Alleviation of low temperature sweetening in potato by expressing *Arabidopsis* pyruvate decarboxylase gene and stress-inducible *rd29A*: A preliminary study

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Abstract The acceptability of potatoes for processing chips and French fries is largely dependent on the color of the finished product. Most potato cultivars and varieties stored at temperatures below 9–10 °C are subjected to low temperature sweetening (LTS) which result in the production of bitter-tasting, dark colored chips and French fries which are unacceptable to consumers. However, storing tubers at low temperatures (i.e., <10 °C) has many advantages such as lowered weight loss during storage, natural control of sprouting, and reduction/elimination of chemical sprout inhibitors. Our earlier research results on LTS suggested a role for pyruvate decarboxylase (PDC) in LTS-tolerance. In the present study, the role of PDC was examined whereby the potato variety Snowden was transformed with *Arabidopsis* cold-inducible pyruvate decarboxylase gene 1 (*AtPDC1*) under the control of promoter *rd29A*. Two transgenic plants were selected and storage studies were conducted on tubers harvested from one of the transgenic lines grown under green house conditions. Transgenic tubers showed higher Agtron chip color score indicating lighter chip and lower reducing sugar and sucrose concentrations compared to the untransformed

tubers during the storage periods studied at 12 °C and 5 °C. These results suggest that overexpression of pyruvate decarboxylase gene resulted in low temperature sweetening tolerance in the transgenic Snowden.

Keywords Cold storage · Low temperature sweetening · Potato · Pyruvate decarboxylase · Transgenic

Introduction

French fries and potato chips constitute the two major processed potato products in the food industry. Numerous factors affect potato quality and many of them relate to the chemical composition of the tuber which are influenced by the environment during growth and storage. A four-year field study conducted in our laboratory to analyze the various compositional factors such as reducing sugars, sucrose, nitrogen, ascorbic acid, protein and dry matter during low temperature storage showed that chip color was highly correlated to reducing sugars (Blenkinsop et al. 2002). Most potato cultivars and varieties stored at temperatures below 9–10 °C are prone to low temperature sweetening (LTS). LTS in potato results in the production of reducing sugars such as glucose and fructose as well as sucrose as a result of starch hydrolysis (ap Rees and Morrel 1990). These reducing sugars act as substrates for the non-enzymatic Maillard browning reaction, which is the major reaction, during frying resulting in the production of bitter-tasting dark colored chips. Other browning reactions such as polyphenol oxidase reaction and caramelization play only a minor role in the final colour of chips and fries. LTS, therefore, is a major concern to the potato processing industry. However, there are many advantages of storing processing tubers at low temperature which include less

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shrinkage and disease loss, control of sprouting, reduction/elimination of the use of chemical sprout inhibitors and extended marketability (Wismer et al. 1995; Sowokinos 2001; Blenkinsop et al. 2004). An Environmental Protection Agency mandate (1996) within the requirements of the Food Quality Protection Act (FQPA) resulted in a reduction in allowable CIPC residue, a chemical sprout inhibitor on fresh potatoes in the USA from 50 ppm to 30 ppm. This mandate coincides with tolerance reductions or restrictions for use of CIPC in other parts of the world (Kleinkopf et al. 2003) with the European member countries having a limit of 5 to 10 ppm. Hence there is a need for alternative methods for sprout suppression, such as cold storage.

Our earlier research with LTS-tolerant and LTS-susceptible varieties, showed consistently higher production of ethanol and lactate in the LTS-tolerant varieties which were negatively correlated to levels of reducing sugars (Marangoni et al. 1997; Blenkinsop et al. 2003). Subsequent research showed that the anaerobic respiratory pathway enzymes such as L-lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH) and pyruvate decarboxylase may have a role in LTS-tolerance (Pinhero et al. 2007).

