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Cold tolerance of potato plants transformed with yeast invertase gene

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Summary

Our study was carried out with potato plants (*Solanum tuberosum* L., cv. Désirée) transformed with the yeast invertase gene under the control of the B33 class I patatin promoter and with the proteinase inhibitor II leader peptide sequence providing for the apoplastic enzyme localization (B33-*inv* plants) and with the plants transformed with the reporter gene encoding bb-glucuronidase under the control of the 35S CaMV promoter (control plants). Exposure to 5°C during 6 days caused an increase in invertase activity and sugar content in B33-*inv* leaves in comparison with the control plants. Cell membranes of B33-*inv* plant cells showed greater cold tolerance under low temperature conditions than control plants that was recorded by electrolyte release. We supposed that higher cold tolerance of B33-*inv* plants was caused by stabilizing effect of sugar on the membranes, because B33-*inv* plants differ from the control plants in higher invertase activity, induced by expression of yeast invertase gene, and high content of sugars.

Key words: *Solanum tuberosum*; transgenic plants; exoosmos of electrolytes; invertase; sugars; low-temperature tolerance

INTRODUCTION

Plants accumulate soluble sugars during adaptation to chilling and freezing, and such accumulation was shown to correlate with the development of cold and frost resistance (Levitt, 1980). The role of low-molecular-weight carbohydrates in plant

tolerance and adaptation to chilling stress was substantiated by the data on sugar participation in osmoregulation and protection of protein and lipid cell components (Yoshida et al., 1997). Invertase (bb-D-fructofuranoside-fructohydrolase, EC 3.2.1.26) plays a key role in changing the composition and ratio of various soluble carbohydrates (Tymowska-Lalanne and Kreis, 1998; Winter and Huber, 2000).

Although the correlation between the content of low-molecular-weight carbohydrates and plant cold tolerance was demonstrated, the importance of hydrolases is poorly studied. Transgenic plants can help in filling this gap. During the last decade, numerous transgenic potato plants were successfully exploited for solving diverse problems. Transgenic plants are increasingly used as models for studying plant responses to stresses and as the sources of tolerant plants (Kuzniak, 2002). At the Max Planck Institute of Molecular Plant Physiology (Golm, Germany), potato plants were transformed with diverse genes, including a yeast-derived invertase gene. Several researchers have studied the effects of the expression of foreign yeast invertase in potato plants (Büssis et al., 1997; Sonnewald et al., 1997; Romanov et al., 1998; Aksenova et al., 2000). High invertase activity in potato plants exhibits the apoplastic enzyme location, in comparison to vacuolar and cytoplasmic enzymes (Büssis et al., 1997). The apoplastic potato plants were used in our research. The objective of this work was to study relation of invertase activity and sugar content to the development of cold tolerance in potato plants expressing foreign yeast invertase.

MATERIALS AND METHODS

Our study was carried out with potato plants (*Solanum tuberosum* L., cv. Désirée) transformed with the yeast invertase gene (*inv*) under the control of the B33 class I patatin promoter and with the proteinase inhibitor II leader peptide sequence providing for the apoplastic enzyme localization (abbreviated below as B33-*inv* plants) and with the plants transformed with the reporter gene encoding bb-glucuronidase (GUS) under the control of the 35S promoter of cauliflower mosaic virus (abbreviated below as the control plants). Transformed plants also harbored the kanamycin resistance gene. All potato plants were obtained from the Max-Planck Institute of Molecular Plant Physiology (Golm, Germany).

Plants were grown *in vitro* at 22°C and illuminated for 16 h with white-light fluorescence lamps (5 klx) during 4 weeks. MS medium (Murashige and Skoog, 1962) containing 0.7% agar, 2% sucrose, 0.5 mg/l thiamine-HCl, 0.5 mg/l pyridoxine, and 60 mg/l *myo*-inositol was used.

Invertase activities before and after 6 daylong exposure to 5°C were assessed by the method described earlier by Deryabin et al. (2003). Sucrose and fructose contents in leaf tissues were determined by the method of Roe et al., glucose was measured by glucose-oxidase method (Turkina and Sokolova, 1971).

Chilling tolerance of potato genotypes was assessed by the electric conductivity of water extracts from plant tissues according to Dexter (Hepburn et al., 1986). Middle leaves were sampled, the petioles were detached and 80 mg of fresh material was placed into glass tube with 10 ml of distilled water. Vacuum infiltration of water was carried out twice in the light. Closed tubes with samples were shaken for 1 h. The content of ions leaked from the tissues was measured in the cell with two flat platinum electrodes (1 cm^2) arranged 6 mm apart using an R 577 bridge (Russia) at room temperature. When the measurement was over, the tubes were placed into a boiling water bath for 20 min and then shaked for 30 min. Thereafter, the measurement was repeated. The electric conductivity was calculated as $ohm^{-1}\ cm^{-1} \cdot 10^{-4}$ using a commonly employed formula (Flint et al., 1967):

$$I = 100(L_t - L_0)/(L_k - L_0),$$

where I is the index of tissue injury, %, L is the electric conductivity of the sample measured before cold injury (L_0), and after it (L_t); L_k is the electric conductivity of the same sample after boiling, $ohm^{-1}\ cm^{-1} \cdot 10^{-4}$. The cold tolerance of the tissue (H) was estimated as the value complementary to I ($H = 100 - I$) and expressed in %.

