Journal of Plant Chemistry and Ecophysiology

Open Access

Austin Publishing Group

Research Article

Constitutively Expressing of the *SUC2* Gene of *Saccharomyces cerevisiae* Encoding of Invertase Apoplastic Localization in Potato Plants Results in Multiple Physiological and Biochemical Changes Associated with Low Temperature Resistance

Deryabin A*, Berdichevets I and Trunova T
Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences, Russian Federation, Russia

***Corresponding author:** Deryabin Alexander, Laboratory of Cold Resistance, K.A. Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences, 35, Botanicheskaya Street, Moscow, 127276, Russian Federation, Russia

Received: November 17, 2017; **Accepted:** February 08, 2018; **Published:** February 16, 2018

Abstract

The first compartment of the cell, which gets Low Temperature (LT) signal and participates in its transduction and response, is the apoplast (extracellular space). The apoplast contains the acid invertase - the key enzyme of carbohydrate metabolism. The aim of this study was to investigate the physiological and biochemical changes associated with formation of resistance of potato (*Solanum tuberosum* L., cv. Désirée) plants to LT and functional role of the apoplastic invertase in this process. For achievement of this purpose used transformed potato plants, which expressed the *SUC2* gene of *Saccharomyces cerevisiae* encoding of invertase apoplastic localization. The *SUC2*-target gene presence and its expression were shown using PCR and RT-PCR. Yeast invertase were identified by MALDI-TOF MS analysis. A soluble form of the yeast invertase was present in the extracellular space, and it was weakly adsorbed onto the cellular wall. Activity of yeast invertase increase the sugars content in the apoplast and leaves of the transformed plants and led to changes in their morphometric parameters. The increase in the essential acid invertase activity in the leaves, revealed during exposure at 5 °C for 3 days indicates significant changes in the carbohydrate metabolism. The exposures at -7 °C for 0.5 h produced a significant less lipid peroxidation activity in the transformed plants, as compared to the control. Our data indicate higher resistance of transformed plants to severe LT conditions. This fact allows us to consider apoplastic invertase as a stress enzyme of carbohydrate metabolism playing an important regulatory role upon forming increased potato plant resistance to LT.

Keywords: *Solanum tuberosum*; Apoplast; Invertase; Low temperature; *SUC2* gene; Sugars

Abbreviations

Inv-IA: Insoluble Acid Invertases; **Inv-N:** Alkaline/Neutral Invertases; **Inv-SA:** Soluble Acid Invertases; **LT:** Low Temperature; **MALDI-TOF MS:** Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry; **MDA:** Malondialdehyde; **PCR:** Polymerase Chain Reaction; **POL:** Peroxidation of Lipids; **RT-PCR:** Reverse Transcriptase-Polymerase Chain Reaction; **TBA:** Thio-Barbituric Acid

Introduction

Low Temperature (LT) is one of the important environmental factor that determines the geographical distribution and productivity of higher plants [1]. According to the response to LT plants are divided into three groups: chilling-sensitive plants, being damaged at a temperature below 8 °C (corn, cotton, cucumbers, pea, rice, soybeans, sunflowers, tobacco, ets); cold-tolerant plants, which can survive at LT, non accompanied by ice formation (carrots, potatoes, radishes, ets); and frost-resistant ones, tolerant to ice formed in intercellular space (shrubs, trees, winter cereals) [2]. An important feature of the majority of plants is their ability to improve their constitutive resistance to LT (≤0 °C) after pre exposure to positive temperature (≥0 °C), i.e., hardening [1]. In nature, the process of hardening starts in the autumn from the onset of dormancy, continues at the temperatures near zero and then at light frost. This process ensures functional and structural reorganization of the cells preventing the formation of intracellular ice and elevating the resistance to intercellular ice to the effect of the temperatures below zero.

It was established long ago that plants accumulate low-molecular-weight soluble sugars (sucrose, fructose, glucose, ets) during hardening, and such accumulation was shown to correlate with the development of cold and frost resistance [1,2]. One of the prerequisites for the formation of plant resistance to LT is the changes in carbohydrate metabolism, in particular in the activity of invertases (EC 3.2.1.26) [3]. Invertase, an enzyme known as invertin including β-fructosidase, glucosucrase, etc., catalyzes the irreversible hydrolysis of sucrose into the two monosaccharides (fructose + glucose). Hydrolysis of sucrose

Citation: Deryabin A, Berdichevets I and Trunova T. Constitutively Expressing of the *SUC2* Gene of *Saccharomyces cerevisiae* Encoding of Invertase Apoplastic Localization in Potato Plants Results in Multiple Physiological and Biochemical Changes Associated with Low Temperature Resistance. J Plant Chem and Ecophysiol. 2018; 3(1): 1019.