Several plant species exposed to environmental stresses such as water deficit, SO₂ fumigation, ozone exposure and low temperature produce considerable amounts of acetaldehyde and ethanol at ambient or even at elevated oxygen concentration (Kimmerer and Kozlowski 1982). This observation suggests that the anaerobic pathway has a general function in aerobic metabolism under stress conditions which damage the intricate mitochondrial ATP-generating machinery. It has been reported that Arabidopsis PDC and ADH genes are induced by cold and dehydration suggesting that anaerobic pathway plays a role under environmental stress (Tadege et al. 1999). Other reports suggest that overexpression of PDC1 or PDC2 in Arabidopsis resulted in improved plant survival under hypoxic conditions whereas ADH1 overexpression had no effect on flooding survival (Ismond et al. 2003). Ismond et al. (2003) reported that an increased PDC activity resulted in increased carbon flow through the ethanol pathway as reflected by an increase in both acetaldehyde and ethanol pool sizes. Similarly, Quimio et al. (2000) found that enhancement of PDC levels in transgenic rice overexpressing PDC corresponded with an increase in submergence tolerance. Biochemical tests have indicated that ADH does not limit fermentation but PDC has a fine control on fermentation pathway due to its low maximum catalytic capacity (Drew 1997; Morrell et al. 1990). Ethanol fermentation requires only two dedicated enzymes, ADH and PDC. Experiments with isogenic maize lines differing in ADH activity, over an approximately 200-fold range, indicated that ADH activity does not limit the capacity for energy production by ethanol fermentation unless there is a reduction in activity

to less than 1 % of wild-type levels (Roberts et al. 1989), which suggests that ADH is present in large excess and may not be a regulatory enzyme of the ethanolic pathway. ADH gene has been induced by cold in rice, maize and Arabidopsis (Christie et al. 1991). The induction of expression of ADH gene by cold suggested a role for PDC. It has been reported that ethanolic fermentation is part of a general response to environmental stress and PDC as the key regulatory first enzyme of ethanolic fermentation (Kurstainer et al. 2003). Studies have also shown that activities of ADH and PDC increased during hypoxia in potato (Biemelt et al. 1999). In Arabidopsis, of the six putative PDC gene family members, only PDC1 was induced by cold to about 8–10-fold. The level of PDC is lower than that of ADH by a factor of 17–65 (Morrell and Greenway 1989; Ismond et al. 2003) and its activity is very close to the rate of ethanolic fermentation in vivo (Morrell et al. 1990; Drew 1997). These results as well as our previous work (Pinhero et al. 2007) support the role of PDC as the control step in ethanol fermentation. Therefore, we hypothesized that genetic modification of potato varieties with desired chipping qualities by over-expressing PDC1 from Arabidopsis under the control of a cold-regulated promoter will regulate the ethanolic fermentative pathway in the transgenic potato during low-temperature storage. This in turn will divert the excess reducing sugars and offer LTS-tolerance. In order to validate our hypothesis we developed a transgenic potato which overexpressed Arabidopsis PDC1 under the control of a cold inducible promoter *rd29A*. The results obtained from this study are presented herein.

Materials and methods

Plant material

Potato (*Solanum tuberosum* L.) variety Snowden was used for this study. Tissue cultured plantlets were obtained from New Liskeard Agricultural Research Station, University of Guelph, New Liskeard, Ontario. Plantlets were multiplied and 6 week-old plantlets were used for the transformation studies. Stem cuttings and leaf discs were used as explants for transformation.

Cloning and plant transformation

Arabidopsis cDNA was synthesized from total RNA according to the protocol of RETROscript Reverse transcription Kit for RT-PCR (Ambion). The full-length coding regions of the PDC 1 of Arabidopsis (AtPDC1, Accession number NM_119461, left primer, AtPDC1L: 5'-ATGGAC ACCAAAATCGGA-3' and right primer AtPDC1R, 5'-CTACTGAGGATTGGGAGGACG-3') was amplified by

