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*Review*

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# Modification of Carbohydrate Metabolism in Transgenic Potato

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## Abstract

**Carbohydrates serve three different principal functions in the metabolism of plants. They are the primary products of energy fixation, they are important transport metabolites, and they are deposited as structural or storage compounds. Modification of carbohydrate metabolism therefore covers approaches to modify yield, to change sink/source relationships and thereby alter the ratio of harvestable material, and to improve the quality of crop plants. The scope of this article is to summarize research done at the Max-Planck-Institute related to the first two fields and to present in some detail what we learned, when we established a new carbohydrate storage form in potato.**

## Introduction

Limitations of plant production, if not imposed by abiotic factors, could arise at three different metabolic levels: (1) Under certain conditions, for example at elevated $CO_2$ concentrations or low temperatures, down-regulation of photosynthesis in the sink organs, which is the green leaves and stem, occurs due to feed back inhibition.

(2) The role of transport of photosynthates in this process is up to now not completely understood, but there is evidence that the sucrose transporter plays an important role in restricting delivery of assimilates to the sink organs. (3) There is debate on whether demand for carbohydrates in the storage and consuming organs can influence production, and the discussion focuses on aspects of sucrose unloading from the vascular system, sucrose metabolism in sink organs, and production of storage carbohydrates like starch.

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## Source capacity

Concerning regulation of photosynthesis, we have to ask which are the rate limiting steps in photosynthate production and what are the key enzymes to be regulated.

Of further importance is whether there are metabolites that regulate the activity of enzymes.

During photosynthesis, photosynthates are exported from the chloroplast as triose phosphates that are condensed to hexoses and ultimately converted to sucrose (Figure 1). A central step is the dephosphorylation of Fructose-bisphosphate to Fructose-6-Phosphate (F6P). Three enzymes are involved in the production of F6P, one of them being Fructose-Bisphosphatase. This key enzyme is regulated by F-2,6-PP (Stitt et al., 1987), which is formed when F6P concentrations exceed a threshold concentration. Overexpression of the enzyme is therefore not likely to result in an increased enzyme activity.

Phospho-fructo-phosphatease catalyzes the reversible formation of F6P, and the direction towards or from it is defined by the phosphate-to-pyrophosphate ratio. But attempts to force the reaction in the direction of sucrose synthesis by cleaving pyrophosphate into inorganic phosphate led to severe growth retardation instead of improved production, because pyrophosphate is needed in phloem companion cells in reactions that fuel sucrose export from the leaf (Geigenberger et al., 1998).

F6P is consumed for sucrose formation in a reaction catalyzed by Sucrose-Phosphate-Synthase (SPS), which is the second key enzyme in the play. SPS is heavily regulated with respect to substrate affinity and turn-over rate. Phosphorylation of the enzyme

at Ser 158 can drastically reduce its affinity to UDP-Glc. Consequently, a simple overexpression of the enzyme does not lead to increased activity. It has, however, been demonstrated that expression of a mutant enzyme that cannot be phosphorylated can override the inactivation (Toroser et al., 1999).

## Transport

The fact that sucrose accumulation in the mesophyll cell occurs at saturation of photosynthesis argues that not the source capacity but the removal of photosynthates from the sources restricts assimilate delivery to the storage organs.

In the literature, two possible sites of active transport are demonstrated: at the entry of the phloem companion cells (CC), which surround the sieve elements (SE), and between companion cells and sieve elements, The CC-specific one has been demonstrated for *Arabidopsis thaliana* (Stadler and Sauer, 1996), the other one for potato (Kuehn et al., 1997). The reason for this discrepancy is unknown.

Both transporters are working as proton/sucrose-symporters, which means that they rely on a proton gradient between apoplast and cytosol of CC and SE, respectively. The proton pump is possibly located at the CC and consumes ATP for every proton that is pumped out. Irrespective of where the transporter is located, all sucrose must pass the apoplast to be loaded into the phloem.

