Research Article

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Suppression of the vacuolar invertase gene delays senescent sweetening in chipping potatoes

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Abstract

BACKGROUND: Potato chip processors require potato tubers that meet quality specifications for fried chip color, and color depends largely upon tuber sugar contents. At later times in storage, potatoes accumulate sucrose, glucose, and fructose. This developmental process, senescent sweetening, manifests as a blush of color near the center of the fried chip, becomes more severe with time, and limits the storage period. Vacuolar invertase (VInv) converts sucrose to glucose and fructose and is hypothesized to play a role in senescent sweetening. To test this hypothesis, senescent sweetening was quantified in multiple lines of potato with reduced *VInv* expression.

RESULTS: Chip darkening from senescent sweetening was delayed by about 4 weeks for tubers with reduced *VInv* expression. A strong positive correlation between frequency of dark chips and tuber hexose content was observed. Tubers with reduced *VInv* expression had lower hexose to sucrose ratios than controls.

CONCLUSION: VInv activity contributes to reducing sugar accumulation during senescent sweetening. Sucrose breakdown during frying may contribute to chip darkening. Suppressing *VInv* expression increases the storage period of the chipping potato crop, which is an important consideration, as potatoes with reduced *VInv* expression are entering commercial production in the USA.

Supporting information may be found in the online version of this article.

Keywords: vacuolar acid invertase; reducing sugars; potato tuber processing quality; RNA interference; chipping potato storage quality; senescent sweetening

INTRODUCTION

Most of the potato (*Solanum tuberosum* L.) tubers produced in the USA are processed to make chips and other fried products.[1] Consumer preference requires the production of light-colored, flavorful products; these traits depend primarily on the glucose and fructose contents of raw tubers. These reducing sugars react with free amino acids in a non-enzymatic Maillard reaction[2] during frying, producing a mixture of pigment and flavor compounds that affects product appearance and taste. Tubers with high reducing sugar contents produce dark-colored, bitter-tasting fried products[3] that are undesirable to consumers. The Maillard reaction also produces acrylamide, a suspected carcinogen,[4,5] and decreasing reducing sugar contents has been found to be an effective way to mitigate acrylamide production in fried potato products.[6–8]

Potato tubers in storage undergo a developmentally controlled, age-dependent shift in metabolism that results in an accumulation of sucrose, glucose, and fructose. This process is referred to as senescent sweetening; it is distinct from cold-induced sweetening, which occurs in response to a low-temperature storage environment.[9] Senescent sweetening negatively affects potato chip color and is manifested as a blush of dark color that begins in the center of the chip and progresses outward as sweetening becomes more severe; it does not appear until after chips are fried.[10] Sugar accumulation in tubers undergoing senescent sweetening is influenced by tuber maturity[11] and is greater if sprout growth is prevented.[12] Senescent sweetening develops sooner after harvest in tubers that are stored at higher rather than lower storage temperatures within the range 7–20 °C.[9] This has been interpreted to indicate that senescent sweetening depends on tuber physiological age. Unlike cold-induced sweetening, senescent sweetening cannot be mitigated by increasing the storage temperature. Moving tubers to warmer temperatures increases the severity of senescent sweetening, presumably by accelerating the physiological aging of tubers. Thus, unlike cold-induced sweetening, senescent sweetening is an irreversible tuber quality defect.

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Senescent sweetening limits the duration of the storage period for chip and fry processing potatoes, since product quality decreases as the extent of senescent sweetening increases. Thus potato varieties with later onset of senescent sweetening are required for long-term storage of processing potatoes, and this trait is highly valued by the processing industry. Different potato cultivars undergo senescent sweetening at different times after harvest.[13] However, since environmental factors during the growing and storage periods influence tuber physiological age, it is often difficult to predict the time at which sweetening will begin.[14] This increases the financial risk to growers and introduces uncertainties about the supply of high-quality raw product to processors.

