The star indicates the date on which slowly cooled tubers reached 3 °C. Bars represent mean expression in three independent samples ± standard error

rate or temperature during the cooling phase. Previous studies have found varying responses of *INH* expression to rapid cooling. In one case, CIS-resistant varieties had increases in *INH* expression, while this was not observed in CIS-sensitive varieties (Brummell et al. 2011). In other studies, there was a decrease in invertase inhibitor transcript accumulation or protein amount (Pressey 1969; Galani Yamdeu et al. 2015). These differences may be more dependent upon potato variety than upon cooling rate.

Expression of *SuSy* was similar for the first eight weeks after cooling began for RC and SC tubers of both varieties. Differences between treatments were observed at later times, though whether these differences were caused directly by imposed storage temperature treatments or indirectly through differences in tuber physiological age is not clear. These observations are in contrast to previous studies, which found increases in *SuSy* expression and SuSy activity shortly after tubers were cooled rapidly to 4 °C (Pressey 1970; Bagnaresi et al. 2008). Like *INH* expression, responses of *SuSy* expression to cold treatment may have depended more upon variety than upon cooling rate.

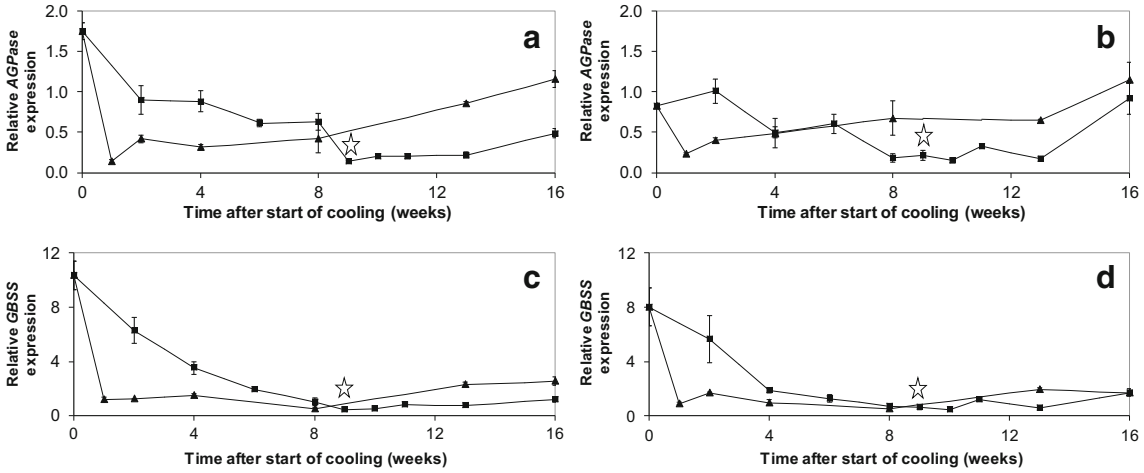

Fig. 7 *AGPase* (**a**, **b**) and *GBSS* (**c**, **d**) expression relative to actin in MegaChip (**a**, **c**) and Snowden (**b**, **d**) tubers during and after rapid (triangles) or slow (squares) cooling to 3 °C, 2013–14. The star indicates the date on which slowly cooled tubers reached 3 °C. Bars represent mean expression in three independent samples ± standard error

Potato variety also had a large effect on expression of genes involved in starch breakdown or sucrose synthesis, although in some cases cooling rate played a significant role as well. For example, *β-amylase* expression showed early and late peaks in RC Sno tubers, while RC MC tubers exhibited only the latter peak (Fig. 6a, b). RC tubers in previous studies generally showed increased *β-amylase* expression within a few days of being placed at low temperatures and decreased expression thereafter (Bagnaresi et al. 2008; Zhang et al. 2014). Expression of *SPS* showed no significant changes over time in SC tubers of MC or Sno, but RC tubers of both varieties showed a peak in *SPS* expression two weeks after cooling began (Fig. 6e, f). Increased *SPS* expression after rapid cooling has also been observed in other varieties (Deiting et al. 1998; Bagnaresi et al. 2008). The transient, early accumulation of *SPS* transcript indicates that the rate of cooling may have affected *SPS* expression.

RC and SC tubers of both varieties studied here had similar chip colors and sugar contents by the end of the sampling period when stored under conditions that strongly promote CIS. However, the timing and extent of changes in chip color, tuber sugar contents, and expression of genes central to carbohydrate metabolism were found to depend strongly on the rate of cooling, the time in storage, and the potato variety in question. Of particular note was the observation that cold-induced increases in tuber sucrose and reducing sugar contents were substantially smaller in SC than in RC tubers throughout most of the storage period. The data presented here demonstrate that care must be taken when comparing data from one cooling treatment to another, as well as when comparing responses of one potato variety to the storage environment to responses of another variety.

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