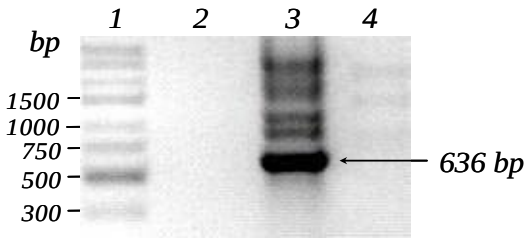

Figure 1: Results of PCR analyse of WT-potato plants and transformed potato plants (B33-inv-plants) by the vector containing the *SUC2*-targeted gene of *S. cerevisiae* using gene specific primers. 1 -GeneRuler TM Express DNA Ladder Set (Fermentas, Lithuania), 2 - Purity control of the reaction, 3 - B33-inv-plants; 4 -WT-plants.

plays a key role in changing the composition of soluble carbohydrates in various compartments of the plant cells. It should be noted that disaccharide sucrose is a metabolically inactive carbohydrate. In order to be included in the metabolic processes, sucrose must be cleaved into monosaccharides, for example, by invertase. In higher plants, an invertase has several types, which differs in the biochemical properties and subcellular localization [4]. Alkaline/neutral invertases (Inv-N) are soluble proteins with optimum activity at pH 7.0-7.8 and are localized in the cytoplasm, mitochondria, and chloroplasts. Acid invertases have an optimum activity at pH 4.5-5.0 and are localized as soluble proteins in the vacuole (Inv-SA) and apoplast (extracellular space). Importantly, invertases localized in the apoplast are usually ionically bound to the cell wall (Inv-IA) [4]. It is known, that apoplastic invertase is a key enzyme of carbohydrate metabolism involved in important physiological processes, including phloem unloading, cell differentiation control, sucrose level regulation in apoplast, and sucrose transport across the plasmalemma [5].

Adaptation to LT has been investigated in the group of frost-resistant plants. Modulation of the activity of invertases was studied mainly based on the example of storage organs of plants (root, tubers, etc.) during their formation, maturation and winter storage. The data concerning the participation of the individual types of invertases in the formation of plant resistance to LT are rare and ambiguous. There is almost no data on the effect of apoplastic invertase activity in the formation of the cold-tolerant plants resistance to LT. A typical representative of the group of cold-tolerant plants is *Solanum tuberosum* L. subsp. *tuberosum*. In comparison with *S. commersonii* (LT_{50} = -4.5 °C) and *S. acaule* (LT_{50} = -6 °C), *S. tuberosum* subsp. *tuberosum* has a lower resistance to LT (LT_{50} = 3 °C). The value LT_{50} is the temperature causing the death of 50% of the plants. The study of the formation of resistance of potato plants to LT has essential fundamental and practical importance, especially for ecology and agriculture.

New opportunities provided by genetic engineering approaches, such as the use of transformed plants expressing genes of heterologous organisms. This approach allows detecting the significance of the products of the target genes in the formation of plant resistance to LT [6]. Of particular interest is a potato line whose carbohydrate metabolism is altered by the integration of the *SUC2*-target gene. *SUC2* gene encodes the *Saccharomyces cerevisiae* invertase (EC 3.2.1.26) under the control of the patatin B33-promoter of class I with an N-end-connected potato proteinase II inhibitor signal peptide, which provides apoplastic localization of yeast invertase. Several researchers who worked with transformed potato plants focused their attention on the role of apoplastic insertase in carbohydrate regulation of growth, development and tuber formation [7-9]. We have assumed, that the transformed potato plants with altered carbohydrate metabolism is a convenient tool to study the functional role of the apoplastic invertase and the products of its activity in formation of tolerance to LT. The aim of this study was to investigate the physiological and biochemical changes associated with formation of resistance of potato plants to LT using potato plants which expressed the *SUC2* gene of *S. cerevisiae* encoding invertase with apoplastic localization.

Materials and Methods

Plant growth conditions

The materials used in this study were non-transformed potato plants (*Solanum tuberosum* L., cv. Désirée) (hereinafter wild-type plants, WT-plants) and the potato line transformed with a vector containing the *SUC2* gene under the control of the patatin B33-promoter of class I (hereinafter B33-inv-plants). Plants were grown *in vitro* at 22 °C with dinnual 16-hour light (illuminating intensity of 100 μmol photons/(m^2.s)) and 8-hour dark for 5 weeks on Murashige and Skoog (MS) medium [10] containing 2% sucrose, 0.7% agar, 0.5 mg/l pyrydoxine, 0.5 mg/l thiamine-HCl, and 60 mg/l *myo*-inositol, pH 5.8. Leaves taken from the middle of the plants were used as the research materials.