The cellular mechanisms underlying senescent sweetening are largely unknown. One hypothesis supported by experimental data posits that senescent sweetening results when reactive oxygen species damage the amyloplast membranes in cells of aging tubers. Increased membrane permeability allows catabolic enzymes from the cytosol to access starch granules. This, in turn, results in more rapid rates of starch breakdown and reducing sugar accumulation.[14]

The enzyme responsible for accumulation of reducing sugars during senescent sweetening has not been identified unequivocally. One candidate enzyme is vacuolar acid invertase (VInv; EC 3.2.1.26), but available data do not exclude the involvement of cell wall invertase or sucrose synthase in this process. Vacuolar invertase activity determines the hexose to sucrose ratio in potato tubers, with higher activity corresponding to higher ratios of hexose to sucrose.[15] Reduction of *VInv* expression through RNA interference (RNAi) has been shown to improve chip and fry color in cold-stored potato tubers by lowering reducing sugar contents.[16–23] The present study was conducted to determine whether reduced *VInv* expression could also mitigate the loss of potato processing quality caused by senescent sweetening. This area of research is particularly timely given ongoing efforts to commercialize potato lines in which *VInv* has been silenced using RNAi.[18,23,24]

MATERIALS AND METHODS

Plant materials and chipping analysis

Tubers from untransformed cultivar controls, referred to here as wild type (WT), of 'Atlantic' (ATL),[25] 'Dakota Pearl' (DKP),[26] and 'MegaChip' (MC),[27] and RNAi lines in which *VInv* expression after 30 days at 4 °C was reduced by more than 80% (ATL lines 1, 3, and 8; DKP line 3; MC lines 3 and 7), between 65 and 80% (DKP line 8; MC line 11), or less than 35% (DKP line 11) relative to WT,[19] were planted at the University of Wisconsin Hancock Agricultural Research Station in Hancock, Wisconsin during the 2013 growing season. All required regulatory approvals for field growth of transgenic plants were obtained from the US Department of Agriculture Animal and Plant Health Inspection Service before planting. Seed tubers were planted in a randomized complete block design with five blocks of eight-hill replicates of each WT control and RNAi line. Standard cultivation and management practices for irrigated potatoes were used. Tubers were mechanically lifted and hand harvested on 19 September and stored at 13 °C for skin set and wound healing. Tubers were treated with isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) on 6 November to prevent sprouting. Cooling of tubers to their final storage temperature of 9 °C began on 4 December. Cooled at a maximum rate of 0.06 °C per 8 h, tubers reached this temperature on 29 December 2013.

Twenty tubers of each line were selected at random for chip color analysis every 2 weeks from 13 March through 30 July 2014 (25, 27, 29, 31, 33, 35, 37, 39, 41, 43, and 45 weeks after harvest (WAH)). For chipping analysis, tubers were cut in half lengthwise through the stem scar, and a 1 mm thick slice was cut from one half for frying. Slices were fried for 2 min 10 s in cottonseed oil at 188 °C. Presence or absence of the chip darkening characteristic of senescent sweetening was assessed visually (Suppl. Fig. 1). Tubers were categorized as exhibiting senescent sweetening when they produced chips with dark blemishes consistent in appearance with senescent sweetening.

Sugar content and gene expression analysis

At two sampling times when blemishes consistent with senescent sweetening were apparent in many chips, 41 and 43 WAH, tissue samples from a position halfway between the apical and basal ends of the tubers were collected as previously described[22] for more detailed analysis. One tissue sample from each tuber was processed for sucrose, glucose, and fructose quantification by high-performance liquid chromatography as previously described.[28] Hexose content was calculated as the sum of tuber glucose and fructose contents. To evaluate the relationship between hexose content and senescent sweetening, sugar data from all WT and RNAi tubers sampled 41 and 43 WAH were sorted from low to high hexose content and apportioned into groups of 50 tubers and their corresponding chips. For *VInv* expression analysis, tissue samples from ten tubers were selected from each group of 20 tubers, and these were divided into five pairs that were ground together under liquid nitrogen to make five pooled samples for each line and sampling time. RNA extraction, DNase treatment, reverse transcription, and quantitative polymerase chain reaction (PCR) were carried out as described previously.[22] Primer sequences and final concentrations are listed in Table 1. *VInv* expression was calculated relative to the geometric mean of the expressions of 60S ribosomal protein, actin, and elongation factor 1α.

