Invited Review

Biochemical and Molecular Control of Cold-Induced Sweetening in Potatoes

Joseph R. Sowokinos

University of Minnesota, Dept. of Horticultural Science, USDA-ARS Potato Research Worksite, 311 5th Avenue NE, East Grand Forks, MN 56721.
Tel: 218-773-2473; Fax: 701-795-8348; e-mail: sowok001@umn..edu
University of Minnesota, Agricultural Experimental Station Scientific Journal Series No: 001210061.

ABSTRACT

The benefits of being able to process potatoes directly into chips or fries from cold storage (2 to 4 C) include less shrinkage, retention of dry matter, decreased disease loss, extended marketability, and the elimination of the need for dormancy-prolonging chemicals. Unfortunately at low temperature, potato tubers undergo a phenomenon known as cold-induced sweetening where the rate of conversion of starch to reducing sugars (i.e., glucose and fructose) is accelerated. As raw potatoes are sliced and cooked in oil at high temperature, the accumulated reducing sugars react with free amino acids in the potato cell forming unacceptably brown- to black-pigmented chips or fries via a non-enzymatic, Maillard-type reaction. Potatoes yielding these unacceptably colored products are generally rejected for purchase by the processing plant. All commercial potato cultivars presently used for the production of potato chips and fries accumulate excess free reducing sugars when exposed to cold stress. If a "cold-processing potato" was available, energy savings would be realized in potato-growing regions where outside storage temperatures are cool. In regions where outside temperatures are moderately high, increased refrigeration costs may occur. This expense would be offset, however, by removal of the need to purchase dormancy-prolonging chemicals, by a decreased need for disease control and by improvement of long-term tuber quality. The primary goal of this review is to describe recent research of a biochemical and molecular nature that relates to the underlaying mechanisms regulating post harvest, cold-induced sweetening in potato tubers. No attempt was made to outline the extensive research conducted on the genetic manipulation of carbon metabolism between starch and free sugars during photosynthesis and/or during potato development in relation to source/sink interactions.

RESUMEN

Los beneficios que se obtienen al procesar papas fritas o en houjuelas de manera directa, que hayan estado almacenadas en cámaras frigoríficas a temperaturas que van de 2 a 4°C, incluyen menor encogimiento, retención de sustancia seca, disminución de enfermedades, un amplio potencial para el mercado y la eliminación de la necesidad de prolongar el estado de dormancia mediante químicos. Desgraciadamente, a bajas temperaturas, los tubérculos de la papa sufren un fenómeno conocido como indución al endulzamiento en frío, según el cual se acelera el rango de conversión al almidón para reducir azúcares (ej., glucosa y fructosa). Cuando se rebanan las papas crudas y se cocinan en aceite a altas temperaturas, los azúcares reductores acumulados reaccionan liberando aminoácidos en la célula de la papa, formando inaceptables pigmentaciones marrones a negras en las papas en hojuelas o fritas, debido a una reacción no enzimática del tipo Maillard. Las plantas procesadoras, generalmente no

Accepted for publication December 27, 2000.
ADDITIONAL KEY WORDS: Potato, cold-sweetening, gene expression, allele, enzyme, sugar.

Abbreviations:
AcInv, acid invertase; AGPase, adenosine diphosphate glucose pyrophosphorylase; ATP-PFK, adenosine triphosphate dependent fructose-6-phosphate 1-phosphotransferase; FBPase, fructose-1,6-bisphosphatase; Fru-1,6-P_2, fructose-1,6-bisphosphate; Fru-2,6-P_2, fructose-2,6-bisphosphate; Fru-6-P, fructose-6-phosphate; GFP, glucose forming potential; Glc-1-P, glucose-1-phosphate; Glc-6-P, glucose-6-phosphate; PEP, phosphoenolpyruvate; PK, pyruvate kinase; PPi, inorganic pyrophosphate; PPi-PFK, pyrophosphate dependent fructose-6-phosphate 1-phosphotransferase; QTL, quantitative trait locus; RT-PCR, reverse transcriptase polymerase chain reaction; UDP-Glc, uridine diphosphate glucose; UGPase, uridine diphosphate glucose pyrophosphorylase; SPS, sucrose phosphate synthase.

aceptan comprar papas con estos colores. Todos los cultivares comerciales de papa usados para la producción de hojuelas y papas fritas acumulan excedentes de azúcares reductores libres al ser expuestos al estrés del frío. Si una "papa procesada en frío" está disponible, la energía ahorrada puede ser aprovechada en aquellas regiones de crecimiento de papa donde las temperaturas de almacenamiento exterior son bajas. En las regiones donde las temperaturas son ligeramente altas, pueden incrementarse los costos de refrigeración. Sin embargo, este gasto se compensaría al eliminarse la necesidad de comprar químicos que producen dormancia, y los que sirven para controlar enfermedades y mejorar la calidad del tubérculo en el largo plazo. La meta primaria de esta revisión es describir la investigación reciente de naturaleza bioquímica y molecular relacionada con los mecanismos que regulan la poscosecha y el endulzamiento inducido en frío en los tubérculos de papa. No se ha hecho ningún esfuerzo para explicar la investigación realizada sobre la manipulación genética del metabolismo del carbono entre el almidón y los azúcares libres durante la fotosíntesis y/o durante desarrollo de la papa respecto a las interacciones de la fuente.

INTRODUCTION

The control of sugar accumulation in potatoes is a complex metabolic process affected by several levels of cellular control. For example, the regulation of cold sweetening, which results in a relatively slow sweetening process (Ewing *et al.* 1981) may initially act through hormone alterations (Doucette and Pritchard 1993; Isherwood 1973, 1976). These changes may influence membrane permeability and composition (Isherwood 1976; Knowles and Knowles 1989; O'Donoghue *et al.* 1994; Spychalla and Desborough 1990; Yada *et al.* 1990). Changes in membrane structure and function can result in cellular adjustments in the compartmentalization of key ions, substrates, and enzyme effector molecules (Isherwood and Kennedy 1975; Sowokinos 1990a). Sweetening also involves the transport of metabolites across the amyloplast membrane. Ultimately, sweetening may result from altered gene expression and/or modulation of post-translational activity of key enzymes influencing the flux of carbon toward sugar formation (Sowokinos 1990b). Despite general agreement on the enzymes thought to be involved in the cold-induced starch-hexose conversion, little is known about their relative contribution and their *in vivo* regulation (Greiner *et al.* 1999).

During the last decade, molecular tools using an *Agrobacterium*-mediated gene transfer system in potatoes have become available to precisely manipulate and analyze gene expression in potatoes (Stitt and Sonnewald 1995). Genetically manipulated plants have provided a tool to measure the contribution that an individual enzyme has in regulating the rate of flux through a metabolic pathway. It would be to our advantage, if the variation of a single parameter could be achieved that directly correlates to a wide range of sweetening potentials experienced within a group of transgenic potato progeny. Transformation experiments directed toward elucidating the pathway and mechanism of the cold sweetening process in potatoes have lead to new discoveries related to the control of metabolic pathways in plants.

VARIATION IN SWEETENING CAPACITY

The sweetening of potato tubers induced by cold-stress was first observed by Müller-Thurgau in 1882. The amount of free sugar a potato accumulates is cultivar dependent (Burton 1969; Coffin *et al.* 1987; Richardson *et al.* 1990; Samotus *et al.* 1974; Sowokinos 1999; Vliet and van Schriemer 1960; Zrenner *et al.* 1996). During cold-induced sweetening in stored potatoes, starch degradation occurs primarily through the action of starch phosphorylase and eventually reducing sugars (i.e., glucose and fructose) accumulate through the enzymatic reactions illustrated in Figure 1 (Mares *et al.* 1985; Morrell and ap Rees 1986a). Even though glucose and fructose are produced in equimolar amounts, glucose has been shown to be present at a higher concentration in potato cells during growth and in storage at 4 C (Merlo *et al.* 1993). The authors suggested that this may be due, in part, to high concentrations of fructokinase found in potato extracts that could cycle fructose back into the pool of hexose-phosphates. Fry color of Russet Burbank and Shepody potatoes has been shown to be more closely associated with glucose concentration than with fructose, total reducing sugars, sucrose, or total sugars (Pritchard and Adam 1994). Coleman *et al.* (1993) indicated that chip color was correlated with tuber glucose content regardless of detection method, cultivar, growing site, or storage temperature. For these reasons, glucose is the only hexose whose concentration is given in Table 1. A wide variation in sugar accumulating ability among potato clones after seven months cold storage (5 C) is evident in Table 1. The potato clones ND3828-15 and Picasso differed greater than 100-fold in their glucose content (0.4 μmol g^{-1} and 47.1 μmol g^{-1} FW, respectively). At the same time, the concentration of sucrose was low in all clones evalu-