Construction of the expression vector and plant transformation

B33-inv-plants were obtained from the Max-Planck Institute of Molecular Plant Physiology (Golm, Germany). The apoplastic invertase construct was prepared using an *Asp718/Sal*I fragment prepared from the PI-3-INV plasmid. Fragment containing the sequence of the *SUC2* gene encoding the mature invertase protein fused to the signal sequence of proteinase inhibitor II of potato. Fragments were cloned between the B33-promoter and Octopine Synthase (OCS) terminator in binary vector derivatives of pBin19 [11]. Potato plants were transformed using the Agrobacterium system and selected *in vitro* on kanamycin containing MS medium, tested for transgene expression using the Northern-blot hybridization as described in reference. [8].

Isolation of plant DNA and RNA

Genomic DNA was isolated from leaves by using cetyl trimethyl ammonium bromide [12]. Total RNA was extracted using the Plant Total RNA Kit Spectrum following the manufacturer's recommendations (Sigma, USA).

Polymerase Chain Reaction (PCR) analysis

The presence of the invertase-encoding *S. cerevisiae SUC2* gene in the B33-inv-plant genome was confirmed by PCR. Primers were designed using the Vector NTI program based on the *S. cerevisiae SUC2* gene sequence, presented in the NCBI database (www.ncbi.nlm.nih.gov): 5'-TCCAAGACAAAGATGCGTTGCG-3' (F) and 3'-TGAAGGAACCGCCAGCAGGT-5' (R). PCR was performed in a My Cycler TM Thermal Cycler (Bio-Rad, USA) under the following conditions: predenaturation at 94 °C for 4 min followed by 30 cycles consisting of denaturation at 94 °C for 45 s, annealing at 61 °C for 1 min, and synthesis at 72 °C for 1 min; then there was a final

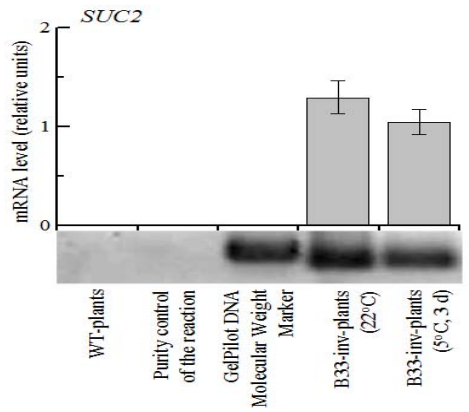

Figure 2: Results of RT-PCR analyse of expression of *SUC2*-targeted gene in B33-inv-plants at 22 ºC and in response to LT (5 ºC, 3 days) at 16 h photoperiod (light intensity of 100 µm of photons/(m^2.s)). Quantified results of RT-PCR are presented by bars.

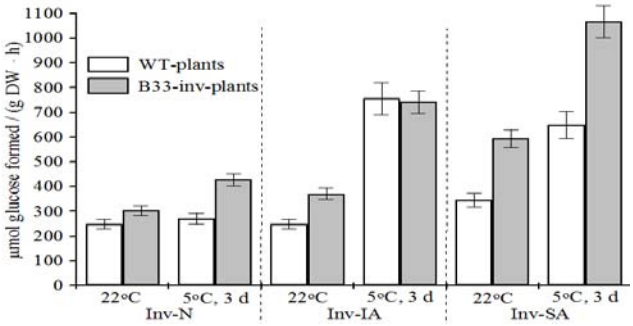

Figure 3: The effect of cooling exposure (5 ºC, 3 days) of the WT-plants and potato plants with the *SUC2*-targeted gene (B33-inv-plants) on activity of invertases (Inv-N - alkaline/neutral invertase; Inv-IA - insoluble acid invertases; Inv-SA - soluble acid invertases) in the leaves.

elongation at 72 °C for 5 min. The amplified DNA fragments were separated using 1.5% agarose gel electrophoresis in a Tris-acetate buffer, identified by staining with ethidium bromide and visualized under ultraviolet light, using the Gel Doc XR System (Bio-Rad, USA). The GeneRuler TM Express DNA Ladder Set (Fermentas, Lithuania) was used as a molecular-weight size marker. Each experiment was replicated at least three times.

cDNA synthesis and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis

Complementary DNA (cDNA) synthesis was performed with the Agilent Low RNA Input Fluorescent Linear Amplification Kit (Agilent, USA) following the manufacturer's recommendations. PCR was performed in a Mastercycler gradient (Eppendorf, Germany). The amplified DNA fragments were separated using 1.0% agarose gel electrophoresis in a Tris-acetate buffer, identified by staining with ethidium bromide and visualized under ultraviolet light, using the Gel Doc XR System (Bio-Rad, USA). The GelPilot DNA Molecular Weight Marker (Giagen, USA) was used as a molecular-weight size marker. Each experiment was replicated at least three times.