Statistics

Differences in development of senescent sweetening between WT and RNAi lines were assessed using the 'proc glimmix' procedure in SAS (SAS Institute, Cary, NC, USA). Differences between WT and RNAi lines in percentages of chips with darkening attributed to senescent sweetening at each sampling time were assayed using Fisher's exact test. Statistical analyses for glucose, fructose, and sucrose were carried out with Tukey's honest significant difference (HSD) test and two-way analyses of variance using JMP® Pro Version 11.0.0 (SAS Institute). Differences between WT and RNAi lines in ratios of hexose to sucrose were evaluated with Tukey's HSD test. All significant differences are $P < 0.05$.

RESULTS

Assessment of senescent sweetening

Senescent sweetening was observed in chips from all RNAi lines, though in most lines it was delayed relative to that in chips from WT tubers (Fig. 1). All three of the ATL RNAi lines, two of the DKP RNAi lines and one of the MC RNAi lines had significantly slower development of chip darkening than the corresponding WT lines (Fig. 1). In many cases, these differences were quite large. For example, 37 WAH, the percentage of chips exhibiting darkening from senescent sweetening was 15–20% in ATL lines

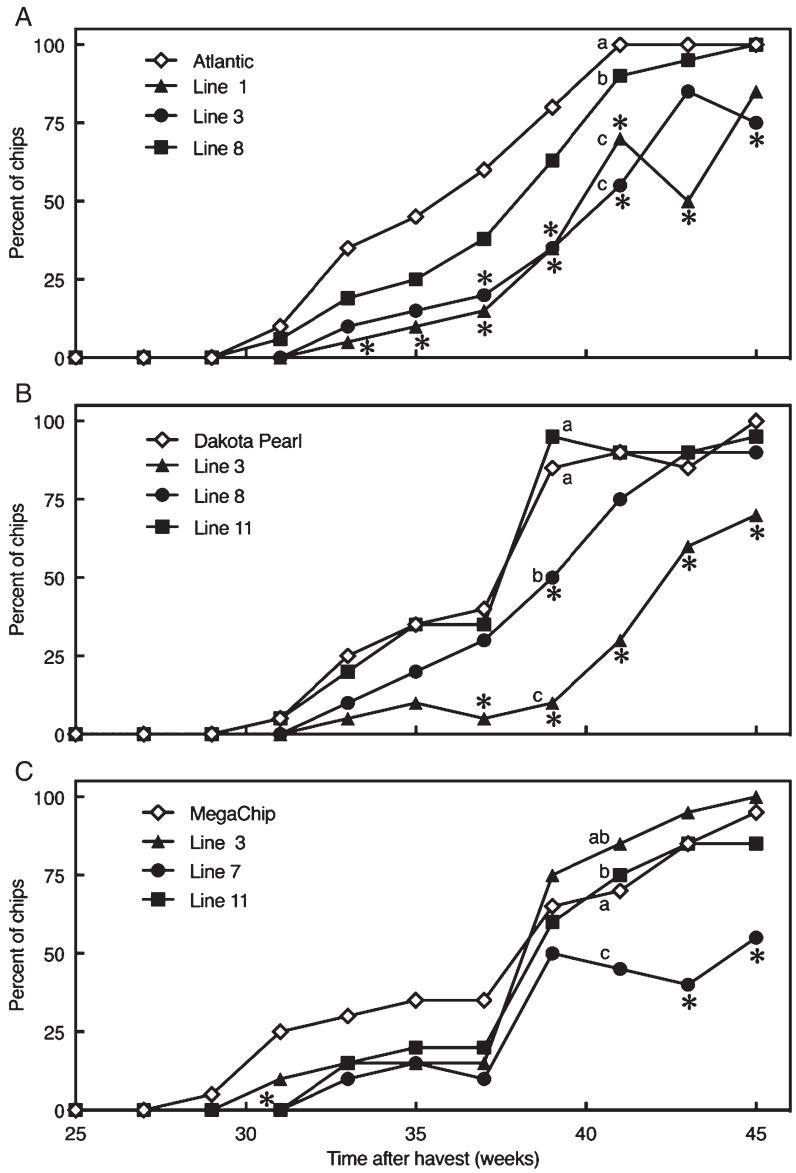

Figure 1. Percentages of chips exhibiting darkening attributed to senescent sweetening in WT and RNAi lines of (A) Atlantic, (B) Dakota Pearl, and (C) MegaChip. Each chip was taken from a different tuber and 20 tubers were sampled for each data point. Within each panel, lines with different lowercase letters