PCR and cloned according to standard protocols (Sambrook et al. 1989). Similarly, *rd29A* promoter was amplified by PCR from *rd29A:mgfp5ER* in pBI 121 using left primer, Hind III + *rd29A* (5'-CCCAAGCTTGAGCCATAGATGCAATTC-3') and right primer, BamHI + *rd29A* (5'-CGGGATCCAATAGAAGTAATCAAACC-3'). AtPDC1 and *rd29A* were cloned following the protocol of Sambrook et al. (1989) between the SacI and Hind III sites in the plant vector pBI121 after introducing restriction sites for AtPDC1. The explants, stem petioles of 1 cm in length and leaf pieces cut into half were precultured for 2 days on MS basal medium (Murashige and Skoog 1962) without vitamins containing 3 % sucrose, 0.5 μ M indolacetic acid, 3 μ M zeatin riboside and 0.7 % agar at 22 ± 2 °C with a 16 h photoperiod and 50 μ mol m⁻² s⁻¹ light intensity. *Agrobacterium tumefaciens* strain LBA 4404 was used to transform the stem petioles and leaf pieces by immersing in *Agrobacterium* culture (bacterial culture diluted 1:10 with 1x MS solution containing 375 μ M acetosyringone) for about 10 min (Rooke and Lindsey 1998). The explants were co-cultivated with *Agrobacterium tumefaciens* for 24 h in the preculture medium. Transformed cells were selected and regenerated in the presence of 50 μ g/mL kanamycin through a three-step regeneration method. After co-cultivation, tissues were transferred to callus-induction medium which contained the preculture medium composition supplemented with vitamins, cefotaxime 400 mg/L and kanamycin. After 2–3 weeks, the callusing sites of each explant were kept separate and were transferred to shoot-induction medium. The shoot-induction medium consists of essentially the components of callus-induction medium, except the indole acetic acid was replaced by 0.3 μ M gibberellic acid. When regenerated shoots reached a height of 5–10 mm, these shoots were excised and transferred to rooting medium in Magenta boxes, which is MS basal medium with 3 % sucrose, 0.6 % agar and cefotaxime 200 mg/L and kanamycin 50 mg/L. Primary transformants were selected by PCR screening of npt II gene and micro-propagated. Small scale genomic DNA was extracted for PCR following the protocol of Purelink plant total DNA purification kit from Invitrogen. The PCR primers of npt II gene are: left primer 5'-CTG AAT GAA CTG CAG GAC GA-3', right primer: 5'-AGA ACT CGT CAA GAA GGC GA-3'

Southern blot analysis of transgenic plants

Genomic DNA was extracted from 1 g of leaves of 2 month old control and transgenic lines L1 and L2 plants at a large scale using Qiagen DNeasy maxi kit (Qiagen, Canada). For Southern analysis, three separate genomic DNA digests with Hind III, Nsi I and EcoR I were set up using 10 μ g of DNA for each digestion. Southern blot analysis was carried out following the standard protocols and DIG-labeled

350 bp DNA probe (left primer: 5'-ATGGACACCAAAA TCGGA-3', right primer: 5'-ACGGTGAAGGTAACAA CGCA-3') synthesized from AtPDC1 by PCR following the DIG system user's guide for filter hybridization and DIG-luminescent detection kit (Boehringer Mannheim/Roche).

RNA extraction and Northern hybridization

Northern hybridization was used to detect the expression of AtPDC1 in the transgenic potato plants. Forty four day old plantlets were stored at 4 °C and leaf samples (100 mg) were obtained prior to and after 4 and 6 h of cold exposure. Samples were ground in liquid nitrogen, and total RNA was extracted using RNeasy Plant Mini kit for purification of total RNA from plants (Qiagen, Canada) according to the manufacturer's instructions. Aliquots (5 μ g) of RNA were fractionated on 0.8 % agarose gels in 3-(N-morpholino) propanesulfonic acid buffer, and then transferred to a positively charged nylon membrane (Boehringer, Germany) using 20x SSC. DIG-labeled DNA probe used for Southern blot was used as probe. The hybridization signals were detected following the DIG system user's guide for filter hybridization and DIG-luminescent detection kit (Boehringer Mannheim/Roche).