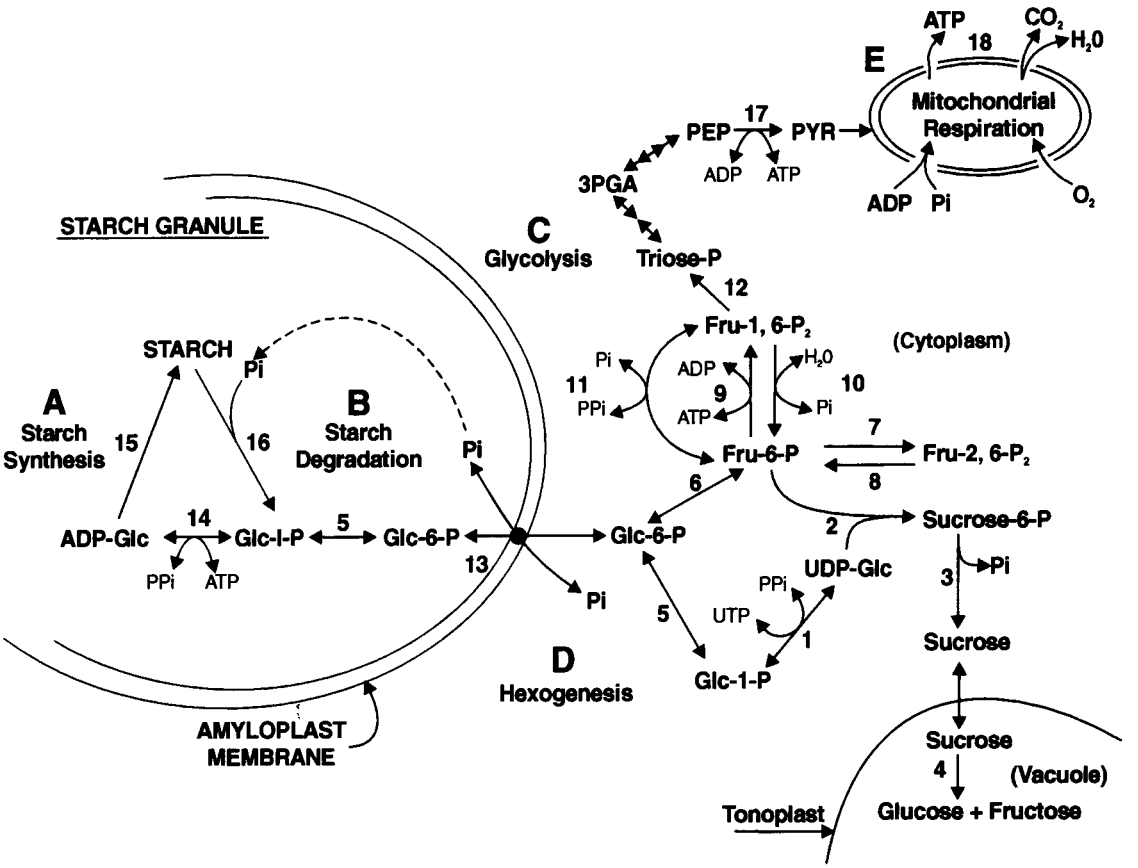

FIGURE 1.
Related carbohydrate pathways in potato tubers A. Starch synthesis, B. Starch degradation, C. Glycolysis, D. Hexogenesis, and E. Mitochondrial respiration. Enzymes represented include (1) UDP-glucose pyrophosphorylase, (2) sucrose-6-phosphate synthase, (3) sucrose-6-phosphate phosphatase, (4) acid invertase, (5) phosphoglucomutase, (6) phosphohexoseisomerase, (7) fructose-6-phosphate 2-kinase, (8) fructose-2,6-bisphosphatase, (9) ATP-phosphofructokinase, (10) fructose-1,6-bisphosphatase, (11) PPi-phosphofructokinase, (12) aldolase, (13) glucose-6-phosphate/phosphate translocator protein, (14) ADP-glucose pyrophosphorylase, (15) starch synthase, (16) starch phosphorylase, (17) pyruvate kinase and (18) mitochondrial electron transport and oxidative phosphorylation reactions.

ated (i.e., 2.3 μmol g^{-1} FW or less). This illustrates that potatoes can differ markedly in their GFP in cold storage (Sowokinos *et al.* 1989). The clone Picasso had a GFP that was approximately 500-fold greater than that for the clone ND3828-15. One explanation for the vast difference in the amount of glucose observed in cold-stored potato clones could be due, in part, to variation in acid invertase activity. This and other explanations for the possible variation in reducing content observed among cold-stressed potatoes will be discussed in this review.

INVESTIGATIONS INTO SUGAR FORMATION:

For basic information describing the general nature of the cold-sweetening process in potatoes, the reader is referred to review and research articles by ap Rees *et al.* (1981), Sowokinos (1990b), and Hill *et al.* (1996). Reviews by Willmitzer *et al.* (1994) and by Stitt and Sonnewald (1995) describe genetic modification approaches to investigate carbon metabolism in plants.

Carbohydrate metabolism in developing and stored potatoes has been studied in detail, but the initial stimuli and mechanisms regulating the process of cold sweetening are still poorly understood (ap Rees and Morrell 1990; Sowokinos 1990b). The final content of sugar in a potato tuber, at any given point in time, is influenced by several genes affecting related carbohydrate pathways in a synergistic fashion. These pathways include (A) starch synthesis, (B) starch breakdown, (C) glycolysis, (D) hexogenesis (i.e., formation of glucose and fructose), and (E) mitochondrial respiration. A scheme illustrating the interrelation of these pathways in potato tubers is illustrated in Figure 1.

TABLE 1–*Variable sweetening-potential of potato clones stored for seven months at 5 C.*

Clone	Glucose[1]	Sucrose	GFP[2]	Color Code[3]	Agtron[4]
	µmol g^{-1} FW	µmol g^{-1} FW			
ND3828-15	0.4	0.9	0.4	1.5	63
ND5775-3	0.6	1.4	0.4	1.5	61
Dakota Pearl	0.7	1.3	0.5	1.5	61
ND860-2	0.7	1.8	0.4	1.5	60
MSB076-2	2.4	1.5	1.6	2.0	58
Snowden	2.0	1.1	1.8	2.0	57
Romania	3.4	1.6	2.1	2.5	56
ND2470-27	2.92.3	1.3	2.5	56	
Norchip	7.8	1.6	4.9	3.5	47
Latona	12.7	0.8	15..9	4.0	44
Dali	16.1	1.4	11.5	4.0	40
Innovator	16.5	0.8	20.6	4.0	41
Amadeus	28.1	0.4	70.3	4.0	39
Symfonia	37.6	1.0	37.6	5.0	38
MSE228-11	41.6	1.6	26.0	5.0	38
Fianna	43.7	0.6	72.8	5.0	37
Picasso	47.1	0.2	235.5	5.0	35

[1]Glucose values for acceptable colored chips are approximately 2.0 µmol g^{-1} FW or less.
[2]GFP = Glucose-forming-potential (µmol glucose/µmol sucrose).
[3]Color Code = Relates to the Potato Chip/Snack Food Association's five-code color chart (1- lightest; 5-darkest).
[4]Agtron values (color reflectance) of 60 or higher generally yield acceptably colored chips.

Schott *et al.* (1995) working with liposomes prepared from potato amyloplasts, demonstrated that Glc-6-P was the only C6 or C3 metabolite efficiently transported across the amyloplast membrane. A study involving antisense inhibition of plastidial phosphoglucomutase supported the view that carbon from the cytosol was imported into potato tuber amyloplasts in the form of Glc-6-P (Tauberger *et al.*, 2000). For these reasons, only the metabolite Glc-6-P is illustrated to be transported across the amyloplast membrane in Figure 1.

Two separate metabolic events are critical in determining a potato tuber's ability to produce sugars in the cold storage: the ability to form sucrose and the ability to hydrolyze sucrose to the reducing sugar's glucose and fructose. The control of sucrose synthesis is controlled by several related enzymes while reducing sugar formation is more specifically related to level of acid invertase activity (Stitt and Sonnewald 1995). Initially, the effect that the related carbohydrate pathways (as illustrated in Figure 1) have on the cells ability to form sucrose will be discussed.

Amyloplast Starch Synthesis

Starch synthesis occurs in the plastids (amyloplasts) of potato cells involving AGPase (Figure 1, rx. 14), starch synthase (rx. 15) and branching enzyme to form an α -1,4-glucan macromolecule (Preiss 1982). This process involves more than one synthase (rx. 15) and more than one branching enzyme (rxs. not shown in Figure 1), and the precise pathway is not fully understood (Burrell 1994). The enzyme AGPase is the key regulatory enzyme in the pathway of starch biosynthesis (Preiss 1988). This was confirmed by Müller-Röber *et al.* (1992) who transformed Desirée (cv.) potatoes with a chimeric gene containing the coding region for AGPase B subunit linked in an antisense orientation to the Ca MV 35S promoter. Starch synthesis was abolished in the transgenic tubers and the reduced amount of dry matter present consisted of 30% sucrose and 8% glucose. This experiment confirmed the unique role that AGPase (rx. 14) occupies in the pathway of starch biosynthesis in plants. Further, the activity of AGPase is subject to allosteric regulation by 3-P-glycerate (activation) and inorganic phosphate (inhibition) (Sowokinos and Preiss 1982). A mutated AGPase gene (*glgC16)* from *E. coli* (i.e., decreased response to allosteric effectors) was over expressed in potatoes, and it was reported that transgenic tubers had an increased rate of starch synthesis (Stark *et al.* 1992). During cold storage these tubers had a decreased ability to accumulate reducing sugars. It was argued that the lower sugar content was due to the increased biosynthetic capacity of the transgenic tubers. Sweetlove *et al.* (1996a) produced transgenic potato plants with an over expressed *glgC16* from *E. coli* under the control of a patatin promoter. They indicated that (1) the amounts of *glgC16* protein were linearly related to extractable activity of AGPase, (2) the cellular location of the protein was similar between transgenic and control tubers, and (3) there were no pleiotropic changes in the activities of other related carbohydrate metabolizing enzymes in the transgenic potatoes. They reported that a four- to five-fold increase in AGPase activity had no detectable effect on starch content in developing mature tubers (Sweetlove *et al.* 1996b). Although a greater flux of label from [U-^{14}C]sucrose into starch was observed, the transgenic tubers demonstrated a greater ability to degrade starch.

Lorberth *et al.* (1998) cloned a gene involved in starch metabolism that encoded a protein (R1) capable of introducing phosphate into starch-like glucans. By reducing the activity of the R1 protein using antisense technology, they were able to produce a reduced-phosphate starch in transformed potatoes that was resistant to degradation when compared to starch from wild-type potatoes. As a result, the genetically modified potatoes

accumulated less hexose when placed into cold storage. These genetic approaches may be successful in reducing the concentration of free sugars in cold-stored potatoes, but they are of limited commercial value if either the quality or the quantity of starch is altered (Greiner *et al.* 1999). For a complete review on the enzymes involved in starch synthesis see Smith *et al.* (1997).

Starch Degradation

Starch degrading enzymes decrease the degree of polymerization of starch which contains predominantly α-1,4-linked glucose units (Steup 1990). Through the action of hydrolytic enzymes, starch is degraded to a mixture of limit dextrins, maltose, and glucose (rxs. not shown in Figure 1). Starch phosphorylase (rx. 16) transfers a glucosyl residue from the free non-reducing end of an amylose-like chain to inorganic phosphate forming Glc-1-P. The process of starch degradation during cold sweetening is argued to be principally phosphorlytic rather than hydrolytic in nature (Mares *et al.* 1985; Morrell and ap Rees 1986a). This view is supported by the fact that sucrose is the first free sugar to accumulate during the sweetening process (i.e., catalyzed by the reactions seen in Figure 1) rather than maltose or glucose (Sowokinos 1990b). In potato tubers two starch phosphorylase isozymes, types L and H, have been described and are believed to be responsible for the complete breakdown of starch (Sonnewald *et al.* 1995). Type L is found in amyloplasts, whereas type H is cytosolic in location. For in depth reviews describing enzymes involved with the degradation of starch see ap Rees (1974), Davies (1990), and Steup (1990).

Cochrane *et al.* (1991) indicated that α-amylase, β-amylase, and α-glucosidase activities were higher in tubers stored at 4 C than those stored at 10 C. Cottrell *et al.* (1993) studied changes in three amylolytic activities (i.e., α-amylase, β-amylase, debranching enzyme) during cold sweetening of potatoes. They reported that the activities of all three starch-hydrolyzing enzymes increased during the first weeks of storage at 4 C. At 10 C reducing sugar content and the three enzymes increased only slightly. A cold-inducible β-amylase was purified and characterized from the cultivar Desirée (Nielsen *et al.* 1997). Its activity was low in tubers stored at 20 C and was progressively induced when temperatures were decreased to 5 C and 3 C. The activities of α-glucosidase and endo-amylolytic activities were not influenced by low storage temperature. Claassen *et al.* (1993) suggested that an increase in starch phosphorylase activity (rx. 16) acts as a triggering event in the sweetening of potato tubers during cold storage. Hill *et al.* (1996) investigated the role of starch phosphorylase and amylase enzymes in the cold-sweetening process using zymograms to separate the different isoforms of each enzyme. When Desirée (cv.) potatoes were stored at warm (17-20 C) or cold (4 C) temperatures, no differences were observed in the isoform banding patterns for starch phosphorylase. Down-regulation of plastidic and cytosolic isozymes of phosphorylase by as much as 67% in Desirée tubers did not affect the level of reducing sugars formed in cold storage (Kumar *et al.* 2000). Earlier, Kennedy and Isherwood (1975) found no effect of storage temperature on phosphorylase activity. Hill *et al.* (1996) did observe a new amylolytic activity that appeared after two to four days of cold storage. The appearance of this activity correlated with the time course of sugar accumulation.

