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Low-temperature stress induces transient oscillations in sucrose metabolism in *Solanum tuberosum*

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Abstract

Exposure to low but nonfreezing temperatures induces the net breakdown of starch and the accumulation of sucrose, glucose and fructose in potato tuber tissue, a complex phenomenon known as low-temperature sweetening (LTS). When transferred to 4°C storage, tissue sucrose levels in LTS-sensitive potato tubers (*Solanum tuberosum* cv. Norchip) did not change monotonically to a new steady state, but rather transiently oscillated about the trajectory to the new steady state. The dynamic patterns observed in sensitive tubers grown in 1993 and 1994 were qualitatively similar. Quantitatively, however, the transient oscillation had a period of 11.5 days in 1993, whereas a period of 80 days was observed in 1994. In contrast, the sucrose levels of the LTS-tolerant potato tubers (*Solanum tuberosum* seedling ND860-2) increased monotonically to a higher level upon exposure to low temperatures.

Keywords: Low-temperature sweetening; Transient oscillations; *Solanum tuberosum*; Sucrose metabolism

1. Introduction

After exposure to low temperatures (i.e. < 10°C), potato tubers as well as many other plants and plant parts, often undergo a phenomenon known as low-temperature sweetening (LTS) which results from the conversion of starch to sugars [1]. Although this phenomenon has been well documented, the causes and mechanisms by which LTS occurs are still not established. Fine metabolic control exerted by allosteric enzymes, coarse metabolic control due to enzyme induction, and mass transfer of reaction substrates and products in and out of subcellular organelles may all play a role in the observed metabolic response to stress.

Excitability, periodic oscillations, polyrhythmicity, chaos, bursting or transitions between multiple steady states are possible in metabolic pathways [2,3]. These dissipative structures are evidence of self-organization in destabilized open systems that are far from equilibrium and obey nonlinear kinetic laws. Such nonequilibrium conditions prevail in cells, tissues and whole organisms, which are highly compartmentalized structures where diffusion is coupled with biochemical reactions. Oscillations can therefore occur at all levels where regulation is exerted [3].

A classic example of a metabolic oscillation is

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that of glycolysis. Glycolytic oscillations have been studied in yeast and muscle extracts, and intact yeast cells. They are solely attributed to the positive feedback regulation of 6-phosphofructokinase (an allosteric enzyme) by the reaction product adenosine diphosphate (ADP) and adenosine monophosphate (AMP). AMP is linked to ADP by the activity of adenylate kinase [3].

While the physiological significance of glycolytic oscillations remains unclear, excitability and oscillations in cyclic nucleotides and calcium concentrations are an integral part of the intercellular communication system of the cellular slime mould *Dictyostelium discoideum*. Cyclic nucleotide waves, induced by starvation, are generated and relayed by the individual amoebae, acting as a chemotactic attractant. These chemical waves regulate the aggregation of over 10^5 individual amoebae into a motile multicellular organism, which eventually transforms into a fruiting body with differentiated stalk and spore cells [3].

The glycolytic oscillator is an example of metabolic oscillations in unicellular organisms and in tissue extracts, while the cyclic nucleotide signalling system of *Dictyostelium discoideum* is an example of a metabolic oscillator in a simple multicellular organism. This work presents evidence of a stress-induced transient oscillation in the sucrose metabolism of intact potato tubers. The transient oscillation is an example of a nonmonotone approach of a complex biological system to a new steady state.

2. Materials and methods

2.1. Plant material

Solanum tuberosum cv. Norchip (LTS-sensitive) and seedling ND860-2 (LTS-tolerant) plants were grown from seed during the summers of 1993 and 1994 at the Cambridge Agricultural Research Station, Ontario Ministry of Agriculture, Food and Rural Affairs (Cambridge, Ont.) using standard agronomical practices. Tubers were harvested manually in late September, and stored for two weeks at 15°C prior to storage at 12, 10, 8, 4 and 2°C (1993), and 16, 12, 8, 4, and 2°C (1994). Tubers were not sprout inhibited in either growing season.