Greenhouse studies

L1 and L2 lines were maintained in tissue culture by clonal propagation. Plantlets from the tissue culture were grown in small pots using Mix LC 1, Professional growing mix (Sungro, Sunshine) and acclimatized to the growth conditions by providing shade for the first week. After 1 month, these plants were repotted into large pots. These plants were grown at 16 h light at 21 ± 2 °C and 8 h dark at 16 ± 2 °C for the first 6 weeks and there after at 12 h light until harvest. The plants were fertilized with 50 ppm 15 N-15P-18 K on alternate days. Tubers were harvested after 167 days and cured at 12 °C and 90–95 % relative humidity for about 1 month before storage.

Specific gravity of tubers

Specific gravity of tubers was determined before and after storage from five tubers per treatment for three replicates by the formula given below.

$$\text{Specific gravity} = \frac{\text{weight of tubers in air}}{(\text{weight in air}) - (\text{weight in water})}$$

Storage studies and chip processing

Tubers were stored at 12 °C and 5 °C, 90–95 % relative humidity in the postharvest facility of Plant Agriculture,

University of Guelph and periodically removed for chipping. A composite chip score for the control and transgenic line L1 was determined from three replicates consisting of five tubers each, which were abrasion-peeled and sliced into 1 mm thick slices using a Hobart mechanical slicer (Hobart Corporation, Troy, OH). The tuber slices were fried in vegetable oil at 175 °C. Samples were crushed into small (ca. 10×10 mm) pieces, and chip color was evaluated using an Agtron M30A colorimeter (Chism Machinery, Niagara Falls, ON) as described in (Blenkinsop et al. 2003).

Sugar analysis

Potato dry matter was obtained from the stored tubers after peeling, freeze-drying, grinding and passing through a 250 µm sieve. Sugar was extracted from 3 g potato dry matter in 10 ml of 80 % ethanol at 80 °C for 1 h (Matsuura-Endo et al. 2004). The solution was evaporated to complete dryness and glucose, fructose and sucrose analyses were carried out using Shimadzu model Gas Chromatography-17 after derivatization with STOX Oxime –internal standard reagent and N-(trimethylsilyl)-imidazole using a 6'×0.25" glass column packed with 3 % OV-17 on Chromosorb W (HP) 80/100 Mesh. Duplicate determinations were performed on a composite sample (Long and Chism 1987).

PDC activity analysis

Total protein for PDC (PDC, EC 4.1.1.1) from tubers was extracted and enzyme activity analyzed as described in by Pinhero et al. (2007). A 100 g sample of peeled and chopped tubers ($n=5-8$) was blended in 100 ml of 50 mM NaPO₄, pH 6.2, containing 0.5 % polyvinylpyrrolidone, 10 mM dithiothreitol, 0.1 % BSA and PDC cofactors, namely, 1 mM thiamine pyrophosphate (TPP) and 2 mM MgCl₂. Following a 40 % ammonium sulfate precipitation, the precipitate was resuspended in 50 mM NaPO₄, pH 6.2, containing 0.1 % BSA. The resuspended protein was concentrated using PD10 column (Amersham Biosciences) following the manufacturer's instructions. PDC activity was measured using a coupled assay where pyruvate is decarboxylated by PDC to produce acetaldehyde and then reduced exogenously by adding ADH, and finally oxidizing NADH in the process. The rate of disappearance of NADH is equivalent to the rate of pyruvate decarboxylation. The assay conditions were established for optimal activity of PDC in order to eliminate the activities of endogenous ADH and LDH. These included crude protein preparations to enrich PDC protein by ammonium sulfate precipitation at 40 % level, concentrating the protein using PD10 column and pH optimum for PDC activity (6.2). The assays were carried out in triplicate from the enriched protein fractions.