Cold stress treatments

For hardening the potato plants were cooled in a chamber at 5

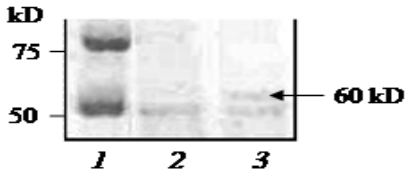

Figure 4: Fragment of Ds-Na-PAGE of apoplast proteins of potato plants. 1 - The Precision Plus Protein TM Standards Set (Bio-Rad, USA); 2 - WT-plants; 3 - B33-inv-plants.

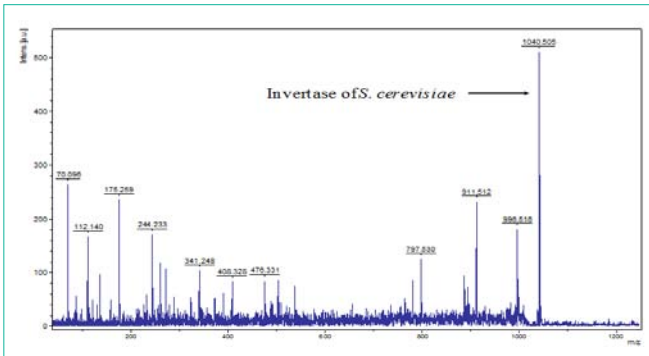

Figure 5: MALDI-TOF mass spectra of proteins isolated from the apoplastic washing fluid of leaves of the B33-inv-plants. Peak labels 1040.505 are the invertase of the yeast *S. cerevisiae*.

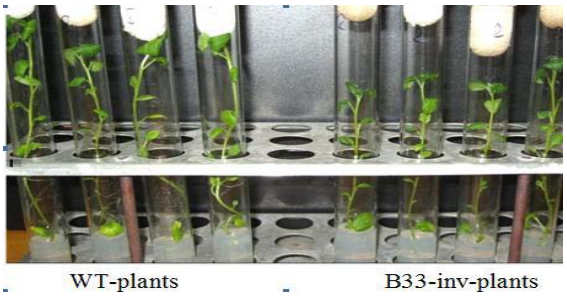

Figure 6: Phenotype of WT-plants and potato plants with the *SUC2* gene (B33-inv-plants). Plants were grown at 22 ºC for 5 weeks on a MS-medium containing 2% sucrose (illuminating intensity of 100 µmol photons/(m^2.s)).

°C for 3 days at 16 h photoperiod and light intensity of 100 µm of photons/(m^2.s).

The degree of cold resistance of potato plants was determined by direct freezing. Five week-old test-tube plants (without a cotton-gauze stopper) were treated at -5 °C or -7 °C for 0.5, 1, 2, and 3 h in a climatic chamber MIR-153 (Sanyo, Japan). The percentage of survival of plants was determined after 1 d of freezing.

Detection of invertase activity

The activity of invertase was estimated by the amount of glucose formed by hydrolysis of sucrose in the incubation medium. Invertase fractions were prepared from leaves as previously described [13]. The enzyme activity was expressed as µmol of glucose formed by hydrolysis of sucrose in the incubation medium per volume of sample taken for analysis.

MALDI-TOF MS analysis

Invertases of *S. cerevisiae* were identified by means of Matrix-

assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. The mass spectra were measured with a Bruker Ultraflex MALDI-TOF mass spectrometer (Germany). Protein identification was accomplished using the Mascot software (www.matrixscience.com). The search was performed using the NCBI (www.ncbi.nlm.nih.gov).

Extraction of apoplastic fluid and SDS-PAGE

The apoplastic washing fluid was obtained from potato leaves by using the infiltration-centrifugation technique [14]. Proteins of apoplastic washing fluid were precipitated with acetone and separated in 12.5% SDS-PAGE. After electrophoresis, the gel was stained with Coomassie R-250 to visualize the proteins. The Precision Plus Protein TM Standards Set (Bio-Rad, USA) was used as molecular-weight size marker.