differed ($P < 0.05$) in the development of chip darkening caused by senescent sweetening. At each sampling time, RNAi lines that differed significantly ($P < 0.05$) from the corresponding WT line in percentage of chips with darkening are indicated by asterisks.

Table 1. Sequences (5′ → 3′) and final concentrations (μmol L^{-1}) of primers used for quantitative PCR. Accessions are from the Potato Genome Sequencing Consortium

Gene (accession)	Forward primer, concentration	Reverse primer, concentration
60S ribosomal protein (PGSC0003DMG400015795)	gcaaagaagaagagagaggagatg, 0.4	ttcaaagccataattgtgtcaagt, 0.4
Actin (PGSC0003DMG400027746)	atgttcccgggtattgctgacaga, 0.4	ctgcctttgcaatccacatctgct, 0.4
Elongation factor 1α (PGSC0003DMG400023270)	ttccacttcaggatgtttacaaga, 0.8	cagcaacattcttaacattgaacc, 0.4
Vacuolar invertase (PGSC0003DMG400013856)	aaacgggttggacacatcat, 0.2	aacccaattccacaatccaa, 0.2

1 and 3 but almost 60% in WT ATL. At the same sampling time, approximately 40% of chips from WT DKP showed evidence of senescent sweetening, whereas fewer than 10% of chips in DKP line 3 showed chip darkening. Senescent sweetening in DKP line 11, where *VInv* silencing is relatively ineffective, occurred at the same rate as in WT DKP.

Tuber sugar contents and *VInv* expression

To explore further the causes of senescent sweetening and the differences between WT and RNAi lines, sugar contents of individual tubers were analyzed at two of the later sampling times, 41 and 43 WAH. Glucose and fructose contents during the late storage period were lower in most ATL and MC RNAi lines than in WT, as well as in