The ordered crystalline-structure of starch granules observed in the cold-resistant potato clone ND860-2 has been suggested to be a key factor for its reduced sweetening potential in cold storage (Barichello *et al.* 1990a). After exposing granules to hydrolysis with α-amylase, scanning electron microscopy revealed that ND860-2 granules were more intact than the granules taken from the cold-sensitive cultivar Norchip. Bright field light microscopy showed that granules of ND860-2 displayed concentric rings that were absent in the Norchip granules. It was not reported, however, if starch granules from other cold-resistant potato clones had the same structural features and resistance to α-amylase as the starch granules from ND860-2.

Glycolysis

It was initially theorized that the cold-lability of glycolytic enzymes led to an increase in hexose- phosphate concentration resulting in a stimulation of sucrose synthesis (ap Rees *et al.* 1981, 1988; Dixon and ap Rees 1980a 1980b; Dixon *et al.* 1981; Hammond *et al.* 1990; Pollock and ap Rees 1975a; Viola and Davies 1994). A few of the glycolytic enzymes that have been investigated are described here.

Fructose-1,6-bisphosphatase. It is illustrated in Figure 1 that the cytosolic enzyme FBPase (rx. 10) provides one of the substrates (i.e., Fru-6-P) for the synthesis of sucrose-6-phosphate via the action SPS (rx. 2). The other substrate for SPS (i.e., UDP-Glc) is formed via a reaction catalyzed by the enzyme UGPase (rx. 1). Discussion of the involvement of SPS and UGPase in the sweetening process is presented in section title "Hexogenesis." FBPase is regulated by substrate availability and is inhibited by the signal metabolite Fru-2,6-P_2 (Stitt 1990; Stitt *et al.* 1987). In addition, Fru-2,6-P_2 serves to activate the reversible glycolytic enzyme PPi-PFK (rx. 11). The cellular level of Fru-2,6-P_2 is regulated by the activity of a specific kinase and phosphatase. Inorganic phosphate and Fru-6-P stimulate the formation of Fru-2,6-P_2 by activating Fru-6-

P, 2-kinase (rx. 7) and inhibiting fructose 2,6- bisphosphatase (rx. 8). For a review of Fru-2,6-P_2 as a regulatory molecule in plants see Stitt (1990). Results have suggested that cold-induced sweetening may be stimulated by low concentrations of Fru-2,6-P_2 (Stitt 1987). Morrell and ap Rees (1986a) have shown that the content of Fru-2,6-P_2 remains low or even decreases during the conversion of starch to sucrose as potatoes sweeten in the cold. In leaves of transgenic tobacco plants, genetic manipulation was used to increase the concentration of Fru-2,6-P_2 and a decreased rate of sucrose synthesis was observed (Scott *et al.* 1995). Claassen and Budde (1996) examined the possible involvement of FBPase in cold-induced sweetening in potato tubers. They concluded that there was no direct relationship between coarse control of FBPase and cold-induced sweetening. When there was a rapid increase in sucrose and reducing sugars, the specific activity of FBPase showed no change at 2 C.

Adenosine Triphosphate Linked-6-Phosphofructokinase—The enzyme ATP-PFK (rx. 9) catalyzes the irreversible conversion of Fru-6-P to Fru-1,6-P_2 and was initially regarded as the enzyme that regulates the entry of carbons into glycolysis and, therefore, the availability of carbon for respiration (Bryce and Hill 1993). The accumulation of sucrose and reducing sugar in potato tubers at 2 C occurred with a concomitant decrease in maximum activity of ATP-PFK (Bredemeijer *et al.* 1991). Biphasic Arrhenius plots of plastidic and cytosolic ATP-PFK revealed that these enzymes are inactivated at temperatures approaching 2 C. They suggested that during cold storage of potato tubers, diminished ATP-PFK activity played a role in the reduction of the rate of glycolysis causing sugars to accumulate. Four distinct forms of ATP-PFK have been shown to occur in potato tubers that differ slightly in their subunit composition (Kruger *et al.* 1988). Investigations into the temperature coefficients of three of the isoforms of ATP-PFK at cold temperatures (2 to 6 C), led Hammond *et al.* (1990) to suggest that inactivation of this enzyme (rx. 9) resulted in the accumulation of hexose phosphates (i.e., Fru-6-P, Glc-6-P, Glc-1-P) leading to an increased synthesis of sucrose. Cold storage of potato tubers, therefore, could decrease the portion of metabolized glucose that could enter glycolysis resulting in an accumulation of hexose-phosphates (Pollock and ap Rees 1975b) and subsequently lead to an increase in reducing sugar content (ap Rees *et al.* 1981). Burrell *et al.* (1994) used genetic manipulation to determine whether ATP-PFK influenced the rate of respiration in potato tubers. Transgenic plants were produced that contained the coding sequence of the *E. coli pfk*A gene linked to a patatin promoter. Expression of this chimeric gene resulted in a 14- to 21-fold increase in the maximum catalytic activity of ATP-PFK without affecting the activities of the other glycolytic enzymes. Their results indicated that a substantial increase in ATP-PFK activity did not significantly alter the flux through glycolysis, but the pool sizes of glycolytic intermediates increased three- to eight-fold. There was also no evidence that the genetic manipulation of ATP-PFK activity had caused a redistribution of flux between the pentose-phosphate-pathway (PPP, rxs. not shown in Figure 1) and glycolytic pathways. It was concluded that respiration of potato tubers was not limited by ATP-PFK.

Pyrophosphate Linked-6-Phosphofructokinase—The enzyme PPi-PFK (rx. 11) is capable of the reversible phosphorylation of Fru-6-P using PPi as a phosphoryl- donor. Direct evidence that this enzyme can act as a glycolytic enzyme in plants under certain conditions was presented by Hatzfeld *et al.* (1989). Stimulation of PPi-PFK in the forward direction (formation of Fru-1,6-P_2) favors glycolysis, while the reverse reaction (formation of Fru-6-P) favors sucrose formation. Morrell and ap Rees (1986b) reported that the activity of PPi-PFK was about 10 times that of ATP-PFK in potato tubers. Therefore, PPi-PFK could potentially allow glycolysis to proceed regardless of the activity of ATP-PFK. Trevanion *et al.* (1991) reported that PPi-PFK was also cold inactivated. In addition, the concentration of Fru-2,6-P_2 required for half-maximal activation increased about eight-fold as the temperature was lowered from 25 C to 2 C. They concluded that these restrictions of the interconversion of Fru-6-P and Fru-1,6-diP would contribute to the accumulation of hexose-phosphates and would subsequently lead to increased sucrose synthesis in the cold. Claassen *et al.* (1991) measured the maximum activity of PPi-PFK along with the concentration of Fru-2,6-P_2 and PPi in Bintje (cv.) potatoes at both 2 C and 8 C. They reported that PPi-PFK can be fully operational in cold-stored potato tubers and that the lack of an increase in PPi concentration supported the functioning of this enzyme during sugar accumulation. Hajirezaei *et al.* (1994) transformed potatoes with "antisense" constructs to the genes encoding the α- and β-subunits of PPi-PFK driven by the constitutive CaMV promoter. Although expression was decreased 70% to 90% in some transformant lines, the plants did not show any unusual phenotype, reduction of growth, or decrease in tuber yields. The rates of accumulation of sucrose and free hexose at 4 C were similar in antisense and wild-type plants. It was apparent that PPi-PFK did not control the rate of glycolysis and that the tubers contained excessive capacity to phosphorylate Fru-6-P. It is possible, however, that the expression of PPi-PFK in the transformants was not decreased enough to suggest a role for this enzyme in limiting

the rate of glycolysis (see Hajirezaei *et al.* (1994) for other possible explanations).

Pyruvate Kinase—Another enzyme that has been indicated as important in the control of glycolysis is PK (rx. 17) (Copeland and Turner 1987; Dixon and ap Rees 1980a). PK is responsible for the exiting of carbons from glycolysis. It catalyzes the irreversible synthesis of pyruvate and ATP from PEP and ADP. Pyruvate is metabolized by several biosynthetic pathways and can be completely oxidized to energy, CO_2 and water in the mitochondrion (rx. 18). The coding sequence of the cytosolic potato tuber PK was placed under the control of the CaMV 35S promoter and used to transform tobacco (Gottlob-McHugh *et al.* 1992). Leaves from cytosolic PK-deficient transformants had normal rates of photosynthetic O_2 evolution, respiratory O_2 consumption and did not show any change in their adenylate energy charge. This suggested that the conversion of PEP to pyruvate in the cytosol is either not essential for leaf respiration or there is an alternative pathway(s). Dunford (1992) produced transgenic potato plants containing an antisense gene to PK, and these plants also developed normal tubers. It was concluded from these results that the regulation of plant respiratory metabolism is subject to a high degree of flexibility *in situ.* (Hajirezaei *et al.* 1994). Flexibility of metabolism may be essential if plants are expected to withstand sudden changes in their environment, without the buffer of a homeostatic mechanism to maintain a constant cellular environment (Gottlob-McHugh *et al.* 1992).

At this point in time, it appears that no concrete evidence has been put forward to support the theory that a cold-labile step in glycolysis is involved in triggering or regulating cold-induced sugar accumulation in potatoes (Hill *et al.* 1996).

Hexogenesis

A more direct approach to inhibit sweetening in potatoes would be to "shut down" the steps involved directly within the sweetening pathway itself. The immediate reactions leading to the production of free reducing sugars are illustrated in Figure 2. Several researchers have conducted genetic modification experiments with these enzymes with varying levels of success. This review will address the role of these enzymes both individually and collectively to gain insight into their importance in controlling the flux of carbon to free sugars.