Each replication represented a sample collected from 3-4 plants. The significance of differences between the treatments was estimated using the Student's criterion. The results significant at $P = 0.95$ are discussed.

RESULTS AND DISCUSSON

As it was shown earlier (Deryabin et al., 2003), activity of different isoforms of invertase and sugar contents in the leaves of potato plants transformed with yeast invertase gene (B33-*inv* plants) were higher than those in control plants the plants transformed with the reporter gene encoding GUS when cultivated at 22°C. Contributing to classical point of view, i.e. the increase in invertase activity as an unspecific plant response to unfavorable environment we could suppose considerable influence of low temperatures on the invertase activity and soluble sugar content in the leaves of chilled plants. And really, long chilling influence (6 days at 5°C) induced a higher activity of most invertase isoforms, mainly in experimental plants (Fig. 1).

Low temperature caused no significantly increased activity of these forms of invertase in the leaves of control plants.

Therefore, cell wall acid invertase was 2.2 times more active after long chilling in B33-*inv* leaves than in control plants, whereas soluble fraction invertase increased more than three-fold. Alkaline invertase in B33-*inv* plants was less activated by chilling. However, alkaline invertase in cold-treated plants was more active after chilling than in control plants both in cell wall (86%) and soluble fraction (68%), as compared to the absence of significant activation in control plant leaves.

