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Transformation of the *Solanum tuberosum* with *Saccharomyces cerevisiae* Gene Suc2 Encoding Cell-Wall Invertase Influences on Sugars Distribution in Plants

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Abstract

Introduction: Invertase is the key enzyme of carbohydrate metabolism, which catalyzes the sucrose hydrolysis. In this study, we investigated a functional role of the apoplastic invertase (cell-wall invertase) on sugars distribution in vegetative organs and cell compartments of potato plants in vitro.

Methods: Our study was carried out with potato (S. tuberosum L., cv. Désirée) plants and the line which expressed the suc2 gene of S. cerevisiae under control of the tuber-specific patatin B33-promoter of class I with an N-end-connected potato proteinase II inhibitor signal peptide, which provides apoplastic localization of yeast invertase. The suc2 gene expression were shown using RT-PCR. The results of Ds-Na-PAGE of apoplast proteins from leaves and MALDI-TOF MS analysis indicate the presence of the yeast invertase in the apoplastic space of the transgenic potato plants. The content of fructose and sucrose was determined according to Roe. The glucose content was determined by the glucose oxidase method.

Results: The integrated target suc2 gene encodes the invertase of yeast with an N-end-connected potato proteinase II inhibitor signal peptide, which provides apoplastic localization of foreign invertase.

Conclusions: Transformation of potato plants resulted in accumulation of fructose in the apoplast, sucrose and glucose in the leaves, and especially, glucose in roots and microtubers. It is indicative of regulatory function of cell-wall invertase that could be found some application in medical, biological and pharmaceutical engineering.

INTRODUCTION

Fundamental insights into how plants transport and store food reserves are critical to improving crop productivity. New opportunities provided by genetic and biological engineering approaches, such as the use of transgenic plants expressing genes of heterologous organisms. Of particular interest is a potato line whose carbohydrate metabolism is altered by the integration of the *suc2* gene of *S. cerevisiae*. This gene encodes the invertase (EC 3.2.1.26, β-*D*-fructofuranosidase) under the control of the tuber-specific patatin B33-promoter of class I with an *N*-end-connected potato proteinase II inhibitor signal peptide, which provides apoplastic localization of yeast invertase [1]. Invertase, an enzyme known as invertin, beta-fructosidase, glucosucrase, etc. is used in basic research and various industrial applications especially in the pharmaceutical and food industry. Invertase catalyses the irreversible hydrolysis of sucrose to free hexoses (glucose + fructose). The production of non-crystallizable sugar syrup from sucrose is one of the major applications of invertase. Invertase being a powerful anti-microbial agent and an antioxidant aids in the prevention of bacterial infestations and gut fermentation due to oxidation [2]. In higher plants an invertases has several isoforms: 1) alkaline/neutral invertases are localized in the chloroplasts, cytoplasm, and mitochondria; 2) acid invertases are localized in the apoplast (cell-wall invertase) and vacuole [3]. Cell-wall invertase is a key enzyme of carbohydrate metabolism involved in important physiological processes, including cell differentiation control, phloem unloading and sucrose level regulation in apoplast. Moreover, the potato plant cell-wall invertase coordinates donor-acceptor bonds between leaves and tubers [3, 4]. Researchers who worked with transgenic potato plants expressing the yeast *suc2* gene encoding apoplastic invertase, focused attention to a role cell-wall invertase in carbohydrate regulation of process tuberization [1, 5] and adaptation to unfavorable environ-

ment [6]. Analysis of the different forms of invertase activity in potato plants showed increased activity of cell-wall invertase in transgenic plants by 30%, respectively [7]. We have assumed, that the transgenic potato plants expressing *suc2* gene encoding the apoplastic invertase yeast is a convenient tool to study a functional role of the cell-wall invertase on sugars distribution in vegetative organs (leaves, roots, microtubers) and cells compartments.

METHODS

Plant Materials

Our study was carried out with potato (*S. tuberosum* L., cv. Désirée) plants (abbreviated below as WT-plants) and the line transformed with a vector containing the *suc2* gene, under the control of the class 1 patatin tuber-specific promoter B33 (abbreviated below as transgenic plants). The cell-wall (apoplastic) invertase construct was prepared using an *Asp718/Sal*I fragment (containing the sequence of the *suc2* gene encoding the mature invertase protein fused to the signal sequence of proteinase inhibitor II) prepared from the PI-3-INV plasmid. Both fragments were cloned between the B33 promoter and the octopine synthase (OCS) terminator in pBin19 binary vector. Potato plants were transformed using the *Agrobacterium* system and selected *in vitro* on kanamycin containing Murashige and Skoog (MS) medium [8], in Max Planck Institute of Molecular Plant Physiology (Golm, Germany) [1].