Sucrose Phosphate Synthase—SPS (Figure 2, rx. 2) is the plant enzyme thought to play a major role in sucrose biosynthesis in both photosynthetic and nonphotosynthetic tissues (Huber and Huber 1996). Sucrose-6-P is synthesized from UDP-Glc and

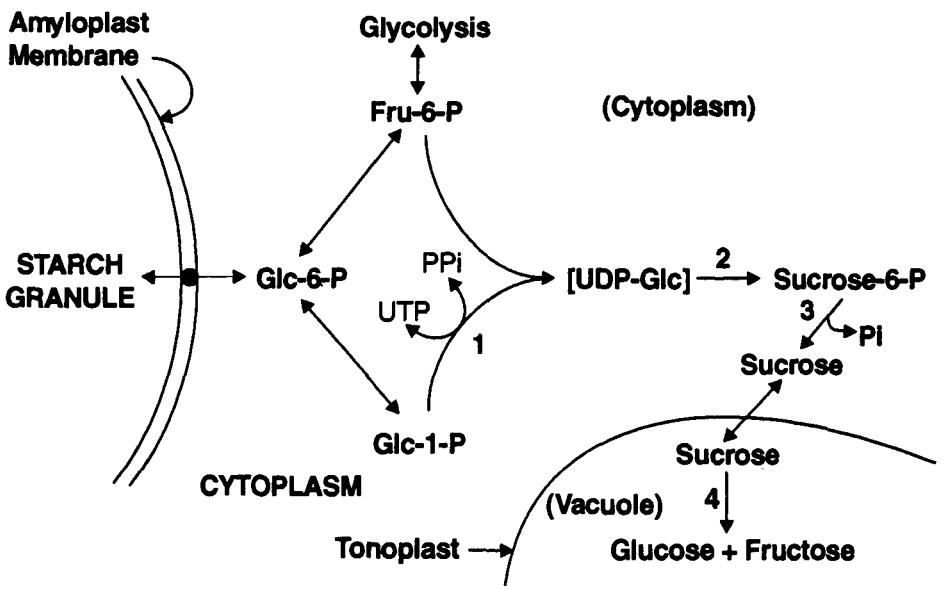

FIGURE 2.
Hexogenic reactions leading directly to the formation of the reducing sugars glucose and fructose in potato tubers. Enzymes represented include (1) UDP-glucose pyrophosphorylase, (2) sucrose-6-phosphate synthase, (3) sucrose-6-phosphate phosphatase, and (4) acid invertase.

Fru-6-P by the action of SPS (Huber and Huber 1992). A specific phosphatase (rx. 3) immediately dephosphorylates sucrose-6-P to free sucrose (Mares *et al.* 1985). SPS has been characterized from leaf tissues (Doehlert and Huber 1983; Stitt *et al.* 1988). SPS activity is modulated by allosteric effectors (e.g., Glc-6-P activates, Pi inhibits) (Doehlert and Huber 1983) and by protein phosphorylation (Huber and Huber 1992; Huber *et al.* 1989). The flux of carbons through SPS reaction is likely regulated during stress (i.e., drought and cold) via alterations in its kinetic properties (Geigenberger *et al.* 1997; Hill *et al.* 1996). During the onset of sweetening in potatoes SPS is activated and a new form of the enzyme appears (Deiting *et al.* 1998; Hill *et al.* 1996; Reimholz *et al.* 1997). These changes coincide temporally with the stimulation of sucrose synthesis and the decline of hexose phosphates (Hill *et al.* 1996). SPS antisense and co-suppression potato transformants were used to analyze the contribution of this enzyme to the control of cold sweetening (Krause *et al.* 1998). A 70% to 80% decrease of SPS expression resulted in a reproducible but non- proportional (10% to 40%) decrease of soluble sugars in cold-stored tubers. Examination of its affinity constants (Km's) of the substrates (Fru-6-P and UDP-Glc) in relation to the physiological concentrations of these metabolites in the cell led investigators to indicate that SPS would be strongly substrate limiting, especially with respect to UDP-Glc (Hill *et al.* 1996; Reimholz *et al.* 1994). In addition, the maximum activity of SPS was found to be 50-fold higher than the net rate of sugar accumulation that can occur in potatoes. It was concluded that alterations in the kinetic properties of SPS, such as those that occur in response to low temperature, would provide a more effective way to stimulate sucrose synthesis than by changes in SPS expression (Krause *et al.* 1998).

UDP-Glucose Pyrophosphorylase—The enzyme UGPase catalyzes the first step committed to the sweetening pathway in potato tubers (Figure 2, rx. 1) through the formation of a high-energy, glucosyl-containing nucleotide, UDP-Glc. UDP-Glc is the key metabolite that contributes a glucose residue to Fru-6-P forming sucrose-6-P, which is immediately dephosphorylated to sucrose. Sucrose is then cleaved via the vacuolar-enzyme acid invertase (rx. 4) to yield the reducing sugar's glucose and fructose. It has been suggested that the availability of UDP-Glc limits the flux of carbon through SPS for the formation of sucrose (Sowokinos *et al.* 2000). Although SPS is activated in the cold, it is still strongly substrate limiting in respect to physiological levels of UDP-Glc present in the cell. Using this scenario, if the intracellular level of UDP-Glc was to fluctuate (even slightly), it should lead to similar change in the concentration of sucrose. In support of this theory, it was reported that following detachment of growing tubers from their mother plant (Geigenberger *et al.* 1994) and following the initiation of cold sweetening (Hill *et al.* 1996) the concentration of UDP-Glc changed in parallel with the concentration of sucrose.

Zrenner *et al.* (1993) used antisense RNA technology for UGPase (rx. 1) and developed potatoes in which activity was suppressed to 4% to 5% of that seen in wild-type plants. Freshly harvested potatoes had no significant difference in their fresh weight, dry weight, starch, hexose-phosphate or UDP-Glc content compared with control tubers. It was shown by ap Rees and Morrell (1990), however, that 4% to 5% of UGPase activity was still in considerable excess compared to the activity of other glycolytic enzymes in the tuber. Antisense constructs may have to reduce UGPase to negligible levels in transgenic potatoes before any phenotypic differences are noted compared to wild-type tubers during growth and development. Storage of mature tubers at low temperature (4 C) for three weeks led to an increase of the steady-state level of UGPase RNA, implicating a role of this enzyme in the process of cold sweetening (Zrenner *et al.* 1993). Longer-term storage studies (six weeks) with potatoes containing antisense constructs for UGPase revealed that the sucrose content of the transformed tubers was significantly correlated with UGPase activity (Borovkov *et al.* 1996; Spychalla *et al.* 1994). Control coefficients of 0.48 and 0.58 (i.e., six weeks of storage at 4 C and 12 C, respectively) suggested that UGPase would exert significant control of the flux of carbon toward sucrose during post-harvest conditions (Spychalla *et al.* 1994). Since different isozymes of UGPase have been shown to exist in potatoes (Sowokinos *et al.* 1997), the degree of inhibition of sucrose synthesis using antisense technologies may depend on the particular UGPase isozyme that is affected.

A relationship has been shown to exist between the allelic polymorphism of UGPase exhibited among potato clones and their ability to accumulate sugars in cold storage (Sowokinos *et al.* 1997). Since UGPase is a single-copy gene in potatoes (Borovkov *et al.* 1996), a tetraploid cultivar has the possibility of containing four different alleles for UGPase. Two UGPase alleles have been previously described in potatoes (Katsube *et al.* 1990; Spychalla *et al.* 1994). They have been designated *Ugp*A and *Ugp*B and are differentiated between by the absence and presence of a single *Bam*H1 restriction site, respectively (Sowokinos *et al.* 1997). RT-PCR was used to clone UGPase from 17 potato cultivars (*Solanum tuberosum* L.) that differed in their ability to accumulate reducing sugar in cold storage (3 C). Both alleles were detected in most potato cultivars.

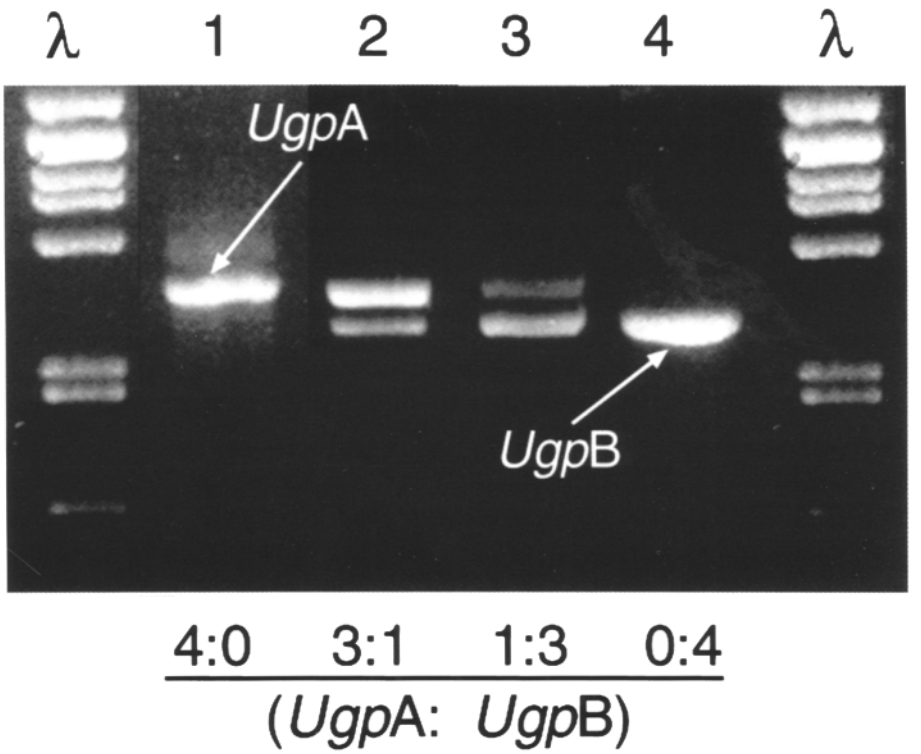

FIGURE 3.
***Bam*H1 digestion of UGPase-cDNAs from two cold-sweetening resistant potato clones (1) Snowden and (2) ND860-2 and from two cold-sensitive potato clones (3) Redsen and (4) Norchip. (see Sowokinos [2001] for experimental methods).**

Tetraploid potatoes with a degree of cold resistance to sweetening demonstrated an allelic ratio (i.e., *Ugp*A:*Ugp*B) of 4:0 or 3:1 in favor of the allele *Ugp*A, while cultivars that were cold sensitive exhibited a predominance for the allele *Ugp*B (i.e., 1:3 or 0:4) (Figure 3) (Sowokinos 2001). Although the existence of UGPase isozymes in potato tubers has been documented (Sowokinos *et al.* 1997), the relationship between UGPase allele composition and isozyme patterns was unclear. To investigate this further 16 potato clones, which differed markedly in their sweetening potential, were examined for their allelic ratios in relation to their respective isozyme profiles (Sowokinos 2001). Activity staining of nondenaturing gels of proteins extracted from potatoes sensitive to cold-induced sweetening displayed two or three acidic isozymes of UGPase designated UGP1, UGP2 and UGP3 (Figure 4A). The most abundant of these forms, UGP3, was purified to homogeneity and characterized from the cold-sensitive cultivar Norchip (Sowokinos *et al.* 1993). Although the activity of UGPase is not highly regulated in the direction of Glc-1-P formation accompanying potato growth, the enzyme exhibits regulatory properties in the direction of UDP-Glc formation (i.e., important in stored potatoes) (rx. 1). The UGPase isozyme UGP3 displayed a "negative cooperative effect" in respect to its substrate's Glc-1-P and UTP (Sowokinos *et al.* 1993). Kinetics of this type are characteristic of enzymes that are sensitive to their environment (Nakae 1971). Most of the cold-resistant potatoes were unique in that they contained up to two additional faster-moving isozymes (slightly more basic in charge) of UGPase designated UGP4 and UGP5 (Figure 4B). Although some polymorphism was observed among clones (Sowokinos 2001), only the cold-resistant clones had the presence of both the slower-moving and faster-moving isozymes of UGPase.

