Short communication

Transgene Coding of a Key Enzyme of the Glycolytic Pathway Helps to Decrease Sugar Content in Potato Tubers

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Abstract: Cold-stored potato tubers gradually accumulate reducing sugars. A proposed reason is a cold-induced blocking of glycolysis. The introduction and expression of the bacterial gene *Lbpfk* coding for cold-tolerant phosphofructokinase might counteract this effect. We have recently introduced this gene into several Czech potato cultivars. The obtained transgenic lines were then tested for three years in field trials. In 17 transgenic lines derived from two of the cultivars we have investigated the accumulation of reducing sugars during two and four months of cold storage. Although in all transgenic lines the sugar content still increased between the 2nd and the 4th month of cold-storage, the level of reducing sugars was in all transgenic lines after both two and four months of cold storage considerably lower than in the original cultivars. The extent of sugar accumulation was also influenced by the parental genotype. No significant differences in sugar accumulation were observed between the transgenic lines from the same parent.

Keywords: *Lactobacillus bulgaricus*; low temperature sweetening; phosphofructokinase; transgenic potato

Potato tubers are processed all the year round also due to efficient cold storage. However, cold storage is accompanied by acceleration of the conversion of starch to reducing sugars (glucose and fructose), the phenomenon known as low temperature sweetening (LTS). The sugar content can exceed 2% of the tuber fresh weight (Isherwood 1973). The acceptable sugar content in tubers of so-called chipping cultivars should not exceed 0.33% of the fresh weight (Duplessis *et al.* 1996). At least five pathways of carbohydrate metabolism could contribute to LTS, i.e. starch synthesis, starch breakdown, glycolysis, hexogenesis and respiration. We decided to solve this problem by an introduction of cold-stable 6-phosphofructokinase from the bacterium *Lactobacillus bulgaricus* (Navrátil *et al.* 2007).

Recently we have introduced the *Lbpfk* gene, the sequence of which was modified to improve its translation, into several Czech potato cultivars (Navrátil *et al.* 2007). The transgenic lines were confirmed by PCR analysis. Here we present the observed sugar accumulation in cold stored tubers obtained from field trials with transgenic lines, derived from the cultivars Vladan and C70/2 (breeder Selekta Pacov, Czech Republic). The cultivars and the corresponding transgenic lines were grown for three years in field trials at Velhartice (altitude 580 m, total yearly precipitation 725 mm, brown soil), with 15 tubers planted from each line. The

harvested tubers were stored in a cold chamber at a constant temperature of +4°C and one part was subjected to reconditioning before the sugar content analysis.

The biochemical analysis of the sugar content was performed in tubers before their storage and then in cold-stored tubers immediately after sampling and also after additional reconditioning. The samples were taken after two and four months of storage. Ten tubers of each sample were used to prepare a homogenate (as well as the chips). Stabilized and filtered samples prepared from 30 g of the homogenate were stored at –80°C until analysed by HPLC using the column WATREX IEX Ca^{2+} form (Watrex, Praha, Czech Republic) and an RID Shodex 101 refractometric detector (Schowa Denko K.K., Kawasaki, Japan). The amount of two reducing sugars (glucose, fructose) was calculated as the percentage of the tuber fresh weight. The frying colour of the chips fried at a temperature of +175°C (20 per each sample) was measured using a Tristimulus D25L/DP9000 colorimeter (Hunter Associates Laboratory, Reston, USA). Each sample was measured five times.

The agronomic characteristics were evaluated for all transgenic lines throughout field trials. The appearance of plants and the yield of tubers were decisive for further biochemical analyses of tuber samples. Most of the transgenic plants had the same appearance as their nontransgenic counterparts and in some cases the yield was lowered (to 84% and 92% of the control depending on the year). The reducing sugar content (RSC) in tubers varied in seasons due to different climatic conditions, being the highest in 2008 already before storage. Next year the values were low again and the difference was found more profound in the cultivar C70/2 than in the cultivar Vladan. As expected, the sugar accumulation during cold storage in 2009 was lower among Vladan transgenic lines (after 4 months on average 0.27% FW). In the same season the C70/2 transgenic lines accumulated reducing sugars in cold stored tubers to a higher extent (after 4 months on average 0.79% FW). The average values from three seasons (Figures 1 and 2) demonstrate that the transgenic Vladan lines exhibited a greater reduction in RSC than the C70/2 lines in comparison with their respective nontransgenic controls. Nevertheless, the lines with low RSC in cold stored tubers are present in both sets of transgenic lines (e.g. V96-26, C70/2-20; both with significantly different RSC after 4 months of cold-storage – see Figures 1 and 2) and moreover such lines show more stable values throughout the seasons. Statistical significance of relative reducing sugar concentrations were calculated using one-paired one-sided Student's *t*-test at the 5% significance level. We estimated the copy number of the transgene among the C70/2 lines using RT-