2.2. Sugar extraction and analysis

Sucrose, glucose and fructose concentrations (mg/g dry weight) were determined by high performance liquid chromatography (HPLC) as described by Wilson et al. [4]. Sugars were extracted by blending 10 g of potato tissue from randomly-selected tubers from a batch of over 1000 tubers with 8 ml of HPLC grade methanol in a Waring Commercial Blender for 90 s. 0.5 g of charcoal was added for each 10 g of potato tissue, and samples were shaken for 20 min and then refrigerated for a minimum of 1 h. Samples were vacuum filtered and supernatants collected and stored at 4°C until analysis. Prior to HPLC analysis, supernatants were cleaned by passing through a SepPak Alumina A cartridge followed by a 0.45 micron nylon filter. HPLC analysis was performed on three individual samples and the values averaged. Standard errors for the sucrose determinations were under 10%. Sampling periods varied from 2 to 4 days throughout the sampling period.

2.3. Data analysis

Preliminary statistical analysis showed that data were normal according to skewness, kurtosis and w statistic criteria. Sucrose accumulation patterns were modelled using Fourier series analysis [5]. The general form of the Fourier series used was:

$$[\text{Sucrose}]\,(\text{mg/g d.w.}) = A_o + \sum_{k=1}^{n} A_k \sin(k\omega t + \phi_k)$$

where A_0 is the average initial sucrose concentration, t is the time in days, A_k corresponds to the amplitude of the harmonic of frequency $k\omega$, where k is an integer ranging from 1 to n, while ϕ_k corresponds to the phase angle of the particular harmonic. The Fourier series were fitted to the experimental data using nonlinear least-squares methods with robust data weighting to exclude outliers, using the software package GRAFIT [6]. This software program uses the Marquart–Levenburg method [7] with a numerical second-order method to calculate partial differentials. The criterion for convergence was a less than 0.01% change in the reduced chi-square (χ^2) value upon variation of parameters. Robust

data weighting was performed using the method of Mosteller and Tukey, as implemented by Duggelby [8]. An additional 'bisquare' weight, b, is incorporated into the analysis and is calculated as:

$$b_i = \begin{cases} \left(1 - u_i^2\right)^2 & \text{if } |u_i| \leq 1 \\ 0 & \text{if } |u_i| > 1 \end{cases}$$

where, with z being the residual weighted by the a priori weight and N the number of observations:

$$u_i = \frac{z_i}{6\Sigma|z_i|/N}$$

The experimental points for the LTS-sensitive Norchip tubers grown in 1993 and stored at 4°C were initially fitted to a Fourier series with a period equal to the full data length T ($k = 1$). Additional, higher-order harmonics were then attempted by increasing sequentially the value of k, where period components are given by T/k. Each subsequent harmonic added to the $k = 1$ series was evaluated by testing for significance of improvements ($p \leq 0.05$) in the reduced chi-square value (χ^2) for the fit (F-test). The F-test was performed by comparing the ratios of reduced chi-square values. Nonlinear fits using simple weighting and robust weighting were identical; hence, none of the data points were considered outliers in our analysis. A higher-order Fourier series ($k > 1$) was adopted only when the fit to the data was significantly improved compared to its $k = 1$ counterpart ($p \leq 0.05$). The process was then repeated using the new higher-order Fourier series as the starting point for comparisons. Each subsequent harmonic added to the series was evaluated by testing for significance of improvements ($p \leq 0.05$) in the reduced chi-square values (χ^2) for the fit (F-test) as described before.

A seven-harmonic series fitted the data significantly better than a one-harmonic series ($p \leq 0.05$) in 1993. No significant improvements in the fit to the data ($p > 0.05$) were observed by adding a greater number of harmonics. The one-harmonic fit to the data was used to detrend this nonstationary time series.

In 1994, a seven-harmonic series also fitted the data significantly better than a one-harmonic series ($p \leq 0.05$). No significant improvements in the fit to the data ($p > 0.05$) were observed by adding a greater number of harmonics. For the 1994 data, the detrending procedure was slightly different. A linear fit (using robust data weighting) through the data, from day 6 onwards, was subtracted from the experimental points, resulting in a stationary time series. The aim of the detrending procedures was to produce stationary time series.

The 1993 and 1994 LTS-tolerant cultivar (ND860-2) data at 4°C were transformed by simply subtracting the average steady-state value of sucrose from each data point, usually the average of all values after day 2. Its dynamic behaviour was not oscillatory in nature.