Growth Conditions of Plants

Plants were propagated by the cuttings method using stem cuttings with one axillary bud, grown in test tubes, sealed with cotton-gauze plugs, in a phytotron chamber at 22°C and 16-h long light day (illuminating intensity of 100 μmol photons/(m^2·s)) on MS-medium containing 0.7% agar and 2% sucrose, pH 5.8. The standard liquid tuberization medium contained MS-medium salts and 80 g·l^{-1} sucrose. Plants were in darkness at 21±1°C within 10 weeks.

Isolation of Plant RNA and cDNA Synthesis

Total RNA was extracted from leaves using the Plant Total RNA Kit Spectrum (Sigma, USA). Complementary DNA (cDNA) synthesis was performed with the Agilent Low RNA Input Fluorescent Linear Amplification Kit (Agilent, USA) following the manufacturer's recommendations.

PCR Analysis

Polymerase chain reaction (PCR) was performed in a Mastercycler gradient (Eppendorf, Germany). 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' (forward primer (F)) and 3'-TGAAGGAACCGCCAGCAGGT-5' (reverse primer (R)). 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.

Extraction of Apoplastic Washing Fluid

The apoplastic washing fluid was obtained from leaves by using the infiltration-centrifugation technique [9].

Ds-Na-PAGE

Proteins of apoplastic fluid were precipitated with acetone and separated by denaturing electrophoresis in 12,5% Ds-Na polyacrylamide gel (Ds-Na-PAGE). After denaturing electrophoresis, the gel was stained with Coomassie R250 to visualize the proteins. The Precision Plus Protein TM Standards Set (Bio-Rad, USA) was used as molecular-weight size marker.

MALDI-TOF MS

Yeast invertases 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 database.

Determination of Sugars

The glucose content in leaf tissues, apoplastic washing fluid, roots and microtubers was determined by the glucose oxidase method using the Olvex diagnosticum Kit (Vital Diagnostics, Russia). The content of fructose was determined according to Roe by the reaction of ketoses with resorcinol with subsequent recalculation of the sucrose content [6].

Statistics

Data were statistically processed using the T-tests software (ISI, USA). The figure and table show the mean values of the typical experiment and their standard errors. We discuss only the differences significant with a 95% significance level.

RESULTS

The use of the *suc2* gene of *S. cerevisiae* was chosen for two reasons: 1) yeast invertase is foreign to the potato, therefore, its activity is not inhibited by plant inhibitors, and 2) yeast invertase has a wider pH range than plant invertase [10]. In the beginning of research with transgenic plants it was necessary to confirm presence and expression a target gene *suc2*. The constitutive of *suc2* gene expression in the transgenic potato plants genome was shown using RT-PCR. PCR-positive transformants showed the *suc2* gene expression (Fig.1a). In order to confirm extracellular localization of yeast invertase, 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 transgenic plants, but not in the WT-plants (Fig. 1b). The *S. cerevisiae* yeast invertase in the transgenic potato plants was identified using MALDI-TOF mass spectrometry (Fig. 2). It is known, that the yeast invertase is mainly a

homodimer and consisted of two identical subunits (Mr = 60 kDa) [11]. Thus, the results are consistent with the published data and indicate the presence of the invertase of *S. cerevisiae* in the apoplastic space of the transgenic potato plants.

According to the main heterotrophy of the plants grown *in vitro*, disaccharide of sucrose (2-3%) as a carbohydrate source is used the most often. However, in the plant cells sucrose can't be utilized for metabolism. Before using it must be split into gexoses by, for example, the enzyme - invertase. To reveal a role of the cell-wall invertase in changing the composition of soluble carbohydrates in various vegetative organs and cell compartments we have assessed the contents of sucrose, glucose and fructose in leaves, roots, microtubers and the apoplastic washing fluid from leaves of potato plants (Table 1).