Determination of carbohydrates

The content of fructose and sucrose was determined with the method to Roe by the reaction of ketoses with resorcinol [15]. The glucose content was determined by the glucose oxidase method using the Olvex diagnosticum Kit (Vital Diagnostics, Russia) following the manufacturer's recommendations. A Genesys 10UV spectrophotometer (Termo Electron Corporation, USA) was used in the study.

Measurement of Malondialdehyde (MDA) Content

The MDA content was determined by the Thiobarbituric Acid (TBA) reaction as described by Heath and Packer [16] with slight modifications. Each replication represents a sample collected from 4-6 plants.

Statistical analysis

Data were statistically processed using the T-tests software (ISI, USA) and visualized using the Microcal Origin (Microcal Software Inc., USA). The figures show the mean values of the typical experiment and their Standard Errors (SE). We discuss only the differences significant with a 95% significance level.

Results and Discussion

The presence of *SUC2*-target gene in the B33-inv-plants genome was detected using PCR with gene-specific primers and subsequent products were separated using agarose gel electrophoresis. A 636 bp fragment of *SUC2* gene was amplified from DNA isolated from B33-inv-plants (Figure 1). The fragment was not amplified from DNA of the WT-plants. To detect the *SUC2* gene expression in the transformed plants, we used RT-PCR and quantitative analysis to measure the invertase activity in the incubation medium. RT-PCR confirmed the *SUC2*-target gene expression in PCR-positive B33-inv-plants (Figure 2).

Subsequent analysis of the invertase activity in the potato plant leaves at 22°C showed the increased activity of Inv-IA and Inv-SA forms in the B33-inv-plants by 50% and 80%, respectively (Figure 3). A slight increase (on 20%) in the Inv-N activity in the B33-inv-plants was probably due to the residual invertase activity of *S. cerevisiae* in the incubation medium with pH 7.5. That the yeast invertase has a wider pH range as compared with the plant invertase [17]. The invertase of *S. cerevisiae* is foreign to the potato plants, and its activity is not inhibited by common plant inhibitors [17]. The observed increase in the Inv-SA activity confirmed our assumption on the partial adsorption of invertase of *S. cerevisiae* in the cell wall of the B33-inv-plants (Figure 3). Thus, obtained data indicated that a higher activity of the Inv-SA in the B33-inv-plants compared to that in the WT-plants was a result of the *SUC2* gene expression. However, there was a need for some specific experiments to confirm the presence of *S. cerevisiae* invertase in the apoplast of the B33-inv-plants. This need was determined by the following the increased activity of Inv-IA and Inv-SA (Figure 3). The apoplastic washing fluid from leaves was obtained. The SDS-PAGE electrophoresis of apoplastic proteins showed the presence of a protein band with a molecular weight of about 60 kDa in the B33-inv-plants, but not in the WT-plants (Figure 4). The yeast invertase is mainly a homodimer with a molecular weight of 270 kDa [18]. The removal of the enzyme carbohydrate revealed that the invertase of *S. cerevisiae* consisted of two identical subunits with a molecular weight of 60 kDa [19]. The *S. cerevisiae* invertase in the B33-inv-plants was identified using MALDI-TOF mass spectrometry (Figure 5). Thus, the results of analysis are consistent with the published data [18,19] and indicate the presence of the yeast invertase in the apoplastic (extracellular) space of the B33-inv-plants. We confirmed insertion of the complete sequence of the subcloned transgene encoding the acid invertase of *S. cerevisiae* in the genomic DNA of the B33-inv-plants. It was shown that yeast invertase synthesized by the *SUC2*-target gene due to the presence of the signal peptide of the inhibitor of potato proteinase II is transported to the extracellular space and is present in this compartment (apoplast) in a soluble form slightly adsorbing on the cell wall. It is important to note that potato invertases localized in the apoplast are bound covalently to the cell wall [5].