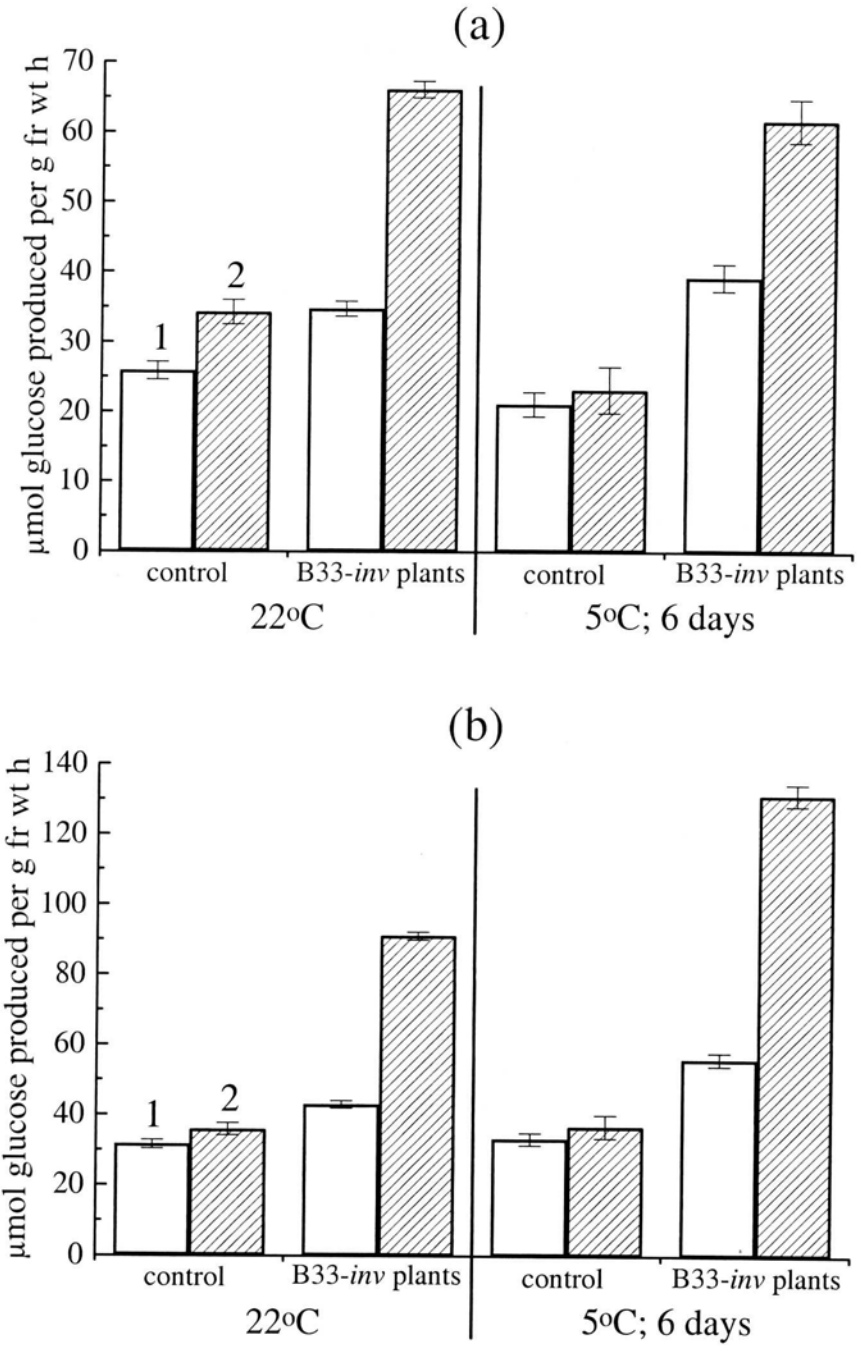

Fig. 1. Acid (1) and alkaline (2) invertase activities in cell wall fraction (a) and supernatant (b), extracted from potato plant leaves before and after 6 days of chilling at 5°C.

We supposed that long chilling influence caused the changes in both sugar contents in leaves and their composition in different forms, as a result of invertase activation. As we see in fig. 2, total contents of sugars became 2.4 times higher after chilling in B33-*inv* leaves and only 1.6 times higher in control plants. Monosaccharide content, that was equal in both transgenic plants at 22°C, rose more rapidly in B33-*inv* and became 30% higher than that in control plants. Fructose was not so abundant as glucose at 22°C (3769 mg/g fresh weight vs. 9187 mg/g fresh weight), but after chilling it raised more than ten-fold, while glucose level rose only 1.5 times. After chilling, sugar content also was higher in control plants, for example sucrose increased 1.6 times, glucose 1.2 times, and fructose 4.8 times, in comparison to unaffected plants. Thus, long influence of low positive temperature on leaves of potato plants transformed with yeast invertase gene caused a great activation of various invertase isoforms, and higher monosaccharide and sucrose contents in comparison to nonchilled plants.

We cannot exclude that rapid fructose elevation was correlated with its defensive function under stress conditions (chilling in our case), unlike glucose that is actively involved in metabolic processes. High sucrose concentration in the leaves after chilling is also due to its influx from nutrient medium, because the transformed plants were growing *in vitro* on nutrient medium with 2% sucrose.

We suppose that faster sugar accumulation of B33-*inv* plants under chilling would lead to higher cold tolerance of their cell membranes, in comparison to control plants. One of the main functions of soluble sugars accumulated under stress conditions, is their antidenaturating effect on dehydrated protein-lipid components of the membranes. The targets of unfavorable environmental conditions (chilling, for example) are the cell membranes that result in higher permeability, the phenomenon easily recorded by rapid release of soluble substances. Therefore, the higher cold tolerance is, the lower is membrane degradation after chilling and electrolyte release from leaf cells.

To study low temperature influence on the membrane integrity by electrolyte leakage we tried to record the appearance of membrane damage. Unlike freezing resistant wild type, capable to tolerate very low temperature without ice crystal formation, cultivated potato plants *Solanum tuberosum* L. are chilling-resistant (Seppanen et al., 1998). In chilling-resistant plants, temperature lowering to 0°C does not cause significant changes in ion permeability through the cell membranes and therefore in electrolyte release. We aimed at short-term effects of those low temperatures that cause no formation of ice crystals in plant cells but induce initial damage.

After the plants were chilled at –12°C for 10, 20 and 50 minutes, significant differences in cold resistance between treated and control plants were determined (Table 1).

Table 1
Cold tolerance (*H*, %) of potato plants under different temperature conditions

Temperature, °C and chilling longevity, min	Plant	
	control	B33-*inv*
22° C	57	59
−8° C; 30 min	64	63
−12° C; 20 min	27	40
−12° C; 30 min	16	59
−12° C; 50 min	11	13

Chilling resistance of B33-*inv* plants was 1.5 higher than in control plants, and significant difference was recorded after 10 and 20 minutes of chilling. Prolonged (50 min) chilling seemed to cause ice formation in the leaves and severe cell damage in all plants, showing equally higher electrolyte release. After that, we tried to lower chilling temperature to –14°C (20 min). Such combination was suitable to reveal the difference in chilling resistance of genotypes studied. Resistance of B33-*inv* plants was 2 times greater than resistance of control plants. It should be remembered, that our temperature test does not reflect absolute values of resistance, because it was used only to elucidate the difference between the transgenic plants, so we deal with relative resistance of plants. Experiments repeated many times confirmed that their membrane system (B33-*inv* plants, in the first place) was more resistant to low temperature in comparison to control plants.

