Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S1-06

Volume 46(3-4):15-16, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Altered fructose-2,6-bisphosphatase levels cause phenotypic changes and shift development in plants

Ottó Toldi[1*], Gabriella Kovács[1], Erzsébet Kiss[2], Seppo Sorvari[3], Peter Scott[4]

[1]Agricultural Biotechnology Center, Gödöllő, Hungary, [2]Department of Genetics and Plant Breeding, Saint Stephanus University, Gödöllő, Hungary, [3]Horticulture MTT, Piikkiö, Finland, [4]School of Biological Sciences, University of Sussex, Brighton, UK

Fructose-2,6-bisphosphate (F2,6P2) is an important intracellular signal metabolite in the control of carbohydrate metabolic fluxes in eukaryotes. Although the specific mechanism of F2,6P2 action varies between species and between tissues, most involve the allosteric activation of P_i-dependent fructose-6-phosphate kinase (PFP) and inhibition of cytosolic fructose-1,6-bisphosphatase (FBPase). These highly conserved enzymes regulate the fructose-6-phosphate / fructose-1,6-bisphosphate cycle, and thereby, determine the carbon flux. In animals and humans the hepatic F2,6P2, synthetised and degraded by the liver 6-phosphofructo-2-kinase / fructose-2,6-bisphosphatase bifunctional enzyme (6PF2K/F2,6P2ase), plays critical role in the control of fuel homeostasis by regulating the hepatic glucose output. The presence of F2,6P2 has also been found essential in tissues possessing a continuously high rate of glycolysis such as placenta, brain, tumour cells, skeletal and heart muscles (Okar and Lange 1999).

Similarly to fungal and animal systems, the kinase and bisphosphatase activities are also present on a bifunctional enzyme in higher plants in the majority of cases, but some plants contain a separate monofunctional fructose-2,6-bisphosphatase (Larondelle et al. 1986). In plant systems, F2,6P2 activates the cytosolic P_i-dependent fructose-6-phosphate kinase (PFP) and the plastidic ADP-glucose pyrophosphorylase (AGPase) as well, which is an important regulatory enzyme of starch synthesis. At the same time, F2,6P2 inhibits the cytosolic fructose-1,6-phosphatase, which catalyses an irreversible regulatory step of sucrose synthesis in photosynthetically active tissues. Theoretically it means that by down regulation of the endogenous F2,6P2 level sucrose flux can be elevated, while synthesis of primary starch become enhanced by its up regulation. The introduction of two modified forms of the rat liver-originated bifunctional enzyme 6PF2K/F2,6P2ase to the C3 plant tobacco (Scott et al. 1995) and to the CAM plant *Kalanchöe* (Truesdale et al. 1999) resulted in dramatic changes in the carbon flux without resulting in alterations in the phenotypes. Therefore, we decided to produce transgenic plants (strawberry, carrot and carnation) with altered F2,6P2 levels that are known to be developmentally sensitive to the elevated or decreased amounts of the available sucrose. We hipothetized that the detection of phenotypic changes and developmental differences can be realistic applying a highly standardized sampling procedure.

*Corresponding author. E-mail: toldi@abc.hu

Materials and Methods

Strawberry and carrot plants were tissue cultured and transformed according to Dr. Seppo Sorvari's unpublished laboratory protocol (Horticulture MTT, FIN-21500 Piikkiö, Toivonlinnantie 518., Finland). Carnation was tissue cultured and transformed following the protocol published by Kiss et al. (2000). Metabolite concentrations and enzyme activity assays were performed as described by Scott et al. (1995), Fernie et al. (2001) and Theodoru and Kruger (2001).

Results and Discussion

The redistribution of radioactivity into various photosynthetic end products was used to estimate metabolic fluxes (Table 1).

Increasing or decreasing the level of F2,6P2 had no significant effect on the net rate of CO_2 fixation, but dramatically altered the partitioning of fixed carbon. The proportion of total metabolised ^{14}C entering starch increased by 15-28% when the level of F2,6P2 was increased by 35-86% in transgenic plant line P-86. At the same time, our results provide direct evidence that a decrease in F2,6P2 promotes the partitioning of photoassimilate into sucrose relative to starch. In leaf disks the percentage of $^{14}CO_2$ metabolised to sucrose is significantly greater in transgenic plants (F-23) than that of the untransformed control lines. These transgenic plants produced 60 clones on 11 runners on average, while wild type plants developed 11 clones on 4 runners during their life history. The key factor in this striking difference was the "leaking" of the sucrose flux in the "bisphosphatase" construct expressing plants (F-23) through the vigorous and early development of runner-clone systems. We suppose that this made it possible that the accumulation of the extra sucrose could not down regulate the photosynthetic activity.

Data of Table 2 proved our assumption that differences between the transgenic and wild type plants in the sugar metabolite profiles (Table 1) were resulted in by the knowingly engineered sugar metabolism. The increased activity of the key enzymes of sucrose synthesis (FBPase, SPS) caused an enlargement of the cytosolic sucrose pool and an elevated sucrose transport in the "bisphosphatase" construct

Table 1. Leaves from different srawberry plants were illuminated for 1 h at 200 μmol s^{-} m^{2-}. Leaf disks were isolated and illuminated at the same light intensity in an oxygen electrode in the presence of 1 M $NaH^{14}CO_3$ (specific radioactivity 100 Ci mol^{-}). After 20 min. the leaf disk was extracted and the distribution of radioactivity between different components was determined. Abbreviations: wt – wild-type control plants; P-86 – transgenic strawberry line containing the "kinase" construct; F-23 – transgenic strawberry line containing the 'bisphosphatase' construct.