Results and discussion

Ethanol fermentation during aerobic metabolism under stress conditions such as cold and dehydration is a possible adaptation mechanism, as evidenced by the induction of Arabidopsis PDC and ADH genes and the functional activation of the ethanolic pathway in several plant species when exposed to environmental stresses (Tadege et al. 1999). It has been reported that use of stress-inducible *rd29A* promoter minimized the negative effects of constitutive 35S CaMV promoter in plant growth while providing greater stress tolerance to salinity, freezing and drought (Kasuga et al. 2004). Our earlier studies suggested a role of anaerobic pathway in LTS tolerance (Blenkinsop et al. 2003; Pinhero et al. 2007). Therefore, in the present study the AtPDC1 gene under the control of *rd29A* promoter was overexpressed in potato variety Snowden. Only the stem explants were transformed after several transformation experiments from which three transformants were obtained and two transgenic lines were selected for further characterization. LTS-tolerance study based on storage was conducted on only one transgenic line L1 due to the lack of sufficient tubers for L2 as a result of pest infestation. The results of these experiments are discussed below.

Transformation of potato with AtPDC1 gene

In order to test our hypothesis of the direct effect of PDC in LTS-tolerance, our initial transformation studies with AtPDC1 were carried out with Snowden. Three regenerants were obtained with stem explants from Snowden variety after several *Agrobacterium*-mediated transformations. Transformations with leaf explant were unsuccessful. The stem explant regenerants selected on kanamycin were preliminarily evaluated by the polymerase chain reaction for the *npt II* gene along with a non-transgenic control plant. These were designated as L1, L2 and L3. We attempted transformation studies with some other commercial varieties such as Monona, but were unsuccessful. Recently, however, we were successful in obtaining 3 transgenic plants from Dakota Pearl and 17 transgenic plants with another variety, Atlantic. It should be noted that transformation was successful with only leaf explants in Atlantic compared to stem explants from Snowden and Dakota Pearl and our experiments are continuing with these transgenics. It was reported that variety as well as explants affect transformation efficiency in potato (Dale and Hampson 1995; Visser et al. 1989). Even though several transformations were conducted throughout the year, transformations initiated during the spring were successful. Similar seasonal effect and very low efficiency of transformation was reported earlier (Visser et al. 1989).

Fig. 1 Southern hybridization of *Arabidopsis thaliana* PDC1 transformed potato. 10 µg genomic DNA from leaves was digested with HindIII (lanes 1, 4, 7) EcoRI (lanes 2, 5, 8), and NsiI (lanes 3, 6, 9). A DIG-labeled AtPDC1 gene DNA fragment of 350 bp was used as a probe

Southern analysis

Southern analysis of L1 and L2 were carried out after digesting the genomic DNA with restriction enzymes HindIII, EcoRI and NsiI using DIG-labeled AtPDC1 specific probe of 350 bp. The probe was designed after conducting sequence alignment with potato pyruvate decarboxylase genes to exclude the conserved domain and select the domain specific to DNA of AtPDC1 to avoid cross hybridization with potato pyruvate decarboxylase genes. Southern analysis confirmed the presence of the AtPDC1 gene in the transgenic plants (Fig. 1, lanes 4, 5, 6, 7, 8 and 9). No signal was observed in the control plant (Fig. 1, lanes 1, 2 and 3). The absence of signal in the control non-transgenic plant validated that no cross hybridization had occurred and that the probe selected was specific to AtPDC1. The restriction enzymes, NsiI and EcoRI, were selected so as to not cut the gene. From the Southern analysis, it appeared that L1 and L2 had three copies of the gene. There was no clear reason for different number of bands using EcoRI and NsiI since AtPDC1 did not have the appropriate restriction cut sites and may be due to incomplete introgression of the transgene, i.e., the restriction enzyme can find more than one restriction site at which to cut the genome (Behnam et al. 2006). L1 and L2 showed the characteristic restriction pattern, indicating that it was an independent transformation.

Northern hybridization

Total RNA was extracted from the control and transgenic lines before and after cold exposure. Northern analysis suggested the expression of AtPDC1 after cold exposure for

which an approximately 2.2 kb transcript was detected. The transgene expression was higher in L1 compared to L2 (Fig. 2, lanes 5 and 8 versus lanes 6 and 9). No expression was observed in the control plants during all time periods studied as well as L1 and L2 before cold exposure (Fig. 2, lanes 1, 2, 3, 4, and 7). The lack of expression of the transgene in L1 and L2 before cold exposure was expected since *rd29A* promoter is a stress-inducible promoter. Little difference was observed in the gene expression levels between 4 and 6 h of cold exposure in L1 and L2 suggesting that maximum expression was reached within that period (Fig. 2, lanes 5, 6, 8 & 9).