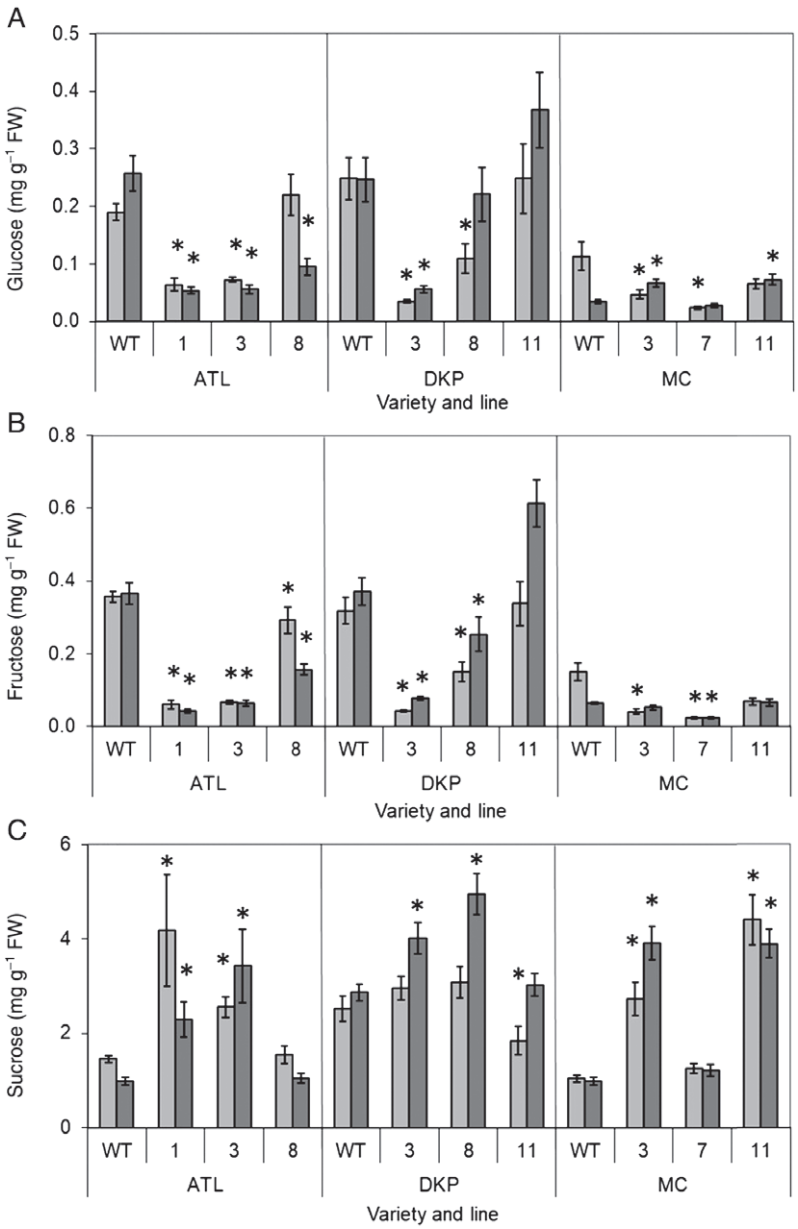

Figure 2. Potato tuber (A) glucose, (B) fructose, and (C) sucrose concentrations in Atlantic (ATL), Dakota Pearl (DKP), MegaChip (MC), and three RNAi lines derived from each WT line. Tubers were sampled 41 (light bars) and 43 (dark bars) weeks after harvest. Bars represent mean concentration in 20 samples ± standard error, with each sample taken from a different tuber. Asterisks indicate values that were significantly ($P < 0.05$) different from those of the WT line of the same variety at the same time.

DKP line 3 compared with WT (Figs 2A and 2B). Conversely, tuber sucrose content was elevated in several of the RNAi lines relative to WT (Fig. 2C). This was especially pronounced in MC lines 3 and 11 and DKP line 8.

The ratio of tuber hexose to sucrose was significantly smaller in all RNAi lines compared with WT lines except for ATL line 8 at 41 WAH and DKP line 11 (Fig. 3). ATL lines 1 and 3, DKP line 3, and MC line 7 had significantly delayed development of senescent sweetening (Fig. 1), and each of these lines had tuber hexose to sucrose ratios that were much lower than those observed for the corresponding WT lines (Fig. 3).

The hexose contents of tubers were strongly correlated with the percentages of chips exhibiting senescent sweetening (Fig. 4). When tuber hexose content was greater than 0.2 mg g^{-1} fresh weight (FW), 80–100% of chips showed darkening consistent with senescent sweetening. Remarkably, 40–60% of chips showed darkening consistent with senescent sweetening when tuber hexose content was less than 0.1 mg g^{-1} FW.

Tubers of all ATL and MC RNAi lines had less *VInv* mRNA accumulation than those from WT lines 41 and 43 WAH (Fig. 5). In 11 of 12 comparisons, these differences were statistically significant. In DKP, however, differences in *VInv* expression between WT and RNAi lines were not observed at these sampling times (Fig. 5).