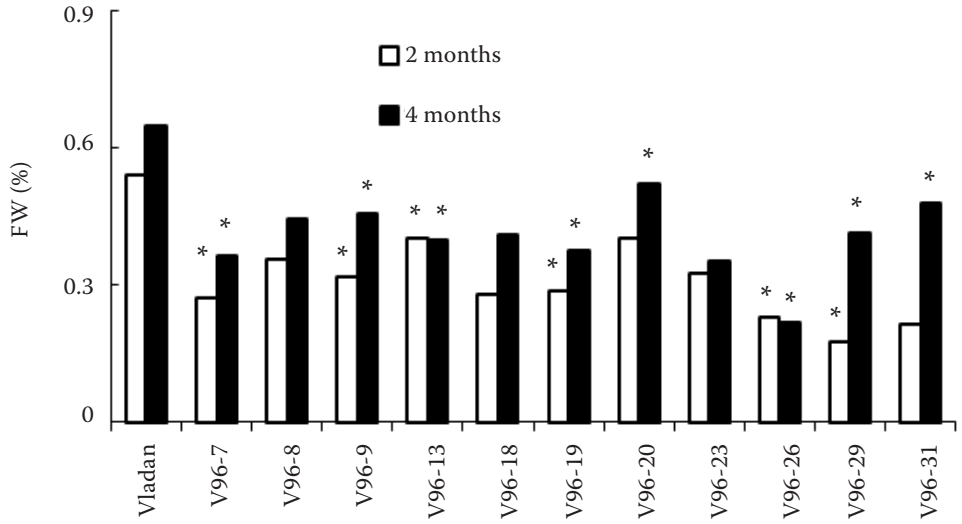

Figure 1. The reducing sugar content in Vladan transgenic lines; the analysed tubers came from seasons 2007 to 2009; the tubers cold-stored for 2 and 4 months were reconditioned before the sample preparation; the figure represents the pooled data on reducing sugar content as mean from three seasons; the values significantly different from the parental cultivar ($P < 5\%$) are marked with an asterisk; the *x*-axis shows the transgenic lines (together with the original cultivar) while the *y*-axis shows the reducing sugar values as a percentage of the tuber fresh weight

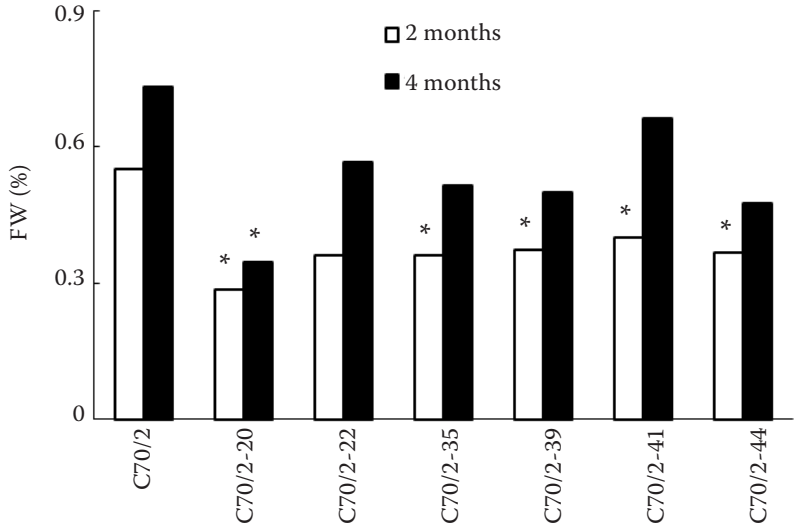

Figure 2. The reducing sugar content in C70/2 transgenic lines; the tubers cold-stored for 2 and 4 months were reconditioned before the sample preparation; the figure represents the pooled data on reducing sugar content as mean from three seasons; the values significantly different from the parental cultivar ($P < 5\%$) are marked with an asterisk; the *x*-axis shows the transgenic lines (together with the original cultivar) while the *y*-axis shows the reducing sugar values as a percentage of the tuber fresh weight

PCR (found from one to six copies per genome, data not shown). The lines having the high copy number (C70/2-20, 39, both having 5 copies per genome) accumulate reducing sugars in tubers to a lesser extent than the lines with one transgene per genome (C70/2-22, 44) (Figure 2). The frying colour of the chips did not improve among transgenic lines substantially (data not shown). Better values were obtained with the Vladan transgenic lines. A substantial improvement in frying colour for all transgenic lines was detected in tubers before storage (e.g. –1.82 for V96-19 chips in 2009, cf. 5.79 for Vladan chips). The transgenic line V96-19 showed the best improvement in frying colour in all three seasons even after the cold storage of tubers.