DISCUSSION

The results indicate that transformation of potato plants with the yeast invertase gene resulted in accumulation of fructose in the apoplast, sucrose and glucose in the leaves, and especially, glucose in microtubers and roots. In transgenic plants compared to that in the WT-plants contents of sugars in leaves and roots was higher by 35-45%, in microtubers - by 270%. We found out that during growth and development of the plants in MS-medium containing 2% sucrose, occurred active assimilation of organic compounds. It is known that sucrose, glucose, and fructose are not only major osmoregulators, plastic and energy substrates, but also signaling molecules and antioxidants [12, 13]. Sugars are involved in changing the balance of plant hormones and regulation of the expression of genes responsible for the growth and development of plants [14]. Earlier we have shown, that increased total sugar contents in the transgenic potato plants expressing *suc2* gene encoding the apoplastic invertase yeast, led to reduce of structural elements of chloroplasts [15, 16] and morphometric parameters, such as, shoot length, developed root system, contents of dried mass [1]. In comparison with the control plants, transgenic potato plants formed a minimal number of tubers per plant, but tubers had a higher mass [17]. It is possible, that glucose accumulated in the extracellular spaces of the transgenic of potato plants, may act as a signal to trigger cell division. It is indicative of a regulatory function of cell-wall invertase. Thus, use of the transgenic potato plants with the yeast invertase gene could be found some application in medical, biological and pharmaceutical engineering [2].

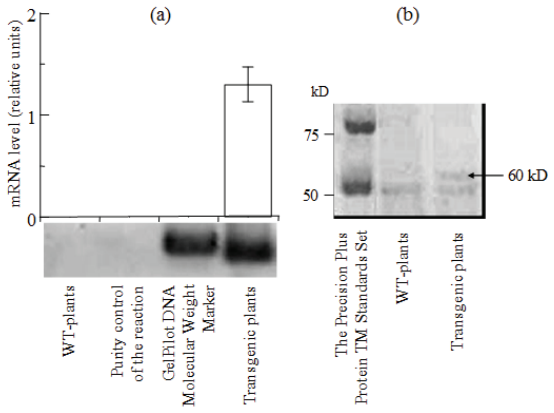

Figure 1: Results of RT-PCR Analyse of Expression of suc2 Gene
(a) Fragment of Ds-Na-PAGE of Apoplast Proteins
(b) From Leaves Potato Plants. Quantified Results of RT-PCR are presented by Bars.

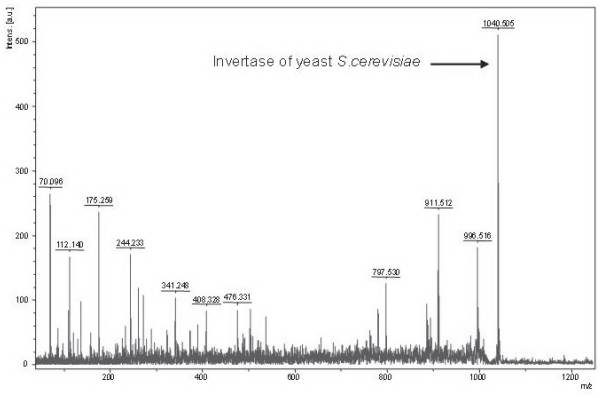

Figure 2: MALDI-TOF Mass Spectra of Proteins Isolated From the Apoplastic Washing Fluid of the Transgenic Potato Plants Expressing *suc2* Gene Encoding the Apoplastic Invertase Yeast. The Arrow Points to the Invertase of the Yeast *S. cerevisiae.*

Table 1: Content of Sugars in Vegetative Organs and Apoplastic Washing Fluid of Potato Plants

Plants and Sugars	Contents of Sugars, mg/g FM			
	Root	Leaves	Apoplast	Microtubers
WT-plants				
Glucose	4.58 ± 0.36	4.26 ± 0.34	0.46 ± 0.03	0.36 ± 0.01
Fructose	0.10 ± 0.01	0.56 ± 0.10	0.07 ± 0.01	0.31 ± 0.01
Sucrose	5.01 ± 0.44	4.06 ± 0.30	0.12 ± 0.01	3.95 ± 0.23
sugars	9.69	8.88	0.65	4.62
Transgenic plants				
Glucose	12.51 ± 0.92	5.44 ± 0.39	0.70 ± 0.05	8.15 ± 0.66
Fructose	0.48 ± 0.03	0.35 ± 0.02	0.17 ± 0.03	0.71 ± 0.05
Sucrose	0.92 ± 0.11	6.16 ± 0.58	0.15 ± 0.01	3.96 ± 0.27
sugars	13.91	11.95	1.02	12.82