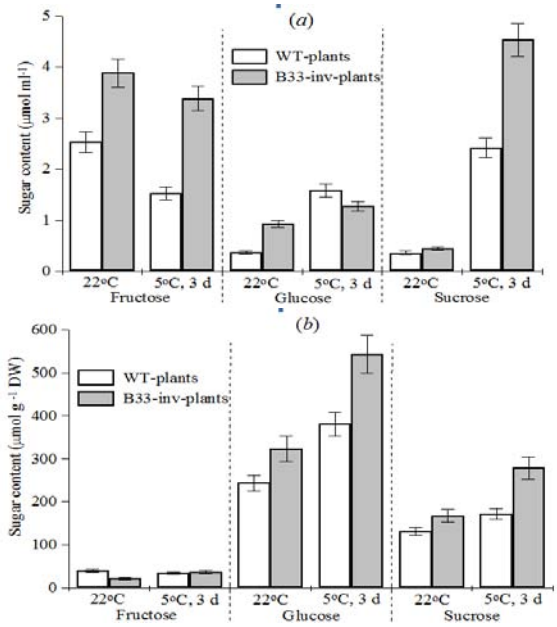

Figure 7: The effect of cooling exposure (5 °C, 3 days) on fructose, glucose and sucrose content in the apoplastic washing fluid **(a)** and leaves **(b)** of WT-plants and potato plants with the *SUC2*-targeted gene (B33-inv-plants).

The morpho-physiological parameters of the potato plants, expressing yeast *SUC2* gene encoding invertase with apoplastic localization and WT-plants under *in vitro* conditions were different. The transformed potato plants exhibited decreased offshoot length

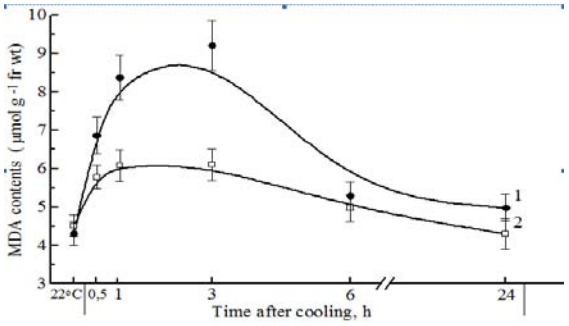

Figure 8: The effect of LT (-7 ºC, 0.5 hours) on contents of MDA in leaves of the WT-plants (1) and B33-inv-plants (2).

and larger water content in tissues (Table 1, Figure 6).

To study the formation of resistance of potato plants to LT, a hardening temperature of 5 ºC and duration of cold exposure for 3 days were chosen. Under the influence of cold the activity of Inv-SA in WT-plants increased by 80%, and in B33-inv-plants, it increased by 85%, in comparison with the activity at 22 ºC (Figure 3). However, at LT exposure in B33-inv-plants activity of Inv-SA on 65% was higher in comparison with WT-plants. This increase was caused by the additional activity of yeast invertase and can serve as one of the arguments proving the participation of apoplastic (cell-wall) invertase in the formation of resistance of potato plants to LT. The increase of Inv-N and Inv-SA the activities observed in the B33-inv-plants was apparently due to the weak adsorption of yeast invertase on the cell wall and present in this compartment (apoplast) in a soluble form. The absence of significant changes in the activity of Inv-N during cold exposure of WT-plants indicates the insignificant participation of this enzyme in the formation of cold resistance.

Apoplast (extracellular space) is the first compartment of the plant cell that gets LT signal and participates in its transduction and response. Analysis of sugar concentration in the apoplast provides additional information on the apoplastic localization of yeast invertase in B33-inv-plants (Figure 7a). Results demonstrated that fructose and glucose concentrations in the apoplast of the B33-inv-plants at 22 ºC, as compared to those in the WT-plants, were by 1.5 and 2.5 time higher, respectively. During 3-day-long exposure at 5 ºC accumulation of sucrose was observed in the apoplast of both potato lines, but the accumulation was more expressed in B33-inv-plants (by 2.0 time).

In leaves of B33-inv-plants the sucrose and glucose levels exceed those in the WT-plants by 35% (Figure 7b). The fructose content in both potato lines was low. The low fructose content resulted from the high activity of fructokinase (EC 2.7.1.4.) that provided maximal use of free fructose in the glycolytic pathway. During the cold exposure (5 ºC, 3 days) of potato plants, accumulation of sucrose and glucose was observed in the leaves of both lines, but the accumulation was more expressed in B33-inv-plants. The high sugar content in the leaves of B33-inv-plants was probably due to the active hydrolysis of sucrose by apoplastic invertases (plant and yeast). Cells use free glucose and fructose for the synthesis of cell wall polymers and/or transport them back into the cell [7]. In the cells, hexoses are phosphorylated by fructokinases and hexokinases (EC 2.7.1.1) and

Table 1: Morpho-physiological parameters of potato plants.

Parameter	B33-inv-plants	WT-plants
Shoot length (cm)	10.6 ± 0.3	13.8 ± 0.3
Fresh leaves (mg/plant)	59.1 ± 8.4	80.2 ± 6.4
Contents of dried mass (% of the fresh plants)	8.98	9.61

Data are mean values ± SE, n = 30-35. Plants were grown *in vitro* at 22 ºC for 5 weeks on a MS-medium containing 2% sucrose (illuminating intensity of 100 μmol photons/(m².s)).