Carbon partitioning in strawberry leaf discs		Plant lines		
		Wild type	P-86	F-23
$^{14}CO_2$ assimilation [μCi min^{-1} (mg chlorophyll) $^{-1}$]		0.80±0.03	0.81±0.07	0.80±0.02
F2,6P2 concentration (pmol g^{-1})		31.2±3.1	57.9±10.1	17.2±2.8
Metabolised ^{14}C (%) recovered in:				
	Starch	60.2±2.1	77.0±5.6	28.2±0.3
	Sucrose	16.1±1.0	9.0±1.0	28.0±0.6
	Amino acids	16.2±2.2	9.2±1.4	28.1±0.9
	Organic acids	7.2±0.3	4.9±0.8	15.7±0.5

Table 2. Monitoring of the photosynthetic carbohydrate flux through the detection of the activities of those enzymes that are involved in the sucrose and starch synthesis or break down. Abbreviations: P-86 – transgenic strawberry line containing the 'kinase' construct; F-23 – transgenic strawberry line containing the 'bisphosphatase' construct; FBPase - fructose-1,6-bisphosphatase; SPS – sucrose phosphate synthase; AGPase – ADP-glucose pyrophosphorilase; SPase – starch phosphorilase; PFP – pyrophosphate: fructose-6-phosphate-1-phosphotransferase; 6PF1K – 6-phosphofructo-1-kinase

Plant lines	Enzyme activity (μmol.min^{-1}.g^{-1} FW)					
	FBPase	SPS	AGPase	SPase	PFP	6PF1K
Wild type	1.12±0.12	7.13±1.51	7.21±2.43	1.04±0.09	0.167±0.018	0.090±0.027
P-86	0.76±0.16	6.71±0.53	8.88±2.72	0.27±0.04	0.140±0.010	0.082±0.012
F-23	4.79±1.30	6.97±1.32	7.01±2.51	1.10±0.07	0.194±0.012	0.090±0.013

expressing plants (F-23). At the same time, the increased starch accumulation in the "kinase" construct expressing plants (P-86) was resulted in either by the higher activity of ADP-glucose pyrophosphorilase (AGPase) or by the lower activity of the starch degrading starch phosphorilase (SPase). On the basis of the increased PFP activity, an elevated carbohydrate flux through the glycolysis was also detected in the sucrose oversynthetising plants (F-23) showing that a part of the extra sucrose is directly utilized for ATP synthesis. This also means that the carbon (fixed in the Calvin cycle) has been recycled rapidly. It may explain why the concentrations of other carbon containing organic molecules (amino acids, organic acids) were also higher in this latter transgenic plant material (Table 1).

The other possible reason for the higher amino acid content of the sucrose oversynthetising plants can be that the extra sucrose means extra energy for such energy demanding transport processes as the amino acid import.

Acknowledgements

This project was supported by a Royal Society (London, UK) Research Grant Award to PS, an Eötvös State Grant Award (Budapest, Hungary), a Széchenyi Grant Award (Budapest, Hungary) and a NATO Research Fellowship Award to OT.

References

Fernie AR, Rocher A, Ratcliffe G and Kruger NJ (2001) Fructose-2,6-bisphosphate activates pyrophosphate: fructose-6-phosphate-1-phosphotransferase and increases triose phosphate to hexose phosphate cycling in heterotrophic cells. Planta 212:250-263.

Kiss E, Veres A, Galli Zs, Nagy N, Tóth E, Varga Á, Hrazdina G and Heszky L (2000) Production of transgenic carnation with antisense ACS (1-aminocyclopropane-1-carboxylate synthase) gene. Int J Hort Sci 6(4):103-107.

Larondelle Y, Mertens E, Vanschaftingen E and Hers HG (1986) Purification of spinach leaf 6-phosphofructo-2-kinase / fructose-2,6-bisphosphatase. Eur J Biochem 161:351-357.

Okar DA and Lange AJ (1999) Fructose-2,6-bisphosphate and control of carbohydrate metabolism in eukaryotes. Biofactors 10:1-14.

Scott P, Lange AJ, Pilkis SJ and Kruger NJ (1995) Carbon metabolism in leaves of transgenic tobacco (*Nicotiana tabacum* L.) containing elevated fructose 2,6-bisphosphate levels. Plant J 7:461-469.

Theodorou ME and Kruger NJ (2001) Physiological relevance of fructose-2,6-bisphosphate in the regulation of spinach leaf pyrophosphate: fructose-6-phosphate-1-phosphotransferase. Planta 213:147-157.

Truesdale M, Toldi O and Scott P (1999) The effect of elevated concentrations of fructose 2,6-bisphosphate on carbon metabolism during deacidification in the crassulacean acid metabolism plant *Kalanchöe daigremontiana*. Plant Phys 121:957-964.