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CONFLICTS OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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AUTHOR S' CONTRIBUTIONS

A. Deryabin designed and performed the experiments. T.Trunova wrote and edited the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Sonnewald U, Hajirezaei MR, Kossmann J, Heyer A, Trethewey RN, Willmitzer L. Increased potato tuber size resulting from apoplastic expression of a yeast invertase. Nat Biotechnol. 1997;15(8):794-7. DOI: 10.1038/nbt0897-794 PMID: 9255797
2. Kulshrestha S, Tyagi P, Sindhi V, Yadavilli KS. Invertase and its applications – A brief review. J Pharm Res. 2013;7(9):792-7. DOI: 10.1016/j.jopr.2013.07.014
3. Roitsch T, Gonzalez MC. Function and regulation of plant invertases: sweet sensations. Trends Plant Sci. 2004;9(12):606-13. DOI: 10.1016/j.tplants.2004.10.009 PMID: 15564128
4. Proels RK, Huckelhoven R. Cell-wall invertases, key enzymes in the modulation of plant metabolism during defence responses. Mol Plant Pathol. 2014;15(8):858-64. DOI: 10.1111/mpp.12139 PMID: 24646208
5. Romanov GA, Sergeeva LI, Golyanovskaya SA, Willmitzer L, Schmlling T, Aksenova NP, 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(3-4):318-24. DOI: 10.1007/s002990050579
6. Deryabin A, Burakhanova E, Trunova T. The involvement of apoplastic invertase in the formation of resistance of cold-tolerant plants to hypothermia. Biol Bull. 2016;43(1):26-33.
7. Deriabin AN, Berdichevets IN, Burakhanova EA, Trunova TI. [Characteristics of extracellular invertase of Saccharomyces cerevisiae in Heterologous expression of the suc2 gene in Solanum Tuberosum plants]. Izv Akad Nauk Ser Biol. 2014;41(1):22-9. PMID: 25735153
8. Murashige T, Skoog F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiol Plant. 1962;15(3):473-97. DOI: 10.1111/j.1399-3054.1962.tb08052.x
9. Hon WC, Griffith M, Chong P, Yang D. Extraction and Isolation of Antifreeze Proteins from Winter Rye (Secale cereale L.) Leaves. Plant Physiol. 1994;104(3):971-80. PMID: 12232141
10. 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(10):3033-44. PMID: 2209536
11. Trimble RB, Maley F. Subunit structure of external invertase from Saccharomyces cerevisiae. J Biol Chem. 1977;252(12):4409-12. PMID: 325007
12. Van den Ende W, Peshev D. Sugars as antioxidants in plants: Crop improvement under adverse conditions. New York: Springer Science+Business Media; 2013.
13. Krasavina MS, Burmistrova NA, Raldugina GN. The Role of Carbohydrates in Plant Resistance to Abiotic Stresses: Emerging Technologies and Management of Crop Stress Tolerance: Elsevier; 2014. 229-70 p.
14. Hummel M, Rahmani F, Smeekens S, Hanson J. Sucrose-mediated translational control. Ann Bot. 2009;104(1):1-7. DOI: 10.1093/aob/mcp086 PMID: 19376782
15. Trunova TI, Astakhova NV, Deryabin AN, Sabel'nikova EP. Ultrastructural organization of chloroplasts of the leaves of potato plants transformed with the yeast invertase gene at normal and low temperature. Dokl Biol Sci. 2003;389:176-9. PMID: 12854424
16. Astakhova NV, Deryabin AN, Sin'kevich MS, Trunova TI. Changes in the chloroplast ultrastructure in potato leaves during long-term micropropagation in vitro. Dokl Biol Sci. 2009;427:362-4. PMID: 19760884
17. Pang H, Du L, Pei J, Wei Y, Du Q, Huang R. Sucrose hydrolytic enzymes: old enzymes for new uses as biocatalysts for medical applications. Curr Top Med Chem. 2013;13(10):1234-41. PMID: 23647545