Table 2: The number of potato plants surviving one day after freezing, %.

Temperature (°C)	Plants	Cooling time (h)			
		0.5	1	2	3
-5	WT	100	100	10	0
	B33-inv	100	100	70	0
-7	WT	90	15	0	0
	B33-inv	100	80	0	0

Note: SE of mean values did not exceed 10%.

involved in metabolism, including the synthesis of sucrose. Thus, the activity of acid invertases of potato plants induced by LT changed the composition and the intracellular concentration of sugars in leaves (Figure 3). The increase in the essential acid invertase activity in the leaves of B33-inv-plants indicates significant changes in the carbohydrate metabolism and regulatory function of this enzyme.

The high sugar (sucrose and glucose) content in B33-inv-plants caused by increased activity of apoplastic (cell-wall) invertase not only inhibited the growth of shoots (Table 1, Figure 6), but also could be a prerequisite for improving their resistance to LT. It is known that decreased growth activity is accompanied by the restructuring of metabolism associated with the inhibition of energy intensive anabolic processes, leading to a nonspecific increase in the stress resistance. Therefore, for abstraction of the processes underlying plant adaptation to low hardening temperatures, we used the method of direct freezing. This method is based on the rapid cooling of plants at low damaging temperatures and allows identification of the differences between potato lines in terms of their constitutive resistance to LT. The results of the comparative analysis of potato plant survival after different freezing modes are presented in the Table 2. Our data indicate higher constitutive resistance of B33-inv-plants to severe LT conditions compared to WT-plants.

To evaluate potato plant tolerance to LT, we employed the method of assessing peroxidation of lipids (POL) by the content of MDA, one of its terminal products. It was shown that both potato lines produced an increase in MDA content with treatment of 3-day chilling at 5 ºC (data not shown). The increase in MDA content in the B33-inv plants was significantly lower than in the WT-plants when potato plants were exposed to -7 ºC for 0.5 h (Figure 8). We observed an increase in the MDA content in the WT-plants by 2.0 times, whereas in the B33-inv plants this index increased only by 35% (in 3 hours after cooling). Consequently, the observed decrease in POL activity in B33-inv-plants under the LT conditions is caused by the effect of soluble sugars. Our data indicate higher constitutive resistance of B33-inv-plants to severe LT conditions compared to WT-plants. It is resulted from the changes in sugar composition in cell of B33-inv-plants produced by the invertase of *S. cerevisiae*.

Soluble sugars are not only major osmoregulators, cryoprotectants, plastic and energy substrates, but also low-molecular-weight reactive

oxygen species scavengers (antioxidants) [20,21]. Increasing the concentration of sugars (mainly glucose, fructose, and sucrose) in the cell depresses the freezing point. Several possible mechanisms of protective action of low-molecular-weight sugars on the biological membranes have been proposed. One of them may depend on the formation of bonds between the O_2 of phosphates within membrane phospholipids and hydroxyls of sugars [22]. This mechanism is consistent with the possibility that in dehydrated cells, water is replaced in biological membranes with sucrose [23]. Sugars can modify plasma membrane ensuring its homeoviscous adaptation [2]. One may suggest that owing to the accumulation of low-molecular carbohydrates in the course of cold acclimation due to activation of acid invertase cold-resistant plants better adapt to temperature drop occurring in nature. Under these conditions, low-molecular-weight reactive oxygen species scavengers, to which soluble carbohydrates presumably belong, play more significant role in the protection against oxidative stress. Thus, the transformed potato plant with the *SUC2* gene (B33-inv-plants) is a convenient tool to study the role of the apoplastic invertase and the products of its activity during growth, development and formation resistance to low-temperature stress.

Acknowledgment

The authors are grateful **Dr. L. Willmitzer** (Max Planck Institute of Molecular Plant Physiology, Germany) and **Prof. G.A. Romanov** (K.A. Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences, Moscow, Russia) for the potato plants provided for the research. We acknowledge **Dr. Ilya Toropygin** (Institute of Biomedical Chemistry of the Russian Academy of Sciences) for MALDI-TOF MS analysis. The work was partially funded by the Russian Foundation for Basic Research.