Previous experiments with two Czech potato varieties Kamyk and Korela and with the original unmodified bacterial gene showed an influence of the transgene on the reducing sugar content in cold-stored tubers (Navrátil *et al.* 2007). For subsequent experiments we modified the transgene in such a way that the gene translation into active protein would be supported. A three-year evaluation of the transgenic lines revealed the influence of the transgene on the reducing sugar content. The effect of the transgene was greater in the cultivar Vladan as compared to hybrid C70/2, although the latter accumulates sugars in harvested tubers to a lesser extent and the bacterial phosphofructokinase could decrease sugars to still lower levels. The better performance of the transgene in Vladan plants probably accounts for differences in the genetic background. A similar effect of the genotype on the transgene expression was observed for the expression of siRNA of vacuolar invertase among four different potato cultivars used (Wu *et al.* 2011). The effect of LbPFK was found not to be very strong and the reason was also discussed by Morandini (2009). He expected that the phosphorus insufficiency in potato tubers as well as the hypoxia of tubers would lead to the shortage of adenosine triphosphate. Both chemicals are needed in a chemical reaction performed by LbPFK. Although Morandini (2009) expected the saturation of the glycolytic pathway, the transgenic experiments with three other glycolytic enzymes show that at least in yeast cells it is not the case and it can be influenced by an additional transgene introduced into a wild type (Wang *et al.* 2011). Nocarova *et al.* (2010) measured the expression and the stability of two transgenes in potato plants during five-year vegetative propagation and found a decrease in the activity of the transgene in some plants (in four plants out of seventeen). In our experiments the transgenic lines were grown for more than five years without any obvious effect on phenotype. The yield of transgenic lines and the general resistance to potato pests did not differ substantially. Although the genetic background of the cold sweetening remains unclear, the activity

of vacuolar acid invertase seems to contribute to this phenomenon to a large extent (Wu *et al.* 2011). The positive results were obtained in pot plants grown just for one season. It is necessary to verify the contribution of the gene silencing in field trial experiments. The suppression of the enzyme activity may have some undesirable effects on other crop characteristics. Roessner *et al.* (2002) showed that the values of different biochemical markers for transgenic and wild type soil-grown tubers formed independent clusters, while the values of the same plants grown in vitro formed clusters very close to each other. In that respect our data from field trials are valuable showing the real potential of transgenic lines to decrease the content of reducing sugars during the cold-storage of potato tubers.

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References

Duplessis P.M., Marangoni A.G., Yada R.Y. (1996): A mechanism for low temperature induced sugar accumulation in stored potato tubers: The potential role of the alternative pathway and invertase. American Journal of Potato Research, **73**: 483–494.

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

Morandini P. (2009): Rethinking metabolic control. Plant Science, **176**: 441–451.

Navrátil O., Fischer L., Čmejlová J., Linhart M., Vacek J. (2007): Decreased amount of reducing sugars in transgenic potato tubers and its influence on yield characteristics. Biologia Plantarum, **51**: 56–60.

Nocarova E., Opatrny Z., Fischer L. (2010): Successive silencing of tandem reporter genes in potato (*Solanum tuberosum*) over 5 years of vegetative propagation. Annals of Botany, **106**: 565–572.

Roessner U., Willmitzer L., Fernie A.R. (2002): Metabolic profiling and biochemical phenotyping of plant systems. Plant Cell Reports, **21**: 189–196.

Wang S.X., Spor A., Nidelet T., Montalent P., Dillmann C., deVienne D., Sicard D. (2011): Switch between life history strategies due to changes in glycolytic enzyme gene dosage in *Saccharomyces cerevisiae*. Applied and Environmental Microbiology, **77**: 452–459.

Wu L., Bhaskar P.B., Zhang R., Bethke P.C., Busse J.S., Jiang J. (2011): Developing cold-chipping potato varieties by silencing the vacuolar invertase gene. Crop Science, **51**: 981–990.

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