That this step is essential could be demonstrated with transgenic plants expressing an invertase in the apoplast of leaf tissue. The sucrose is cleaved into

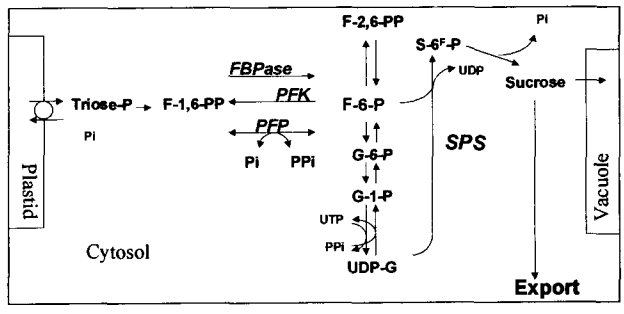


**Figure 1.** Regulation of carbohydrate metabolism in mesophyll cells (source tissue). Metabolites: F6P: fructose-6-phosphate; F-1,6-PP: fructose-1,6-bisphosphate; F-2,6-PP: fructose-2,6-bisphosphate; G1P: glucose-1-phosphate; G6P: glucose-6-phosphate; P: phosphate; Pi: inorganic phosphate; PPi: pyrophosphate; S: sucrose; S-6-P: surose-6-phosphate; Triose-P: triose-phosphates; UDP: uridine-diphosphate; UTP: uridine-triphosphate; UDP-G: UDP-glucose; Enzymes: FBPase: fructose-bisphosphatase; PFK: phosphofructo-kinase; PFP: fructose-6-phosphate 1-phosphotransferase; SPS: surosephosphate-synthase.

**Table 1.** Maximal photosynthetic rate of potato plants in mM $O_2/m^2 \times h \pm$ standard deviation. Control: untransformed wild type (Var. Désirée). aSp13, 43, 5, 34: different transgenic plant lines expressing an antisense RNA to the sucrose transporter transcript.

| | Max Photosynthetic Rate |
|---|---|
| Control | 74±7,2 |
| ASp13 | 71±7 |
| ASp43 | 54±7,5 |
| ASp5 | 46±7,1 |
| ASp34 | 38±12,7 |

hexoses and cannot be transported (Bussis et al., 1997). As a consequence, soluble sugars increase dramatically in the leafs and cause osmotic damage of the tissue.

An indication for the questions whether transport is limiting, comes from a different experiment: Antisense inhibition of the sucrose transporter leads to a similar tailback of sugars as the invertase does and also causes a reduction in assimilation rate (Riesmeier et al., 1994). The proportionality of transporter inhibition and reduction of assimilation strongly argues for the hypothesis that transport is already limiting under normal conditions (Table 1). The prove for this hypothesis would be an increased transport rate of plants overexpressing the transporter. Unfortunately up to now nobody succeeded in functional overexpression of this transporter, and therefore this questions remains unanswered.

### *Sink capacity*

Sink organs are defined as net importers of sucrose (Herbers and Sonnewald, 1998), which is under most conditions cleaved by sucrose synthase (SuSy) into UDP-Glucose (UDP-Glc) and fructose. This reactions conserves the energy of the sucrose glycosidic bond and is therefore reversible. UDP-Glc as well as fructose are ultimately converted to G6P, which is imported into the amyloplast, where it is used for starch synthesis (Figure 2).

If starch synthesis is prevented by inhibition of the key-enzyme ADP-Glucose Pyrophosphorylase, soluble sugars increase and the storage capacity of the organ is drastically reduced (Muller-Rober et al., 1992). As a consequence, many very small tubers with an all together reduced dry weight are produced.

To answer the question, whether sucrose breakdown as entry point for sink metabolism is limiting, a sucrose cleaving activity additional to sucrose synthase, an invertase from bakers yeast, was in-

troduced. This was planned to reduce sucrose concentration in the tubers, thereby attracting more assimilates and increase synthesis of storage carbohydrates. The reduction of sucrose concentration was indeed achieved (Table 2), but starch was reduced and high levels of glucose accumulated (Sonnewald et al., 1997). A possible explanation is the relatively low activity of glucokinase, which would convert glucose to G6P. To overcome this limitation, a glucokinase from *Zymomonas mobilis* was introduced into the invertase-plants (Trethewey et al., 1998). But instead of channeling the carbohydrate to starch synthesis, it went into glycolysis and was lost (Table 3) (Trethewey et al., 1999). The starch content was even further reduced in the glucokinase-plants as compared to the invertase-plants (Table 4).

Interestingly, a completely different result is obtained, when the invertase is located not in the cytosol of potato tubers, but in the apoplast (Sonnewald et al., 1997). Such a configuration leads to a partial hydrolysis of sucrose in the apoplast, and because the hexoses are not imported into the cell, there is an elevated hexose level in the tuber. It is not yet clear whether glucose or fructose might act as a signal, or if increased hexose levels in the apoplast reduce the osmotic gradient between cytoplasm and apoplast, thereby facilitating mass transport to the tuber. The consequence of apoplastic invertase is a strong increase in tuber size and a reduction of tuber number (Table 5). The total tuber yield is not increased, but the ability of a single tuber to attract assimilates is strongly increased.