DISCUSSION

Senescent sweetening, which resulted in chip darkening of WT lines 29–31 WAH, was delayed, though not eliminated, in tubers

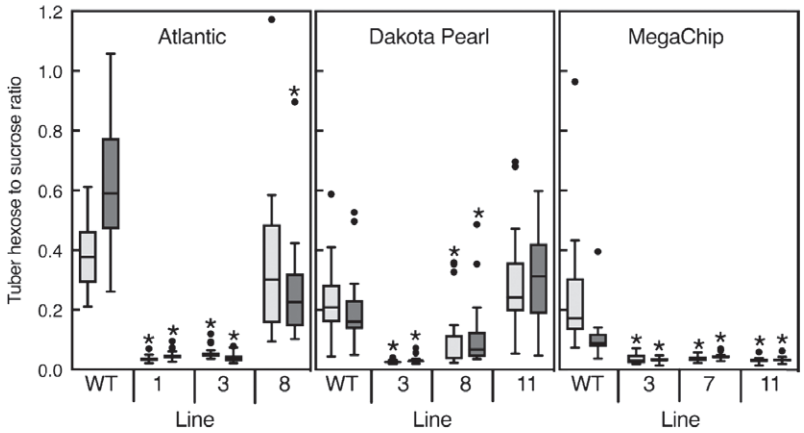

Figure 3. Ratios of potato tuber hexose to sucrose in Atlantic, Dakota Pearl, MegaChip, and three transgenic lines derived from each WT line. Tubers were sampled 41 (light bars) and 43 (dark bars) weeks after harvest. Twenty tubers were sampled from each line at each time, with each sample taken from a different tuber. Horizontal lines in each box plot are median values, boxes define the interquartile range, and whiskers extend to the farthest point within 1.5 times the interquartile range from the median. Asterisks indicate samples that were significantly ($P < 0.05$) different from those of the WT line of the same variety at the same time.

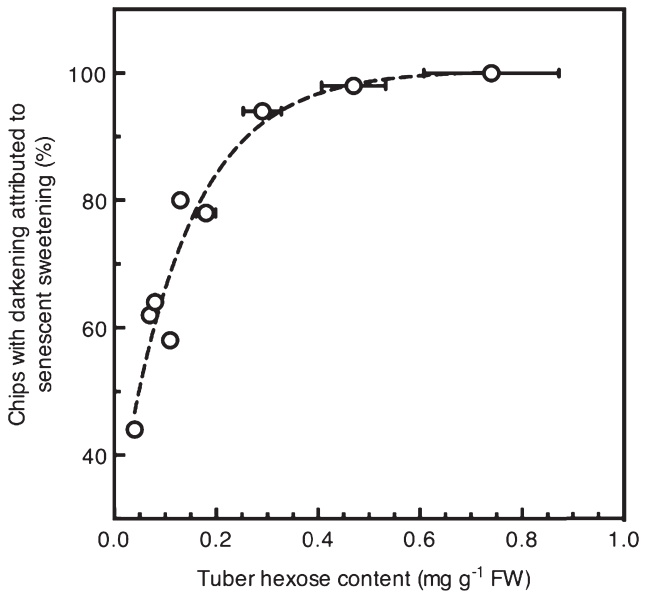

Figure 4. Percentage of chips with darkening attributed to senescent sweetening increased as tuber hexose content increased. Tubers and corresponding chips were assigned to groups of 50 based on measured hexose content. Data points represent percentage of 50 chips with darkening attributed to senescent sweetening. Error bars are standard deviations of mean hexose contents for each group of 50 tubers. In several cases, error bars are smaller than the symbols used for the data points.

with reduced *VInv* expression relative to WT tubers (Fig. 1). The length of this delay varied, being 4–6 weeks for three ATL lines, 2–6 weeks for two DKP lines, and approximately 4 weeks for three MC lines through 37 or more WAH. This is a considerable addition to the useful life of the stored crop.