It was determined that eight of the 16 potato clones, with a predominance of the UGPase allele *Ugp*B (Table 2, top group), developed higher levels of Glc when placed into cold storage compared to the eight clones with a predominance of the allele *Ugp*A (Table 2, lower group). The three-fold greater Glc content in the cold-susceptible potatoes (31.2 ± 2.2 μmol g^{-1} FW) compared to the cold-resistant clones (10.3 ± 2.1 μmol g^{-1} FW) was significantly different at the 99% level of confidence.

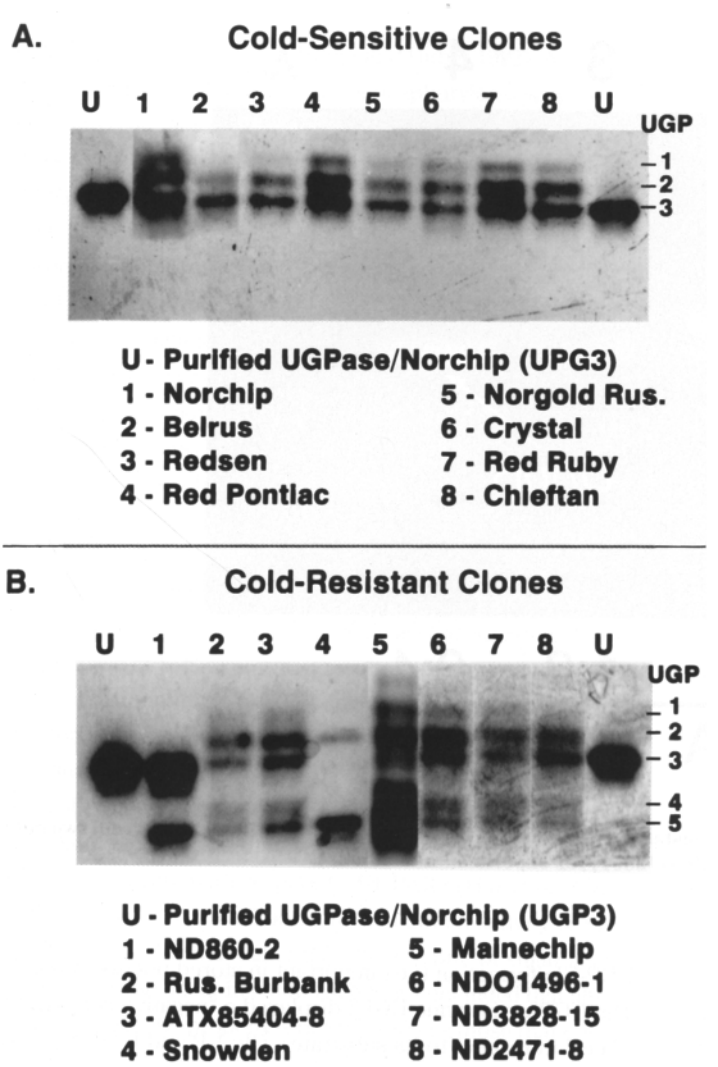

FIGURE 4.
Activity staining of UGPase isozymes following separation on a 7.5% nondenaturing polyacrylamide gel. (A) Eight cold-sensitive potato clones which sweeten markedly when placed into cold storage (4 C), (B) Eight cold-resistant potato clones that resist sugar accumulation. (See Sowokinos [2001] for experimental details.)

The isozymes UGP4 and UGP5 have recently been purified to homogeneity from the cold-resistant cultivar Snowden. It was paramount to determine if these cold-resistant isozymes had catalytic properties that were distinct from those previously reported. Preliminary results have suggested that UGP4 and UGP5 are unique catalytically, compared to UGP3, in terms of (1) pH optimum, (2) substrate affinities for Glc-1-P and UTP, (3) maximum reaction velocity, and (4) the magnitude of product inhibition with UDP-Glc (unpublished data). The differences in the kinetic properties of these cold-resistant UGPase-isozymes may provide, in part, a biochemical basis for explaining the variation in sugar accumulation between potato cultivars.

Acid Invertase—Besides metabolic parameters that influence a cell's ability to form sucrose, the second critical metabolic event limiting a potato tuber's sweetening ability is the efficiency of the vacuolar-enzyme AcInv to hydrolyze sucrose to free reducing sugars (i.e., glucose and fructose) (rx. 4). Sucrose content in potato tubers has been shown to be inversely correlated with AcInv activity (Davies *et al.* 1989; Hatch and Glasziou 1963; Richardson *et al.* 1990; Sowokinos *et al.* 1985, 2000). AcInv activity increases several-fold during storage of potatoes at low temperature resulting in the formation of a high concentration of reducing sugar (Pressey 1969; Sasaki *et al.* 1971). Pressey (1967) reported the presence of an inhibitor for acid invertase in potato tubers. He suggested that the lack of correlation between sugar levels and invertase activity following three months storage at low temperature was due to the presence of an invertase inhibitor (Pressey 1969). Richardson *et al.* (1990) noted that total acid invertase activity (i.e., assayed after destroying the endogenous inhibitor) generally reflected sugar changes more closely that did basal activity (i.e., assayed with the inhibitor present). On the other hand, when potatoes were stressed with a deficit of moisture early following tuberization, basal invertase activity (i.e., assayed in the presence of inhibitor) was shown to be more closely associated with sugar content (Sowokinos *et al.* 2000). A similar observation was also made when 30 genetically distinct potato clones were subjected to cold storage temperatures (unpublished data). To successfully quantitate basal-invertase activity in the presence of inhibitor (using crude extracts), caution must be used to ensure that assays are conducted within the narrow-range of linear reaction kinetics as limited by enzyme concentration (Sowokinos *et al.* 2000). To further complicate the situation, Isla *et al.* (1992) have shown that the proteineous AcInv inhibitor is extra-vacuolar while AcInv is located in the vacuole. Therefore, questions still remain concerning the role of invertase and invertase inhibitor in relation to the *de novo* regulation of sugar accumulation in potatoes (Burrell 1994; Isla *et al.* 1992; Zhou *et al.* 1994).

Genetic modification of acid invertase activity (rx. 4) in potatoes has resulted in decreased concentrations of reducing sugars (Greiner *et al.* 1999; Zrenner *et al.* 1996). Zrenner *et al.*

TABLE 2–*Comparisons of the UGPase-isozyme content and -allelic ratio of potato clones in relation to their sugar content and chip color following cold storage (3 C) for two-months.*

Clones with UGPase Isozymes UGP1,2, &3	Allelic[1] Ratio	Glc Content µmol g^{-1} FW	Color Code[2]
Belrus	0:4	32.2 ± 0.7[3]	5.0
Norchip	0:4	27.7 ± 0.2	4.5
Crystal	1:3	27.5 ± 1.0	4.5
Red Pontiac	1:3	27.4 ± 1.2	5.0
Redsen	1:3	25.7 ± 0.9	5.0
Norgold Russet	1:3	28.2 ± 1.2	5.0
Chieftain	1:3	42.0 ± 1.2	5.0
Red Ruby	1:3	39.2 ± 0.6	5.0
		31.2 ± 2.2[4]	4.8 ± 0.1[5]
Clones with Additional UGPase Isozymes UGP4 & 5			
Mainechip	3:1	7.4 ± 1.0	3.5
ND860-2	3:1	3.0 ± 0.4	2.5
ND3828-15	3:1	5.2 ± 0.4	3.5
ATX85404-8	3:1	11.4 ± 0.7	3.5
NDO1496-1	3:1	8.7 ± 0.6	4.0
ND2471-8	3:1	15.0 ± 0.1	3.5
Russ. Burbank	3:1	21.5 ± 1.6	4.0
Snowden	4:0	10.1 ± 0.9	3.0
		10.3 ± 2.1[4]	3.4 ± 0.2[5]

[1]UGPase allelic ratio = *Ugp*A:*Ugp*B
[2]Color code = Relates to the Potato Chip/Snack Food Association's five-code color chart (1-lightest; 5- darkest).
[3]Mean and standard error, respectively
[4,5]All paired values differ significantly (P<0.01)
(Sowokinos 2001)

(1996) transformed potato with a cold-inducible vacuolar invertase (VI) isoform in an antisense configuration. Although there was almost no correlation between the total amount of AcInv activity and the accumulation of reducing sugars in transgenic tubers, there was a striking correlation between the hexose/sucrose ratio and the extractable soluble AcInv activity. After storage at 4 C, hexose:sucrose ratios were significantly reduced in transgenic tubers. Interestingly, an absolute reduction in cold-induced hexose accumulation (-34%) was observed only in the strongest antisense line, where residual VI activity was <10% as compared with wild-type plants. Based on these results, the authors concluded that other invertase isoforms may contribute to cold-induced hexose accumulation. Alternatively, the results could indicate that the cold-inducible isoform is expressed in excess over cellular demand, thus requiring a greater than 90% reduction in activity before a significant effect is observed. It was concluded that soluble AcInv does not control the total amount of soluble sugars in cold-stored potato tubers, but is involved in the regulation of the ratio of hexose to sucrose. Greiner *et al.* (1999) strongly inhibited the activity of the cold-induced VI in potato plants and circumvented the possible limitations of isoform-specific antisense repression of VI. They investigated the possibility of repressing the action of cold-induced VI by expression of a putative vacuolar invertase inhibitor from tobacco, called Nt-inhh in potato plants under the control of the CaMV 35S promoter. They decreased cold-induced hexose accumulation up to 75% without affecting tuber yield. Transgenic tubers had the same quantity and quality of starch observed as seen in the control tubers. This is an important prerequisite for the biotechnological use of Nt-inhh in potato transformations (Greiner *et al.* 1999). Although the research efforts described above led to a decrease in the concentration of sugar produced during cold-induced sweetening, the level of hexose accumulated was still in excess of what is commercially acceptable for the production of potato chips and fries.