3. Results and discussion

Sucrose concentrations of potato tubers stored at 10°C or higher maintain a steady-state value throughout the sampling period (data not shown). However, when potato tubers are stored below 10°C, sucrose concentrations increase to a new steady state. This was clearly shown for the 1993 and 1994 tubers (Fig. 1). This discontinuity at 10°C may constitute a bifurcation point in sucrose metabolism, where exposure to temperatures below this critical value cause the system, i.e. the potato tuber, to jump into a different state that produces a higher sucrose concentration. The simplest case of a bifurcation is the

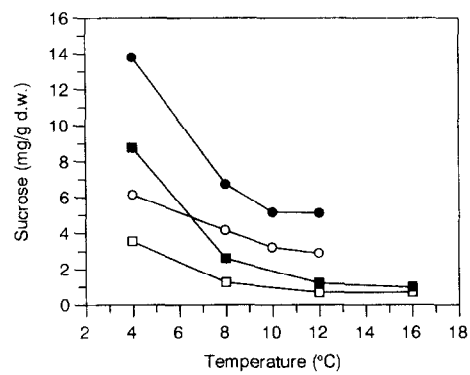

Fig. 1. Sucrose tuber tissue concentrations (mg/g d.w.) as a function of temperature for low-temperature sweetening (LTS)-sensitive *Solanum tuberosum* Norchip tubers grown in 1993 (●) and 1994 (■), and LTS-tolerant *Solanum tuberosum* ND860-2 tubers grown in 1993 (○) and 1994 (□). Below 10°C, sucrose tissue concentrations increase significantly ($p \leq 0.05$) with decreasing temperatures.

transition from one steady state to another. More complex cases include transitions from a steady state to oscillations, or from oscillations to chaos [2].

At a critical temperature change from the apparent bifurcation point at 10°C (ΔT), complex dynamic behaviour in sucrose metabolism of potato tubers was observed. This critical temperature distance depends on the relative tolerance or sensitivity of the tissue to low-temperature stress. For a LTS-sensitive cultivar (Norchip), ΔT is 6°C. The average sucrose concentration values at 4°C for the LTS-sensitive cultivar shown in Fig. 1 are averages of the fluctuating values observed in tissue sucrose levels at this temperature (Fig. 2A). A true steady-state concentration was not reached at this temperature.

Fig. 2A shows the complex pattern of sucrose accumulation in 1993-grown Norchip tubers during a 55-day period at 4°C, while Fig. 3A shows the complex pattern of sucrose accumulation in 1994-grown Norchip tubers during a 73-day period at 4°C. Sucrose concentration did not increase monotonically from one state to another, but rather transiently oscillated towards the new state. The nonmonotone behaviour observed in these two years, for potatoes grown under vastly different environmental field conditions, was strikingly similar. For comparison purposes, the time axes of both patterns were normalized to a relative time. This parameter is defined as the actual storage time divided by the time required to reach the apex of the first large oscillation (27 days in 1993 and 49 days in 1994). When the 1993 and 1994 data were compared in terms of their relative times (Fig. 4), striking pattern similarities were evident; however, the period of the oscillations observed in 1993 was shorter than that observed in 1994. Differences in growing conditions (tempera-