Growth and development

The two transgenic lines L1 and L2 when grown in the greenhouse showed some phenotypic differences from their wild control plant (Fig. 3). Transgenic line L1 was taller than wild, while L2 was robust and shorter than the wild counterpart. L1 was also more resistant to pests such as thrips and mealy bugs whereas L2 was more susceptible than the wild-type under green house conditions (data not reported). An average yield of 1.005 kg/plant was obtained from control and L1 plant whereas L2 was affected by pests and yielded only 314.7 g/plant. It has been reported that transgenic potatoes constitutively overexpressing bacterial PDC showed lesion mimic phenotype in the leaves accompanied by induction of the plant defense response and resistance to *Phytophthora infestans* (Tadege et al. 1998). In their study, the plant defense response was observed only at 18 °C and not at 25 °C and the extent of lesion phenotype was related to the level of PDC expression. We did not observe any lesion mimic phenotype in our experiments other than some resistance to pests. We suggest that the level of pest resistance observed under greenhouse conditions in L1 could be related to such a response since L1 transgene expression was higher than L2. However, the reason for L2 being more susceptible to pests compared to the wild untransformed plant is not known.

Fig. 2 Northern blot analysis of *Arabidopsis thaliana* PDC 1 transcripts. 5 µg of total RNA was loaded into a 0.8 % agarose gel. DIG-labeled DNA fragment of AtPDC1 gene was used as a probe. Lanes 1, 2, and 3 represents wild, L1 and L2, respectively, before exposure; 4, 5 and 6 after 4 h and 7, 8 and 9 after 6 h of exposure to cold at 4 °C. Equal loading of RNA on the gel was confirmed by ethidium bromide staining

Fig. 3 Phenotype of 108 days-old control and transgenic plants. Plantlets developed under tissue culture conditions were first acclimatized in small pots with high humidity and transplanted to large pots in green house. Control is transformed and developed under the same conditions as the transgenic without the gene construct, L1 and L2 are transgenic plants

The following could possibly explain the above observations. It should be noted that the performance of transgenic plants is affected by three phenomena. 1. Insertion mutagenesis. DNA introduced by transformation can cause disruption of the plant gene it is inserted into or a gene close to it likely causing subtle changes in phenotype. T-DNA insertional mutagenesis is unlikely to have any significant influence on the performance of the transgenic plants for two reasons. 1) that the majority of mutations are known to be recessive and, therefore, would not be visible in the transformed generation and 2) in most higher plants, a high proportion of the DNA is not actively coding (Dale and McPartlan 1992). Although there is now evidence that

Fig. 4 Chip color score of potato tubers. A composite chip color score for each treatment was determined from three replicates of five tubers each. The data are presented as mean $\pm$ standard error from three replicates

the position of T-DNA insertion may not be random within the plant genome, any T-DNA copies integrating into inactive DNA would not be expected to have an effect on plant genotype (Sawahel 1994). 2. Pleiotropy. It is known from genetic studies with non-transgenic plants that individual genes can have, apparently unrelated, multiple effects on plant phenotype (Dale and McPartlan 1992; Sawahel 1994). 3) Somaclonal variation. The creation of transgenic plants usually involves a tissue-culture phase and the regeneration of plants from explants with a high capacity for plant regeneration. Plant regeneration from cultured tissues and cells is well known to result in genetic variation among regenerated plants (Karp 1991; Dale and

Fig. 5 A representative sample of chips prepared from the control and transgenic tubers after storage. **a** and **e** represents chips from tubers stored at 5 °C from control non-transgenic potato after 28 and 56 days storage respectively. **b** and **f** are chips from tubers stored at 12 °C from control non-transgenic potato after 28 and 56 days storage respectively. **c** and **g** are chips from tubers stored at 5 °C from L1 after 28 and 56 days storage respectively. **d** and **h** are chips from tubers stored at 12 °C from L1 after 28 and 56 days storage respectively