Mitochondrial Respiration

Potato respiration declines as the storage temperature decreases (Workman *et al.* 1979). Below 5 C there is a brief respiratory burst followed by a subsequent decrease in respiration rate to a new steady state (Amir *et al.* 1977; Isherwood 1973). The initial respiratory burst has been attributed to the combination of cytochrome-mediated and cyanide-resistant respiration (i.e., alternative oxidase) (Sherman and Ewing 1983). Experimental evidence to date has indicated that the cold lability of glycolytic enzymes is not involved in triggering sugar accumulation in potatoes (Hill *et al.* 1996). Burrell *et al.*, (1994) indicated that a substantial increase in ATP-PFK activity in potatoes (rx. 9) did not significantly alter the flux through glycolysis, but that the pool sizes of glycolytic intermediates increased three- to eight-fold. These findings suggest that there may be a metabolic block(s) beyond glycolysis, possibly related to a potato cultivar's ability to completely respire sugars to energy, $C0_2$, and water (rxs. 18). Mitochondrial membrane integrity, which changes in cold storage and during tuber aging (Hiser and McIntosh 1990; Nantes *et al.* 1999; Theologis and Laties 1976), is of paramount importance to the successful functioning of the cytochrome and alternative oxidase respiratory processes. A decreased fluidity and an increased leakiness of cellular membranes is noted when potatoes are subjected to cold stress (Shekhar *et al.* 1979; Workman *et al.* 1979). Nantes *et al.* (1999) have recently shown that a

plant uncoupling mitochondrial protein (PUMP) in potato tuber mitochondria is induced by aging at 28 C and that this induction is strongly stimulated when tubers are stored in the cold (4 C). Since this 32 kDa-protein has the ability to decrease the generation of reactive oxygen suggests that it plays an important physiological role in maintaining the post-harvest respiratory ability of potato tubers. Cultivars that resist sweetening in the cold have shown a higher rate of respiration in storage (M. Ehlenfeldt, personal communication). The cold-resistant potato clone ND860-2 has shown a higher respiration rate throughout storage compared to the cold-susceptible cultivar Norchip (Barichello *et al.* 1990b). The question arises: do certain potato cultivars have a greater genetic potential to maintain the structure and function of their mitochondria when subjected to cold temperatures? To examine this possibility, mitochondria were extracted and purified from four potato clones that differed markedly in their ability to sweeten in the cold (Gounaris and Sowokinos 1992). Cold temperature (3 C) resulted in mitochondria with a lower buoyant density in all cultivars tested. The change in density, however, was more pronounced in clones that accumulated higher amounts of reducing sugars. Two-dimensional gel electrophoresis of mitochondrial proteins revealed a 26 kDa polypeptide only in those clones that sweetened at a higher rate. It was suggested that changes in the buoyant density were due to alterations in the permeability of the inner mitochondrial membrane.

CURRENT STATUS OF COLD SWEETENING RESEARCH

The large variation in GFP that potato clones exhibit in the cold (Table 1) indicates that major differences exist at the genetic and metabolic level. Figure 1 illustrates that more than one carbohydrate pathway exists in the potato cells and that each pathway is located within a distinct cellular compartment. Genetic analysis has determined that three key loci are involved in the process of cold sweetening in potatoes (Thill and Peloquin 1994). Initially, it is important to identify "these key loci" that are important in the regulation of sugar accumulation.

Comparative biochemical analysis of different potato cultivars has provided a powerful tool to facilitate the discovery of factors regulating the cold-induced sugar accumulation for a more specific molecular manipulation (Zrenner *et al.* 1996). The availability and analysis of transgenic potatoes have provided insights into the mechanism of cold sweetening. These experiments have supported the contention of Kacser (1987), which suggested that the control of a metabolic pathway is shared by each of its component steps. Genetic investigations have proven that the potato plant is extremely plastidic in nature, i.e., can self-regulate to accomplish a certain goal through metabolic readjustments (Gottlob- McHugh *et al.* 1992). If you change environmental conditions or genetically alter a particular enzymatic step, another reaction becomes more important in the regulatory process.

Interpretation of results from genetic engineering experiments has been complicated by the fact that several isozymes exist for most of the enzymes involved. Using antisense constructs to reduce the activity of a specific isozyme not having a regulatory function would lead to misinterpretations of the importance that other forms may have on the sweetening process. In addition, it is difficult to assess if biochemical changes accompanying cold sweetening serve to initiate and/or to regulate the process. Most metabolic changes observed in cold tissue may simply be a part of the sweetening process itself, having little or no effect on its initiation or regulation. On the other hand, biochemical modifications that may prove to trigger sweetening (e.g., amylase action) may have little or no effect on the final quantity of reducing sugars accumulated.

Although the process of sweetening is complex (i.e., more than one gene involved), it is noteworthy that the comparison of the UGPase allelic ratio and the isozymes expressed from a single locus should relate so closely with cold-induced sugar accumulation among genetically diverse tetraploid potato clones (Table 2). The kinetic properties of UGPase-isozymes UGP4 and UGP5, which are only present in cold-resistant potato clones, may help to explain, in part, the regulation of hexogenesis in potato tubers (unpublished data). A role for UGPase in the sweetening process is also supported by the observation that a QTL for cold-sweetening resistance in potatoes has been shown to occur on the upper region of chromosome 11, where the locus for UGPase is located (C. Gebhardt, personal communication). Studies of UGPase isozymes in potatoes involving their (1) purification and kinetic characterization, (2) intracellular localization and, (3) post-translational modification system(s), are being conducted. It is anticipated that these experiments will help to clarify the physiological role(s) of UGPase isozymes in relation to the phenomenon of cold-induced sweetening and to potato metabolism in general.

CONCLUSION

Two possible strategies for minimizing hexogenesis in cold stored potatoes would be (1) to prevent sucrose accumulation

and/or (2) to prevent the conversion of sucrose to hexose. Due to the flexibility and interrelation of carbohydrate pathways in potato tubers, It is likely that dual or higher-level antisense constructs accompanied with constructs increasing the expression of other genes may be required to successfully alter a potato's sweetening potential. As stated by Stitt and Sonnewald (1995), the probability of obtaining a desired change would diminish as the number of steps between the manipulation and the goal increases. In this light, the probability of successfully controlling low-temperature sweetening could increase if isozymes involved directly in the process of hexogenesis (rxs. in Figure 2) were subject to genetic manipulation.

Observations that an enzyme activity is highly regulated at the catalytic level does not allow one to assume that it has any significant effect on controlling the flux of carbon toward free sugar (Burrell 1994; Stitt and Sonnewald 1995). SPS (rx. 2) may be an example of such an enzyme. Additionally, enzymes that are freely reversible and are not highly regulated at the catalytic level may still have the potential to limit the flux of carbons through metabolic pathways (Haake *et al.* 1998; Kruckleberg *et al.* 1989; Neuhaus *et al.* 1989). UGPase (rx. 1) appears to represent this type of enzyme. Regulation of hexogenesis through UGPase may manifest itself through the expression of isozymic forms with unique catalytic properties that enable them to take over or to dominate the rate of UDP-Glc formation. The net effect being a decreased rate of UDP-Glc formation resulting in a lower concentration of reducing sugars being formed.

Several molecular, biochemical, and/or compositional properties have been found to be unique to potatoes that resist sweetening when placed into cold storage. Continued research into cold sweetening may reveal which of these properties are indeed involved in initiating and/or controlling hexogenesis. To accomplish this, it is mandatory to show that a significant variation of the "property in question" can be correlated with the wide variation of sweetening capacity observed among genetically diverse potato clones.

LITERATURE CITED

Amir, J., V. Kahn, and M. Unterman. 1977. Respiration, ATP level, and sugar accumulation in potato tubers during storage at 4 C. Phytochemistry 16:1495-1498.

ap Rees, T. 1974. Pathways of carbohydrate breakdown in plants, MTP International Review of Science. Plant Biochemistry 11:89-127.

ap Rees, T., W.L. Dixon, C.J. Pollock, and F. Franks. 1981. Low temperature sweetening of higher plants. *In*: Rhodes, M.J.C. (ed.), Recent advances in the biochemistry of fruits and vegetables. Academic Press, New York. pp.41-61.

ap Rees, T., M.M. Burrell, T.G. Entwistle, J.B.W. Hammond, D. Kirk, and N.J. Kruger. 1988. Effects of low temperature on the respiratory metabolism of carbohydrate by plants. *In*: Woodward, I.F., and S.P. Long (eds.), Plants and Temperature. Company of Biologists, Cambridge, UK. pp.377-393.

ap Rees, T. and S. Morrell. 1990. Carbohydrate metabolism in developing potatoes. Am Potato J 67:835-847.

Barichello, V., R.Y. Yada, R.H. Coffin, and D.W. Stanley. 1990a. Low temperature sweetening in susceptible and resistant potatoes: Starch structure and composition. J Food Sci 55:1054-1059.

Barichello, V., R.Y. Yada, R.H. Coffin, and D.W. Stanley. 1990b. Respiratory enzyme activity in low temperature sweetening of susceptible and resistant potatoes. J Food Sci 55:1060-1063.

Borovkov, A.Y., P.E. McClean, J.R. Sowokinos, S.H. Ruud, and G.A. Secor. 1996 Effect of expression of UDP-Glucose pyrophosphorylase antisense and ribozyme RNAs on the enzyme activity and carbohydrate composition of transgenic potato plants. J Plant Physiol 147:644-652.

Bredemeijer, G.M.M., H.C.J. Burg, P.A.M. Claassen, and W.J. Stiekema. 1991. Phosphofructokinase in relation to sugar accumulation in cold-stored potato tubers. J. Plant Physiol 138:129-135.

Bryce, J.H., and S.A. Hill. 1993. Energy production in plant cells. *In*: Lee, P.J., and R.C. Leegood (eds.), Plant Biochemistry and Molecular Biology. Wiley, Chichester. pp.1-21.

Burrell, M.M. 1994. Control of carbohydrate metabolism in potato tubers. *In*: Belknap, W.R., M.E. Vayda, and W.P. Park (eds.), The Molecular and Cellular Biology of the Potato, 2nd edition, C.A.B. International, Wallingford, UK. pp.45-55.

Burrell, M.M., P.J. Mooney, M. Blundy, D. Carter, F. Wilson, J. Green, K.S. Blundy, and T. ap Rees. 1994. Genetic manipulation of 6-phosphofructokinase in potato tubers. Planta 194:95-101.

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. Eur Potato J 12:81-95.

Claassen, P.A.M., and M.A.W. Budde. 1996. Possible involvement of fructose 1,6-bisphosphatase in cold-induced sweetening in potatoes. Potato Res 39:141-151.

Claassen, P.A.M., M.A.W. Budde, and M.H. van Calker. 1993. Increase in phosphorylase activity during cold-induced sugar accumulation in potato tubers. Potato Res 36:205-217.

Claassen, P.A.M., M.A.W. Budde, H.J. de Ruyter, M.H. van Calker, and A. van Es. 1991. Potential role of pyrophosphate:fructose 6-phosphate phosphotransferase in carbohydrate metabolism of cold stored tubers of *Solanum tuberosum* cv. Bintje. Plant Physiol 95:1243-1249.