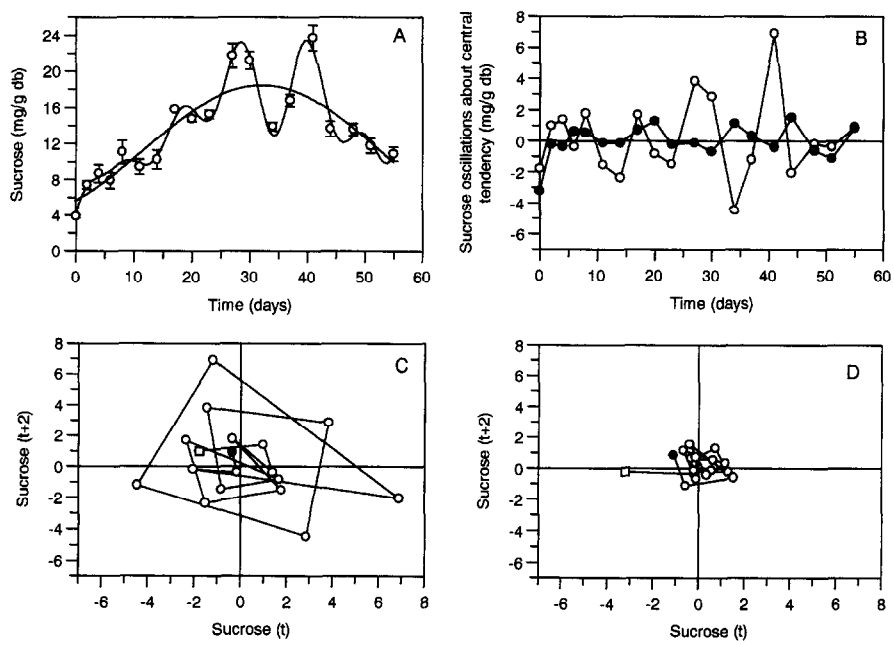

Fig. 2. Tubers grown in 1993. (A) Oscillatory pattern of sucrose accumulation (mg/g d.w.) in potato tuber tissue from the low-temperature sweetening (LTS)-sensitive Norchip cultivar as a function of storage time at 4°C. Each point in time represents the average of three separate experiments and their standard errors. (B) Stationary (detrended) time series for sucrose tuber tissue concentrations in LTS-sensitive (○) and LTS-tolerant (●) cultivars. (C) Transition function portrait for the LTS-sensitive cultivar tuber sucrose concentrations. The sucrose levels of the sensitive cultivar oscillate at 4°C because, unlike the tolerant tuber, it is not able to maintain a steady-state tissue concentration. (D) Transition function portrait for the LTS-tolerant cultivar tuber sucrose concentrations. The tolerant cultivar manages to keep sucrose levels within a narrow margin of concentrations at 4°C. In both transition function portraits, □ and ● represent the beginning and end of the time series, respectively.

ture, precipitation, environmental stresses) between the two years may have influenced initial tuber carbohydrate metabolism.

For proper mathematical analysis, time series have to be detrended; i.e. the underlying tendency of the series must be removed from their oscillatory behaviour [5]. These nonstationary time series were detrended as outlined in Section 2. The resulting stationary time series are shown in Fig. 2B (1993) and Fig. 3B (1994). Our results show that Norchip tuber sucrose levels transiently oscillate about a central accumulation tendency towards the new steady state. In contrast, for a LTS-tolerant (ND 860-2) tuber, the transient oscillations are much smaller, or nonexistent, and sucrose levels increase monotonically to a new steady state. This effect becomes quite obvious in the transition function portraits (iterated map) for both time series (Fig. 2C,Fig. 2D for 1993, and Fig. 3C,Fig. 3D for 1994). The sensitive cultivar loses part of its metabolic control and the tissue sucrose levels transiently oscillate (Fig. 2CFig. 3C), while the tolerant cultivar manages to keep a tight metabolic control over its sucrose tissue levels (Fig. 2DFig. 3D).

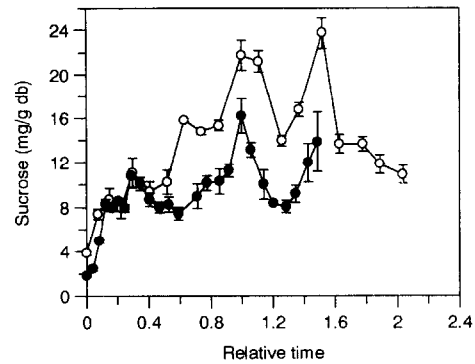

Fig. 4. Oscillations in sucrose tuber tissue concentrations in the low-temperature sweetening (LTS)-sensitive Norchip cultivars at 4°C for 1993 (○)- and 1994 (●)-grown tubers. The relative time corresponds to the time divided by that required to reach the apex of the first oscillation.