Fig. 6 Fructose, glucose and sucrose concentrations of potato tubers before storage analyzed by GC. The data represent mean $\pm$ SE from two replicates of a composite sample

McPartlan 1992; Sawahel 1994). Results of small-scale independent experiments have shown that similar frequencies of abnormal phenotypes are obtained for both modified and non-modified plants from tissue cultures. Therefore it is not surprising to find phenotypic changes that are not caused by the foreign gene or its insertion in modified plants produced by tissue culture. Foreign DNA in modified plants constitutes only a small fraction of the

host cell genome (often a ratio of 1:1 million). The insertional and deletional mutations resulting from foreign gene transfer are, therefore, likely to contribute only a small proportion of the variants seen (Yang 1988; Sawahel 1994). Hence, we believe that the changes in plant height noticed between L1 and L2 could have resulted from somaclonal variation and not from the transgene expression.

Tuber quality and chipping

An important quality of potatoes used for French fries and potato chips is high dry matter, which is measured as specific gravity or percent solids. The specific gravity of the tubers in the transgenic line and control were similar (1.107 and 1.095 respectively), and was not affected significantly during storage, as measured after 14, 28, 42 and 56 days (data not shown).

Specific gravity of tubers influences chip color. It has been reported that tubers with high specific gravity produce lighter chip color (Lyman and Mackey 1961). Good quality potato chips should possess a light color and for most potato cultivars/varieties chipping quality (i.e., color) is affected during cold storage especially after storage at 5 °C. Chip color of the potato chips was, therefore, evaluated

Fig. 7 Fructose (a), glucose (b), sucrose (c) and total reducing sugars (glucose + fructose, d) of potato tubers determined by GC after storage at 12 °C and 5 °C. The data represent mean $\pm$ SE of two replicates from a composite sample

Fig. 8 Pearson Correlation Coefficient for the relationship between chip scores and reducing sugars (a) and sucrose (b) measured for the control untransformed and transgenic L1 tubers stored at 12 °C and 5 °C

after storing the potatoes at 12° and 5 °C. The results of the chip color quality, as determined by Agtron color measurements, showed that the color was consistently higher (a higher score denoting lighter color chips) for the transgenic tubers at 12° and 5 °C compared to the control tubers during 14, 28, 42 and 56 days after storage (Figs. 4 and 5). The chip score was higher for tubers stored at 5 °C after 14 days as compared to samples stored at 12 °C. The chip score decreased during 28 and 42 days after storage at 5 °C, whereas there was little decrease during storage at 12 °C.

Another important determinant of the quality of potatoes used for French fries and potatoes chip processing is the level of reducing sugars which impacts on chip color. In general, the level of fructose in tubers was lower than glucose and sucrose, and sucrose was higher compared to glucose and fructose combined (Figs. 6 and 7). The fructose and sucrose contents were higher in the control tubers before storage compared to the transgenic line, while glucose concentration was higher in transgenic line (Fig. 6). The levels of glucose, fructose, glucose + fructose and sucrose increased steadily as the storage period

increased. The fructose, glucose and sucrose contents were always higher in the tubers stored at 5 °C compared to 12 °C. Fructose, glucose and sucrose contents were higher in the control tubers compared to the transgenic line during all the storage periods studied except 42 days after storage, where the sucrose content was higher in the transgenic line (Fig. 7). The results obtained in our study associated with lighter chips reflecting lower reducing sugars and sucrose from transgenic potatoes stored at 5 °C, exhibiting LTS-tolerance, are in agreement with published results indicating the accumulation of reducing sugars during cold storage below 9–10 °C (Blenkinsop et al. 2002). There is a general agreement that the level of reducing sugars such as glucose and fructose is the most important factor that determines the chip color (Blenkinsop et al. 2002; McCann et al. 2010; Roe et al. 1990; Sowokinos 1990; Sowokinos 2001; Wismer et al. 1995). This effect was seen in all tubers (i.e., control and transgenic tubers) stored at 12 °C where the level of reducing sugars and sucrose were lower than the tubers stored at 5 °C which also produced lighter chips (Figs. 4, 5 and 7).