Probably, xeromorphic ultrastructure of their cells played a positive role in chilling resistance. It was shown previously that B33-*inv* plants were characterized by lower number and smaller size of the structural elements of chloroplasts (starch grains; grana; thylakoids per granum; total number of thylakoids). This was in agreement with the lower growth rate and smaller leaf size in these plants, as compared to the plants transformed with the reporter gene encoding GUS. However, exposure of potato plants to hypothermia (6 days at a temperature of 5°C) caused the reduction of the membrane structures, which was more pronounced in B33-*inv* plants (Trunova et al., 2003). Hence, the mechanism of survival of cold-tolerant plants under low temperatures consists in the maintenance of the functional activity of their cells at the level corresponding to the temperature regime of the ambient medium. Because of the growth inhibition and lack of specific mechanism of enhancement of the resistance to

ice formation based on high level of cell organization, the cells of cold-tolerant plants require neither large amount of energy nor enhanced supply of photosynthetic products. Their strategy is to maintain the cell structure and functioning at a reliable level at low temperatures causing cold adaptation (Trunova et al., 2003). Our studies have shown that the membrane system in B33-*inv* plants was more resistant to low temperature than in control plants. The differences in resistance could be explained by stabilizing effect of sugars on the cell membranes, because B33-*inv* plants differ from control plants by both high invertase activity induced by expression of yeast invertase gene and high content of sugars.

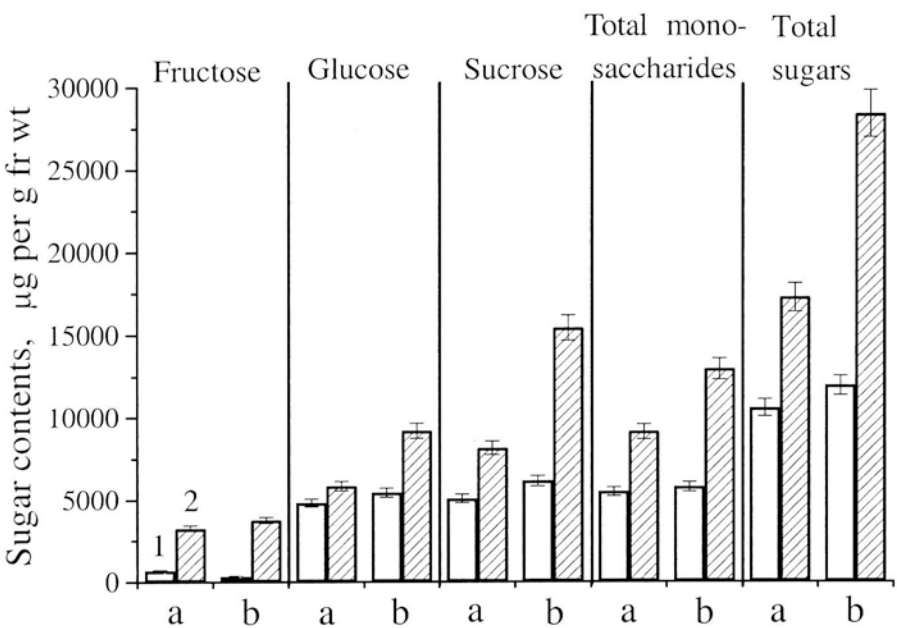

Fig. 2. Sugar contents in leaves of control plant (a) and B33-*inv* plants (b) under 22°C (1) and after 6 days of chilling at 5°C (2).

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Streszczenie

Badania przeprowadzono na roślinach ziemniaka (*Solanum tuberosum* L. cv. Désirée) transformowanych genem inwertazy drożdżowej pod kontrolą promotora patatyny B33 klasy I i inhibitora proteinazy II liderowej sekwencji peptydu umożliwiającej lokalizację apoplastycznego enzymu (rośliny B33-inv) oraz roślinach transformowanych genem kodującym ß-glukuronidazę pod kontrolą promotora 35S CaMV (rośliny kontrolne). Sześciodniowa ekspozycja na temperaturę 5°C powodowała wzrost aktywności inwertazy i zawartości cukru w liściach roślin B33-inv w porównaniu z roślinami kontrolnymi. Błony komórek roślin B33-inv wykazywały większą tolerancję na chłód w warunkach niskiej temperatury niż rośliny kontrolne o czym świadczy zarejestrowany wypływ elektrolitów. Prawdopodobnie wyższa tolerancja na chłód roślin B33-inv wynikała ze stabilizacyjnego wpływu cukru na błony, ponieważ rośliny B33-inv różniły się od roślin kontrolnych wyższą aktywnością inwertazy indukowaną ekspresją genu inwertazy drożdżowej i większą zawartością cukru.