The data for tuber *VInv* mRNA accumulation (Fig. 5), reducing sugar contents (Figs 2A and 2B), and hexose to sucrose ratios (Fig. 3) suggest that the effects of silencing *VInv* lasted through late-season storage. Reducing sugar contents were decreased in RNAi lines of ATL and MC, as well as in DKP line 3, 41–43 WAH. Furthermore, the ratio of hexose to sucrose, which is positively correlated with the activity of VInv in potato,[29] was lower in seven of nine RNAi lines than in WT controls at the sampling times investigated (Fig. 3). Taken together, these data give strong support to the hypothesis that VInv participates in the process of senescent sweetening.

The strong relationship between tuber hexose content and the percentage of chips exhibiting senescent sweetening (Fig. 4) was consistent with prior observations.[29] However, a substantial number of chips from tubers with very low hexose contents (<0.1 mg g^{-1} FW) exhibited darkening attributable to senescent sweetening. Prior research has shown that reducing sugar contents alone are unreliable predictors of chip color,[30] especially when reducing sugar contents are low.[31] It has previously been noted that 'deterioration in potato chip appearance after long-term storage coincided with or occurred shortly before the onset of "senescent sweetening"'.[32] These observations suggest that changes other than an increase in tuber hexose content may contribute to the chip darkening that is observed as potato tubers undergo senescent sweetening. Some of these changes, in other metabolites or in structural properties of membranes or cell walls, might promote chip darkening before reducing sugar contents are appreciably elevated. In particular, sucrose can break down at high temperatures to yield glucose and fructose, and sucrose can contribute to chip color darkening.[3,31,32,34] Most of the tubers in the present study had sucrose contents 41–43 WAH at least twice as high as the maximum desirable for chip processing (1.0 mg g^{-1} FW).[33] In tubers with hexose contents lower than 0.2 mg g^{-1} FW, chips from tubers with visible senescent sweetening had significantly higher sucrose contents than chips from tubers without visible senescent sweetening (2.53 and 2.08 mg g^{-1} FW respectively; $P = 0.01$). In chips with low hexose contents, dark chip color may have resulted from reducing sugars produced by thermal degradation of sucrose during frying rather than by enzymatic breakdown during storage. This may have been particularly important during the late-season storage period of MC lines 3 and 11. Tuber sucrose contents 41–43 WAH in these two RNAi lines were 2.7–4.4 mg g^{-1} FW, whereas WT sucrose contents were approximately 1 mg g^{-1} FW.

Potato cultivars with reduced expression of *VInv* as a result of RNAi have been deregulated in the USA and are entering commercial production. The findings presented here indicate that one potential attribute of these lines may be a delay in the onset of senescent sweetening relative to the parental cultivar. This

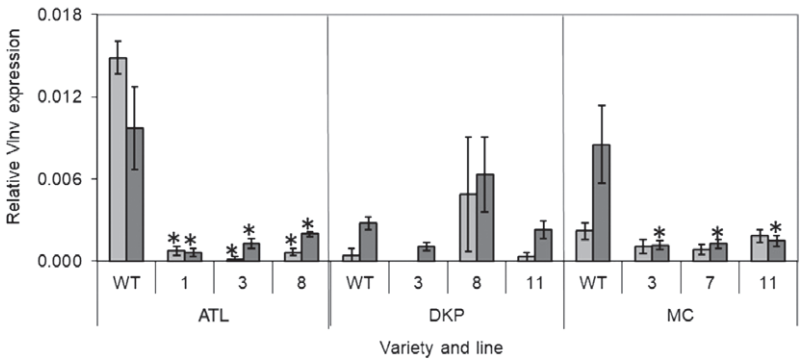

Figure 5. Relative expression of vacuolar invertase gene in Atlantic (ATL), Dakota Pearl (DKP), and MegaChip (MC) tubers sampled 41 (light bars) and 43 (dark bars) weeks after harvest. Bars represent mean values in five samples ± standard error. Asterisks indicate values that were significantly ($P < 0.05$) different from those of the WT line of the same variety at the same time. Expression of vacuolar invertase is relative to that of 60S ribosomal protein, actin, and elongation factor 1α.

trait may be useful to potato growers and processors wishing to maximize the useful storage life of their crop.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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