Correlation of chip score between reducing sugars and sucrose

Chip score and reducing sugars (fructose + glucose) and sucrose of tubers from control and L1 samples were analyzed via Pearson correlation. A significant negative correlation was observed between chip color and reducing sugar concentration (Fig. 8a) as well as sucrose (Fig. 8b). The negative correlation was higher with reducing sugars than sucrose. A similar negative correlation between chip color score and reducing sugars as well as sucrose has been

Fig. 9 Pyruvate decarboxylase activity of tubers after storage. Tubers were analyzed for PDC activity after storage at 12 °C and 5 °C for 28 days. The data are the mean $\pm$ SE from three replicates expressed as percentage of the activity of tubers before storage

reported by others (Sowokinos 1990; Blenkinsop et al. 2002; Song 2004).

Activity of pyruvate decarboxylase

The initial activity of PDC before storage was slightly higher in the control tubers compared to the transgenic tubers. PDC activity increased during storage at 5 °C and 12 °C in both control and transgenic tubers (Fig. 9). No difference in activity was apparent between the control and transgenic tubers at 12 °C. However, PDC activity was higher in the transgenic tubers than the control tubers stored at 5 °C and was similar to that of tubers stored at 12 °C. The extent and pattern of increase in PDC activities noted in our study confers its role in LTS-tolerance. Ismond et al. (2003) reported improved plant survival under hypoxic conditions by overexpressing PDC1 or PDC2 in Arabidopsis. Research results also support that enhancement of PDC levels in transgenic rice overexpressing PDC corresponded with an increase in submergence tolerance (Quimio et al. 2000; Ismond et al. 2003; Kursteiner et al. 2003; Agarwal and Grover 2006). Recently, it has been reported that the increased hypoxia tolerance in *Malus hupehensis* may be partly due to the higher enzyme activity of PDC, ADH and LDH (Li et al. 2010).

Conclusions

Expression of AtPDC1 under the control of *rd29A* promoter in Snowden resulted in its overexpression during cold storage. Transgenic tubers stored under cold conditions resulted in lighter chip color and lowered reducing sugars such as glucose and fructose, as well as sucrose. A negative correlation was observed between the chip color and reducing sugars (glucose + fructose) and sucrose. These results suggest overexpression of AtPDC1 resulted in increased LTS-tolerance. The accelerated accumulation of sucrose, fructose and glucose that occurs during LTS is an extremely complex process, involving the interaction of a number of pathways of carbohydrate metabolism, including starch degradative and synthetic pathways, sucrose synthesis and degradation, glycolysis, the oxidative pentose phosphate pathway, and mitochondrial respiration (Blenkinsop et al. 2004). Other researchers have recently developed LTS-tolerant potatoes by inhibiting the activities of vacuolar invertase by genetically modifying potatoes (Ye et al. 2010). However, our laboratory was the first to propose that anaerobic respiratory pathway is one of the targets responsible for LTS-tolerance in the LTS-tolerant varieties studied (Blenkinsop et al. 2003; Pinhero et al. 2007). We have proposed from our earlier studies that potato tubers may respond to low temperature storage by shifting respiratory

metabolism towards the anaerobic pathway, the cold-tolerant tubers undergoing a greater shift in energy metabolism to ensure maintenance of metabolic control during cold storage, and at the same time, maintain lower levels of reducing sugars (Blenkinsop et al. 2003). Therefore, anaerobic pathway flux may play an important role in affecting reducing sugar accumulation, and consequently processing quality of potato tubers stored at low temperatures. The role of PDC in the anaerobic pathway and in stress response and tolerance has been widely reported (Drew 1997; Morrell et al. 1990; Quimio et al. 2000; Ismond et al. 2003). We suggest a similar mechanism in our transgenic tubers responsible for LTS-tolerance. This result also corroborates our earlier findings of a role of PDC in LTS-tolerance (Blenkinsop et al. 2003; Pinhero et al. 2007).

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