References

1. Levitt J. Responses of plants to environmental stresses. V. 1. Chilling, freezing and high temperature stresses, New York: Academic Press; 1980.
2. Trunova TI. Plant and cold stress (The 64th Timiryazev memorial lecture): Nauka Publ., Moscow; 2007. (In Russian)
3. Nägele T, Heyer AG. Approximating subcellular organisation of carbohydrate metabolism during cold acclimation in different natural accessions of *Arabidopsis thaliana*. New Phytol. 2013; 198: 777-787.
4. Roitsch T, Gonzalez MC. Function and regulation of plant invertases: sweet sensations. Trends Plant Sci. 2004; 9: 606-613.
5. Roitsch T, Balibrea ME, Hofmann M, Proels R, Sinha AK. Extracellular invertase: key metabolic enzyme and PR protein. J Exp Bot. 2003; 54: 513-524.
6. Jewell MC, Campbell BC, Godwin ID. Transgenic plants for abiotic stress resistance. In: Transgenic crop plants: Springer-Verlag Berlin Heidelberg; 2010.
7. Büssis D, Heineke D, Sonnewald U, Willmitzer L, Raschke K, Heldt HW. Solute accumulation and decreased photosynthesis in leaves of potato plants expressing yeast-derived invertase either in the apoplast, vacuole or cytosol. Planta. 1997; 202: 126-136.
8. Sonnewald U, Hajlrezaei MR, Kossmann J, Heyer A, Thethewey RN, Willmitzer L. Increased potato tuber size resulting from apoplastic expression of a yeast invertase. Nat Biotech. 1997; 15: 794-797.
9. Romanov GA, Konstantinova TN, Sergeeva LI, Golyanovskaya SA, Kossmann J, Willmitzer L, et al. Morphology and tuber formation of *in vitro* grown potato plants harboring the yeast invertase gene and/or the *rolC* gene. Plant Cell Rep. 1998; 18: 318-324.
10. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962; 15: 473-497.
11. Dietze J, Blau A, Willmitzer L. *Agrobacterium*-mediated transformation of potato (*Solanum tuberosum*). In: Gene transfer to plants: Springer-Verlag, Berlin. 1995.
12. Lipp M, Brodmann P, Pietsch K, Pauwels J, Anklam E, Börchers T, et al. IUPAC collaborative trial study of a method to detect genetically modified soy beans and maize in dried powder. J AOAC Int. 1999; 82: 923-928.
13. Deryabin AN, Dubinina IM, Burakhanova EA, Astakhova NV, Sabel'nikova EP, Trunova TI. Influence of yeast-derived invertase gene expression in potato plants on membrane lipid peroxidation at low temperature. J Thermal Biol. 2005; 30: 73-77.
14. Hon WCh, Criffith M, Chong P, Yang DSC. Extraction and isolation of antifreeze proteins from winter rye (*Secale cereale* L.) leaves. Plant Physiol. 1994; 104: 971-980.
15. Turkina NV, Sokolova SV. Biochemical methods in plant physiology. Nauka Publ.; Moscow: 1971. (in Russian)
16. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. 1. Kinetics and stoichoimetry of fatty acid peroxidation. Arch Biochem Biophys. 1968; 125: 189-215.
17. Von Schaewen A, Stitt M, Schmidt R, Sonnewald U, Willmitzer L. Expression of a yeast-derived invertase in the cell wall of tobacco and arabidopsis plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. EMBO J. 1990; 9: 3033-3044.
18. Neumann NP, Lampen JO. Purification and properties of yeast invertase. Biochem. 1967; 6: 468-475.
19. Trimble RB, Maley F. Subunit structure of external invertase from *Saccharomyces cerevisiae*. J Biol Chem. 1977; 252: 4409-4412.
20. Tarkowski ŁP, Van den Ende W. Cold tolerance triggered by soluble sugars: a multifaceted countermeasure. Front Plant Sci. 2015; 6: 203-220.
21. Krasavina MS, Burmistrova NA, Raldugina GN. The role of carbohydrates in plant resistance to abiotic stresses. In: Emerging Technologies and Management of Crop Stress Tolerance. Elsevier Inc. 2014; 229-270.
22. Strauss G, Hauser H. Stabilization of lipid bilayer vesicles by sucrose during freezing. Proc Nat Acad Sci USA. 1986; 83: 2422-2426.
23. Caffery M, Tonseca V, Leopold AC. Lipid-sugar interactions relevance to anhydrous biology. Plant Physiol. 1988; 86: 754-758.

Citation: Deryabin A, Berdichevets I and Trunova T. Constitutively Expressing of the *SUC2* Gene of *Saccharomyces cerevisiae* Encoding of Invertase Apoplastic Localization in Potato Plants Results in Multiple Physiological and Biochemical Changes Associated with Low Temperature Resistance. J Plant Chem and Ecophysiol. 2018; 3(1): 1019.