Cochrane, M.P., C.M. Duffus, M.J. Allison, G.R. Mackay. 1991. Amylolytic activity in stored potato tubers. 2. The effect of low-temperature storage on the activities of α- and β-amylase and α-glucosidase in potato tubers. Potato Res 34:333-341.

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

Coleman, W.K., G.C.C. Tai, S. Clayton, M. Howie, and A. Pereira. 1993. A portable monitor for the rapid assessment of processing quality of stored potato tubers. Am Potato J 70:909-923.

Copeland, L., and J.F. Turner. 1987. The regulation of glycolysis and the pentose phosphate pathway. *In*: Davies D.D. (ed.), The Biochemistry of Plants. Academic Press, New York. pp. 107-128.

Cottrell, J.E., C.M. Duffus, L. Paterson, G.R. Mackay, M.J. Allison, and H. Bain. 1993. The effect of storage temperature on reducing sugar concentration and the activities of three amylolytic enzymes in tubers of the cultivated potato, *Solanum tuberosum* L. Potato Res 36:107-117.

Davies, H.V. 1990. Carbohydrate metabolism during sprouting. Am Potato J 67:815-827.

Davies, H.V., R.A. Jefferies, and L. Scobie. 1989. Hexose accumulation in cold stored tubers of potato (*Solanum tuberosum* L). The effects of water stress. J. Plant Physiol 134:471-475.

Deiting, U., R. Zrenner, and M. Stitt. 1998. Similar temperature requirement for sugar accumulation and for the induction of new forms of sucrose phosphate synthase and amylase in cold-stored potato tubers. Plant, Cell Env 21:127-138.

Dixon, W.L., and T. ap Rees. 1980a. Identification of the regulatory steps in glycolysis in potato tubers. Phytochemistry 19:1297-1301.

Dixon, W.L., and T. ap Rees. 1980b. Carbohydrate metabolism during cold-induced sweetening of potato tubers. Phytochemistry 19:1653-1656.

Dixon, W.L., F. Franks, and T. ap Rees. 1981. Cold lability of phosphofructokinase from potato tubers. Phytochemistry 20:969-972.

Doehlert, D.C., and S.C. Huber. 1983. Regulation of spinach leaf sucrose phosphate synthase by glucose-6-phosphate, inorganic phosphate and pH. Plant Physiol 73:989-994.

Doucette, M.S., and M.K. Pritchard. 1993. ABA involvement in low temperature sweetening of potatoes. Acta Horticulturae 343:293-294.

Dunford, R. 1992. Pyruvate kinase and glycolytic control in potatoes. Ph.D thesis. Cambridge University, Cambridge, UK.

Ewing, E.E., A.H. Senesac, and J.B. Sieczka. 1981. Effects of short periods of chilling and warming on potato sugar content and chipping quality. Am Potato J 58:633-647.

Geigenberger, P., L. Merlo, R. Reimholz, and M. Stitt. 1994. When growing potato tubers are detached from their mother plant there is a rapid inhibition of starch synthesis, involving inhibition of ADP-glucose pyrophosphorylase. Planta 193:486-493.

Geigenberger, P., R. Reimholz, M. Geiger, L. Merlo, V. Canale, and M. Stitt. 1997. Regulation of sucrose and starch metabolism in potato tubers in response to short-term water deficit. Planta 201:502-518.

Gottlob-McHugh, S.G., R.S. Sangwan, S.D. Blakeley, G.C. Vanlerberghe, K. Ko, D.H. Turpin, W.C. Plaxton, B.L. Miki, and D.T. Dennis. 1992. Normal growth of transgenic tobacco plants in the absence of cytosolic pyruvate kinase. Plant Physiol 100:820-825.

Gounaris, Y., and J.R. Sowokinos. 1992, Two-dimensional analysis of mitochondrial proteins from potato cultivars resistant and sensitive to cold-induced sweetening. J Plant Physiol 140:611-616.

Greiner, S., T. Rausch, U. Sonnewald, and K. Herbers. 1999. Ectopic expression of a tobacco invertase inhibitor homolog prevents cold-induced sweetening of potato tubers. Nat Biotechnol 17:708-711.

Haake, V., R. Zrenner, U. Sonnewald, and M. Stitt. 1998. A moderate decrease of plastid aldolase activity inhibits photosynthesis, alters the level of sugars and starch, and inhibits growth of potato plants. Plant J 14:147-157.

Hajirezaei, M., U. Sonnewald, R. Viola, S. Carlisle, D. Dennis, and M. Stitt. 1994. Transgenic potato plants with strongly decreased expression of pyrophosphate:fructose-6-phosphate phosphotransferase show no visible phenotype and only minor changes in metabolic fluxes in their tubers. Planta 192:16-30.

Hammond, J.B.W., M.M. Burrell, and N.J. Kruger. 1990. Effect of low temperature on the activity of phosphofructokinase from potato tubers. Planta 180:613-616.

Hatch, M.D. and K.T. Glasziou. 1963. Sugar accumulation cycle in sugar cane. II Relationship of invertase activity to sugar content and growth rate in storage tissue of plants grown in controlled environments. Plant Physiol 38:344-348.

Hatzfeld, W-D., J. Dancer, and M. Stitt. 1989. Direct evidence that pyrophosphate:fructose-6-phosphate phosphotransferase can act as a glycolytic enzyme in plants. FEBS Letters 254:215-218.

Hill,. L, R. Reimholz, R. Schröder, T.H. Nielsen, and M. Stitt. 1996. The onset of sucrose accumulation in cold-stored potato tubers is caused by an increased rate of sucrose synthesis and coincides with low levels of hexose-phosphates, an activation of sucrose phosphate synthase and the appearance of a new form of amylase. Plant, Cell Env 19:1223-1237.

Hiser, C., and L. McIntosh. 1990. Alternative oxidase of potato is an integral membrane protein synthesized *de novo* during aging of tuber slices. Plant Physiol 93:312-318.

Huber, S.C., and J.L.A. Huber. 1992. Role of sucrose phosphate synthase in sucrose metabolism in leaves. Plant Physiol 99:1275-1278.

Huber, S.C., and J.L.A. Huber. 1996. Role and regulation of sucrose-phosphate synthase in higher plants. Annu Rev Plant Physiol Mol Biol 47:431-444.

Huber, J.L.A., S.C. Huber, and T.H. Nielsen. 1989. Protein phosphorylation as a mechanism for regulation of spinach leaf sucrose-phosphate synthase activity. Arch Biochem Biophys 270:681-690.

Isherwood, A. 1973. Starch-sugar interconversion in *Solanum tuberosum*. Phytochemistry 12:2579- 2591.

Isherwood, A. 1976. Mechanism of starch-sugar interconversion in *Solanum tuberosum*. Phytochemistry 15:33-41.

Isherwood, F.A., and M.G.H. Kennedy. 1975. The composition of the expressed sap from cold stored potatoes. Phytochemistry 14:83-84.

Isla, M.I., D.P. Leal, M.A. Vattuone, and A.R. Sampietro. 1992. Cellular localization of the invertase, proteinaceous inhibitor and lectin from potato tubers. Phytochemistry 31:1115-1118.

Kacser, H. 1987. Control of metabolism. *In*: Davies D.D. (ed.), The Biochemistry of Plants. Vol 11, Academic Press, London. pp. 39-67.

Katsube, T., Y. Kazuta, H. Mori, K. Nakano, K. Tanizawa, and T. Fukui. 1990. UDP-glucose pyrophosphorylase from potato tuber: cDNA cloning and sequencing. J Biochem 108:321-326.

Kennedy, M.G.H., and F.A. Isherwood. 1975. Activity of phosphorylase in *Solanum tuberosum* during low temperature storage. Phytochemistry 14:667-670.

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

Krause, K-P., L. Hill, R. Reimholz, T.H. Nielsen, U. Sonnewald, and M. Stitt. 1998. Sucrose metabolism in cold-stored tubers with decreased expression of sucrose phosphate synthase. Plant, Cell Env 21:285-299.

Kruckeberg, A.L., H.E. Neuhaus, R. Feil, L.D. Gottlieb, and M. Stitt. 1989. Decreased-activity mutants of phosphoglucose isomerase in the cytosol and chloroplast of *Clarkia xantiana*: Impact on mass-action ratios and fluxes to sucrose and starch, and estimation of flux control coefficients and elasticity coefficients. Biochem J 261:457-467.

Kruger, N.J., J.B.W. Hammond, and M.M. Burrell. 1988. Molecular characterization of four forms of phosphofructokinase purified from potato tuber. Arch Biochem Biophys 267:690-700.

Kumar, G.N.M., L.O. Knowles, N. Fuller, and N.R. Knowles. 2000. alpha-1,4 glucan phosphorylase activity correlates with senescent sweetening but not low temperature-induced sweetening in potato. Plant Physiol 123(S):126 (abstract no. 596).

Lorberth, R., G. Ritte, L. Willmitzer, and J. Kossmann. 1998. Inhibition of a starch-granule-bound protein leads to modified starch and repression of cold sweetening. Nat Biotechnol 16:473-477.

Mares, D.J., J.R. Sowokinos, and J.S. Hawker. 1985. Carbohydrate metabolism in developing potato tubers. *In*: Li, P.H.(ed.), Potato Physiology. Academic Press, New York. pp.279-327.

Merlo, L., P. Geigenberger, M. Hajirezaei, and M. Stitt. (1993) Changes of carbohydrates, metabolites and enzyme activities in potato tubers during development, and within a single tuber along a stolon-apex gradient. J Plant Physiol 142:392-402.

Morrell, S., and T. ap Rees. 1986a. Control of the hexose content of potato tubers. Phytochemistry 25:1073-1076.

Morrell, S., and T. ap Rees. 1986b. Sugar metabolism in developing tubers of *Solanum tuberosum*. Phytochemistry 25:1579-1585.

Müller-Röber, B., U. Sonnewald, and L. Willmitzer. 1992. Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. EMBO J 11:1229-1238.

Müller-Thurgau, H. 1882. Uber Zuckeranhaufung in Pflanzentheilen in Folge niederer Temperatur. Landwirtsch Jahrb 11:751-828.

Nakae, T. 1971. Multiple forms of uridine diphosphate glucose pyrophosphorylase from *Salmonella typhimurium*. J Biol Chem 246:4404-4411.

Nantes, I.L., M.M. Fagian, R. Catisti, P. Arruda, I.G. Maia, and A.E. Vercesi. 1999. Low temperature and aging-promoted expression of PUMP in potato tuber mitochondria. FEBS Letters 457:103-106.

Neuhaus, H.E., A.L. Kruckeberg, R. Feil, and M. Stitt. 1989. Reduced activity of phosphoglucose isomerase in the cytosol and chloroplast of *Clarkia xantiana* II. Study of the mechanisms which regulate photosynthate partitioning. Planta 178:110-112.

Nielsen, T.H., U. Deiting, and M. Stitt. 1997. A β-amylase in potato tubers is induced by storage at low temperature. Plant Physiol 113:503-510.