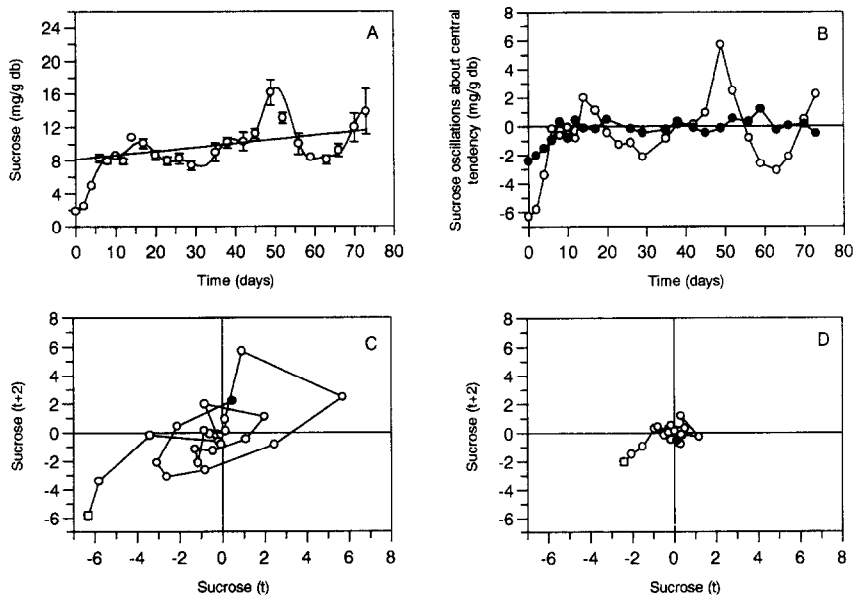

Fig. 3. Tubers grown in 1994. (A) Oscillatory pattern of sucrose accumulation (mg/g d.w.) in potato tuber tissue from the low-temperature sweetening (LTS)-sensitive Norchip cultivar as a function of storage time at 4°C. Each point in time represents the average of three separate experiments and their standard errors. (B) Stationary (detrended) time series for sucrose tuber tissue concentrations in LTS-sensitive (○) and LTS-tolerant (●) cultivars. (C) Transition function portrait for the LTS-sensitive cultivar tuber sucrose concentrations. The sucrose levels of the sensitive cultivar oscillate at 4°C because, unlike the tolerant tuber, it is not able to maintain a steady-state tissue concentration. (D) Transition function portrait for the LTS-tolerant cultivar tuber sucrose concentrations. The tolerant cultivar manages to keep sucrose levels within a narrow margin of concentrations at 4°C. In both transition function portraits, □ and ● represent the beginning and end of the time series, respectively.

At 2°C, tissue sucrose concentrations increased in a sigmoidal, nonoscillatory fashion (data not shown) to an average of 89 mg/g d.w. (Norchip) and 66 mg/g d.w. (ND 860-2) in 1993, and 35 mg/g d.w. (Norchip) and 35 mg/g d.w. (ND860-2) in 1994. This situation possibly represents a complete loss of metabolic control. We therefore postulate that at 4°C, the carbohydrate metabolism of the LTS-sensitive tuber becomes temporarily deregulated, it undergoes oscillations but manages to adapt to the decreased temperature and reach a new steady state. In contrast, at 2°C all metabolic regulation or capacity for recovery are lost.

The sucrose oscillations were analyzed using Fourier series analysis in order to generate a preliminary power spectrum. The power is defined as the square of the amplitude A_k at the corresponding harmonic $k\omega$. The patterns shown in Fig. 5 were generated by subtracting the values of the seven-harmonic Fourier series from the one-harmonic Fourier series fit to the experimental data every 0.25 days. For the 1993 data (Fig. 5A), the resulting stationary time series was best modelled by a one-harmonic Fourier series (a sine wave) with a period of 11.5 days (0.55 rad/day), i.e. the time series displayed only one predominant frequency. No improvements in the fit to the data were observed when higher harmonics were added ($p > 0.05$). For the 1994 data (Fig. 5B), the resulting stationary time series was best modelled by a nine-harmonic Fourier series with a period of 80 days (0.078 rad/day). Each subsequent addition of a harmonic was significant ($p \leq 0.05$); however, the contribution became smaller with the addition of each new harmonic, and therefore the process was stopped at the ninth harmonic.

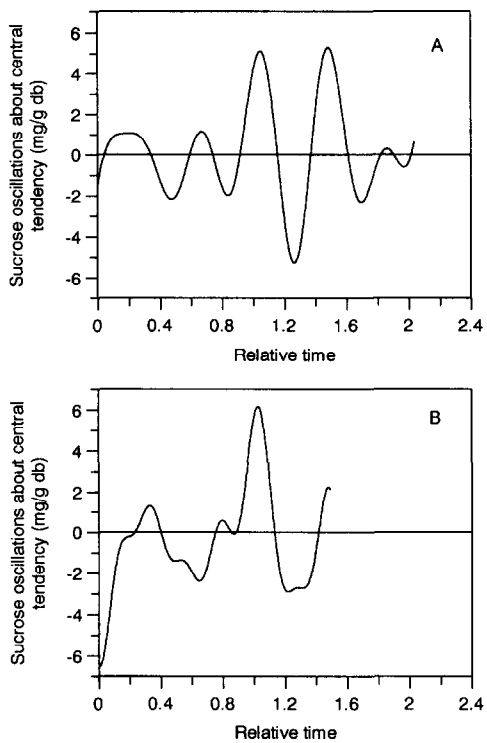

Fig. 5. Oscillations in sucrose tuber tissue concentrations in the low-temperature sweetening-sensitive Norchip cultivar at 4°C for 1993 (A)- and 1994 (B)-grown tubers. Data points were generated every 0.25 days using Fourier series analysis and series data detrending as described in the text. The relative time corresponds to the time divided by that required to reach the apex of the first oscillation.

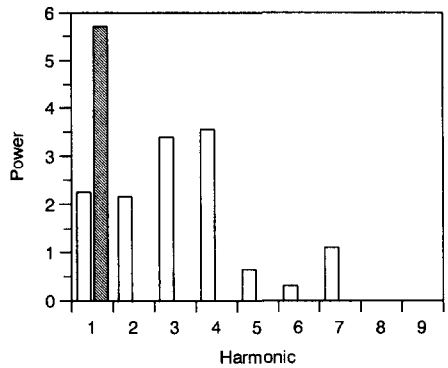

Fig. 6. Power spectra for the 1993 (■) and 1994 (□) sucrose oscillations observed in the low-temperature sweetening-sensitive Norchip potato tubers.

A power spectrum (square of the amplitude of the harmonic vs. harmonic number) was generated for these waves (Fig. 6). For the 1993 data, the power spectrum could only be generated up to three harmonic components because of constraints imposed by the Nyquist theorem — the maximum frequency (minimum period) that can be estimated corresponds to half (twice) the sampling frequency (period). Our minimum sampling period was two days. For the 1994 data, we could have generated a spectrum of up to 20 harmonic components. We doubled the requirements of the Nyquist theorem by estimating only up

to 10 harmonics for oscillations of the Norchip cultivar. No aliasing was observed in any of the analysis.

This analysis clearly demonstrated the presence of only one harmonic in the periodic motion of the 1993 sucrose oscillations. Had the data not been detrended, a power spectrum could have been erroneously generated using the seven-harmonic series, or even worse, assumed to be an infinite number of harmonics as in classic Fourier analysis. For the 1994 data, a more complex oscillation was observed with several frequency components. Even though the oscillations observed in 1993 and 1994 were quite similar when expressed in terms of relative time, subtle differences in the oscillatory patterns were evident. Whether the 1994 oscillation contains random noise or is deterministic in nature is unclear at the moment; however, the pattern observed in Fig. 6 is reminiscent of white noise.

Glucose and fructose are the products of sucrose degradation, and also displayed complex dynamic behaviour in the sensitive cultivar. However, the observed patterns were not periodic and their relationship to the sucrose oscillations is not evident at this stage of our work (data not shown). In addition, the signal-to-noise ratio is smaller for fructose and glucose than for sucrose, and it becomes difficult to discern between true oscillations and random noise.

The question remains as to the cause of these metabolic oscillations. LTS of *Solanum tuberosum* (potato) tubers is a very complex environmental stress-induced phenomenon attributed to the deregulation of some or all carbohydrate metabolic pathways: from starch and sucrose degradation and synthesis; to glycolysis, gluconeogensis and respiration [1,9–11]. LTS-induced sucrose oscillations may be due to the effects of temperature on allosteric glycolytic enzymes, such as 6-phosphofructokinase [12], allosteric sucrolytic enzymes such as UDP-glucose pyrophosphorylase [13], allosteric starch synthetic enzymes such as ADP-glucose pyrophosphorylase [14,15], subcellular decompartmentation due to damage to the amyloplast membrane [16] and vacuolar membrane [10,11], and could also be due to diverse effects on respiration [17]. Recent evidence, however, points to the fact that glycolytic metabolism is greatly affected by low-temperature stress, and that genotypic variation in the partitioning of the excess carbon into starch or sucrose may ultimately determine the extent of LTS in different potato cultivars [18].

In conclusion, we have determined that transient oscillations in sucrose metabolism occur in whole *Solanum tuberosum* (potato) tubers exposed to low-temperature stress, and that this nonmonotone behaviour is more evident in the sensitive cultivar. Future work will be aimed at characterizing this behaviour more thoroughly and establishing its relationship with other metabolic pathways which may be controlling or affecting the observed behaviour.

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