O'Donoghue, E.P., R.Y. Yada, and A.G. Marangoni. 1994. Low temperature sweetening in potato tubers: the role of the amyloplast membrane. J. Plant Physiol 145:335-341.

Pollock, C., and T. ap Rees T. 1975a. Activities of enzymes of sugar metabolism in cold-stored tubers of *Solanum tuberosum*. Phytochemistry 14:613-617.

Pollock, C., and T. ap Rees. 1975b. Effect of cold on glucose metabolism by callus and tubers of *Solanum tuberosum*. Phytochemistry 14:1903-1906.

Preiss, J. 1982. Regulation of the biosynthesis and degradation of starch. Annu Rev Plant Physiol 33:431-454.

Preiss, J. 1988. Biosynthesis of starch and its regulation. *In*: Preiss, J. (ed.), The Biochemistry of Plants, Carbohydrates. Vol 14, Academic Press, San Diego. pp.181-254.

Pressey, R. 1967. Invertase inhibitor from potatoes: Purification, characterization and reactivity with plant invertases. Plant Physiol 42:1780-1786.

Pressey, R. 1969. Role of invertase in accumulation of sugars in cold-stored potatoes. Am Potato J 46:291-297.

Pritchard M.K., and L.R. Adam. 1994. Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody Potatoes. Am Potato J 71:59-68.

Reimholz, R., P. Geigenberger, and M. Stitt. 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.

Reimholz, R., M. Geiger, V. Haake, U. Deiting, K-P. Krause, U. Sonnewald, and M. Stitt. 1997 Potato plants contain multiple forms of sucrose phosphate synthase, which differ in their tissue distributions, their levels during development, and their responses to low temperature. Plant, Cell Env 20:291-305.

Richardson, D.L., H.V. Davies, H.A. Ross, and G.R. Mackay. 1990. Invertase activity and its relation to hexose accumulation in potato tubers. J Exp Bot 41:95-99.

Samotus, B., M. Niedzwiedz, Z. Kolodziej, M. Leja, and B. Czajkowska. 1974. Storage and reconditioning of tubers of Polish potato varieties and strains I. Influence of storage temperature on sugar level in potato tubers of different varieties and strains. Potato Res 17:64-81.

Sasaki, T., K. Tadokoro, and S. Suziki. 1971. Multiple forms of invertase of potato tuber stored at low temperature. Phytochemistry 10:2047-2050.

Schott, K., S. Borchert, B. Müller-Röber, and H.W. Heldt. 1995. Transport of inorganic phosphate and C3- and C6-sugar phosphates across the envelope membranes of potato tuber amyloplasts. Planta 196:647-652.

Scott, P., A.J. Lange, S.J. Pilkis, and N.J. Kruger. 1995. Carbon metabolism in leaves of transgenic tobacco (*Nicotiana tabacum* L.) containing elevated fructose-2,6-bisphosphate levels. Plant J 7:461-469.

Shekar, V.C., W.M. Iritani, and J.R. Magnuson. 1979. Starch-sugar interconversion in *Solanum tuberosum* L. II. Influence of membrane permeability and fluidity. Am Potato J 56:225-235.

Sherman, M., and E.E. Ewing. 1983. Effects of temperature and low oxygen atmospheres on respiration, chip color, sugars, and malate of stored potatoes. J Amer Soc Hort Sci 108:129-133.

Smith, A.M., K. Denyer, and C. Martin. 1997. The synthesis of the starch granule. Annu Rev Plant Physiol Plant Mol Biol 48:67-87.

Sonnewald, U., A. Basner, G. Burkhard, and M. Steup. 1995. A second L-type isozyme of potato glucan phosphorylase: cloning, antisense inhibition and expression analysis. Plant Mol Bio 27:567-576.

Sowokinos, J.R. 1990a. Effect of stress and senescence on carbon partitioning in stored potatoes. Am Potato J 67:849-857.

Sowokinos, J.R. 1990b. Stress-induced alterations in carbohydrate metabolism. *In*: Vayda, M.E., and W.P. Park (eds.), The Molecular and Cellular Biology of the Potato. C.A.B. International, Wallingford, UK. pp.137-158.

Sowokinos, J.R. 1999. Choices for potatoes that chip directly from 42 F without reconditioning. Valley Potato Grower 64(114): 14-17.

Sowokinos, J.R. 2001. Pyrophosphorylase in *Solanum tuberosum* L.: Allelic and isozyme patterns of UDP-Glucose pyrophosphorylase as a marker for cold-sweetening resistance in potatoes. Am J Potato Res 78:57-64.

Sowokinos, J.R., E.C. Lulai, and J.A. Knoper. 1985. Translucent tissue defects in *Solanum tuberosum* L. I: Alterations in amyloplast membrane integrity, enzyme activities, sugars and starch content. Plant Physiol 78:489-494.

Sowokinos, J.R., B. Morgan, M. Sleeper, and I. Shea. 1989. Establishment of glucose-forming-potential (GFP) as a tool to screen breeding clones for processing potential. RRV Potato Res and Reporting Conference pp.145-155.

Sowokinos, J.R., and J. Preiss. 1982. Pyrophosphorylases in *Solanum tuberosum* L. III. Purification, physical and catalytic properties of ADP-Glucose pyrophosphorylase in potatoes. Plant Physiol 69:1459-1466.

Sowokinos, J.R., C.C. Shock, T.D. Stieber, and E.P. Eldredge. 2000. Compositional and enzymatic changes associated with the sugar-end defect in Russet Burbank potatoes subjected to a single, transient, moisture stress period during early tuber bulking. Am J Potato Res 77:47-56.

Sowokinos, J.R., C.T. Thomas, and M.M. Burrell. 1997. Pyrophosphorylases in potato V. Allelic polymorphism of UDP-Glucose pyrophosphorylase in potato cultivars and its association with tuber resistance to sweetening in the cold. Plant Physiol 113:511-517.

Sowokinos, J.R., J.P. Spychalla, and S.L. Desborough. 1993. Pyrophosphorylases in *Solanum tuberosum* L. IV. Purification, tissue localization, and physicochemical properties of UDP- Glucose pyrophosphorylase. Plant Physiol 101:1073-1080.

Spychalla, J.P., and S.L. Desborough. 1990. Fatty acids, membrane permeability, and sugars of stored potato tubers. Plant Physiol 94:1207-1213.

Spychalla, J.P., B.E. Scheffler, J.R. Sowokinos, and M.W. Bevan. 1994. Cloning, antisense RNA inhibition, and the coordinated expression of UDP-glucose pyrophosphorylase with starch biosynthesis genes in potato tubers. J Plant Physiol 144:444-453.

Stark, D.M., K.P. Timmerman, G.F. Barry, J. Preiss, and G.M. Kishore. 1992. Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. Science 258:287-292.

Steup, M. 1990. Starch degrading enzymes, *In*: Lea, P.J. (ed.), Methods in Plant Biochemistry. Vol 3. Academic Press, London. pp.103-128.

Stitt, M. 1987. Fructose 2,6-bisphosphate and plant carbohydrate metabolism. Plant Physiol 84:201-204.

Stitt, M. 1990. Fructose 2,6-bisphosphate as regulatory metabolite in plants. Annu Rev Plant Physiol. Mol Biol 41:153-185.

Stitt, M., S.C. Huber, and P. Kerr. 1987. Control of photosynthetic sucrose synthesis. *In*: Hatch, M.D., and N.K. Boardman (eds.), Biochemistry of Plants. Academic, New York. 10:327-407.

Stitt, M., I. Wilke, R. Feil, and H.W. Heldt. 1988. Coarse control of sucrose phosphate synthase in leaves: alterations of kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. Planta 174:217-230.

Stitt, M., and U. Sonnewald. 1995. Regulation of metabolism in transgenic plants. Annu Rev Plant Physiol Plant Mol Biol 46:341-368.

Sweetlove, L.J., M.M. Burrell, and T. ap Rees. 1996a. Characterization of transgenic potato (*Solanum tuberosum*) tubers with increased ADPglucose pyrophosphorylase. Biochem J 320:487-492.

Sweetlove, L.J., M.M. Burrell, and T. ap Rees. 1996b. Starch metabolism in tubers of transgenic potato (*Solanum tuberosum*) with increased ADPglucose pyrophosphorylase. Biochem J 320:493-498.

Tauberger, E., A.R. Fernie, M. Emmermann, A. Renz, J. Kossmann, L. Willmitzer, and R.N. Trethewey. 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 J 23:43-53.

Theologis, A., and G.G. Laties. 1976. Membrane lipid integrity as a prerequisite of cyanide-resistant respiration in potato slices. Plant Physiol 57:S-93.

Thill, C.A. and S.J. Peloquin. 1994. Inheritance of potato chip color at the 24-chromosome level. Am Potato J 71:629-646.

Trevanion, S.J., and N.J. Kruger. 1991. Effect of temperature on the kinetic properties of pyrophosphate:fructose 6-phosphate phosphotransferase from potato tuber. J. Plant Physiol 137:753-759.

Viola, R. and H.V. Davies. 1994. Effect of temperature on pathways of carbohydrate metabolism in tubers of potato (*Solanum tuberosum* L.). Plant Science 103:135-143.

Vliet, W.F. and W.H. van Schriemer. 1960. The sugar accumulation of potatoes kept at low temperature, as studied in a small section of Dutch varieties. Eur Potato J 3:263-271.

Willlmitzer, L., W.B. Frommer, J. Kossmann, B. Müller-Röber, J. Riesmeier, U. Sonnewald, U-I. Flügge, and H. Heldt. 1994. Transgenic potatoes changed in carbohydrate partitioning and allocation. *In*: Belknap, W.R., M.E. Vayda, and W.P. Park (eds.), The Molecular and Cellular Biology of the Potato. 2nd Edition, C.A.B. International, Wallingford, UK. pp.57-65.

Workman, M., A. Cameron, and J. Twomey. 1979. Influence of chilling on potato tuber respiration, sugar, o-dihydroxyphenolic content and membrane permeability. Am Potato J 56:277-288.

Yada, R.Y., R.H. Coffin, K.W. Baker, and M.J. Leszkowiat. 1990. An electron microscopic examination of the amyloplast membranes from a potato seedling resistant and a processing potato cultivar susceptible to low temperature sweetening. Can Inst Fd Sci Technol J 23:145-148.

Zhou, D., A. Mattoo, N. Li, H. Imaseki , and T. Solomos. 1994. *Plant Gene Register*: Complete nucleotide sequence of potato tuber acid invertase cDNA. Plant Physiol 106:397-398.

Zrenner, R., K. Schuler, and U. Sonnewald. 1996. Soluble acid invertase determines the hexose-to-sucrose ratio in cold-stored potato tubers. Planta 198:246-252.

Zrenner, R., L. Willmitzer, and U. Sonnewald. 1993. Analysis of the expression of potato uridinediphosphate-glucose pyrophosphorylase and its inhibition by antisense RNA. Planta 190:247-252.