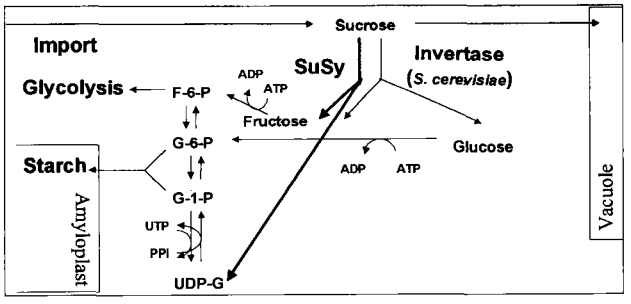


**Figure 2.** Regulation of carbohydrate metabolism in potato tubers (sink tissue). Metabolites: ADP: adenosine-diphosphate; ATP: adenosine-triphosphate; F6P: fructose-6-phosphate; G1P: glucose-1-phosphate; G6P: glucose-6-phosphate; P: phosphate; Pi: inorganic phosphate; PPi: pyrophosphate; UDP: uridine-diphosphate; UTP: uridine-triphosphate; UDP-G: UDP-glucose. Enzymes: SuSy: surose-synthase

**Table 2.** Soluble sugars in potato tubers in $\mu$M/g fresh weight $\pm$ standard deviation. Control: untransformed wild type (Var. Désirée). U-In2-34: plant No. 34 expressing cytosolic yeast invertase. U-In2-17: plant No. 17 expressing cytosolic invertase.

| | Sucrose | Glucose | Fructose |
|---|---|---|---|
| Control | 15.6±0.7 | 2.1±0.4 | 1.1±0.1 |
| U-In2-34 | 1.4±0.3 | 24.2±0.9 | 0.5±0.2 |
| U-In2-17 | 0.6±0.1 | 40.6±3.4 | 0.8±0.1 |

**Table 3.** Release of $CO_2$ from potato tubers in nM C/g fresh weight. Control: untransformed wild type (Var. Désirée). Inv: plant expressing cytosolic yeast invertase. GK-41, 29, 38: cytosolic invertase plants expressing glucokinase from *Zymomonas mobilis*.

| | $CO_2$ Production |
|---|---|
| Control | 18 |
| Inv | 58 |
| GK-41 | 71 |
| GK-29 | 79 |
| GK-38 | 81 |

**Table 4.** Density in g/cm$^3$ of potato tubers as direct measure of starch content. Control: untransformed wild type (Var. Désirée). Inv: plant expressing cytosolic yeast invertase. GK-41, 29, 38: cytosolic invertase plants expressing glucokinase from *Zymomonas mobilis*.

| | Density |
|---|---|
| Control | 1,082 |
| Inv | 1,074 |
| GK-41 | 1,066 |
| GK-29 | 1,061 |
| GK-38 | 1,057 |

**Table. 5** Mean fresh weight per tuber in g and total tuber yield per plant in kg $\pm$ standard deviation of potato expressing apaplastic yeast invertase. Control: untransformed wild type (Var. Désirée). U-In1 3, 41, 33: different transgenic plant lines expressing yeast invertase targeted to the apoplast.

| | Mean fresh weight/tuber (g) | Total yield per plant (kg) |
|---|---|---|
| Control | 143 | 1.21±0.08 |
| U-In1-3 | 192 | 1.30±0.10 |
| U-In1-41 | 179 | 0.91±0.07 |
| U-In1-33 | 209 | 1.23±0.09 |

*Altering carbohydrate composition: starch*

In contrast to approaches towards increasing yield, manipulation of carbohydrate metabolism has proven very successful to alter quality traits. Two projects are described here. The one is changing starch composition of potato, and the other is establishing fructan metabolism in potato.

Starch metabolism is the concerted action of more than a dozen of enzymes, the function of which is not yet fully understood. For example, starch phosphorylases have been implicated as starch degrading enzymes in the text books, but evidence accumulates that they are also involved in build-up of starch granules in potato (Duwenig et al., 1996). To investigate the function of all enzymes, many transgenic lines have been produced, which are reduced in the activity of one or more enzymes (Heyer et al., 1999; Kossmann et al., 1995). Most of the starches that resulted had properties different from wild-type starch. In a comparison of starch gels obtained from transgenic phosphorylase antisense plants a strongly increased turbidity is indicating that not only starch degradation is affected in these plants.

For a long time it is known that GBSSI is essential for synthesis of amylose. Inhibition of this enzyme results in a pure amylopectin starch. On the other hand, inhibition of branching enzyme 1 (BE1) and the so-called R1 enzyme, the function of which is not known yet, leads to high amylose starch (Lorberth et al., 1996; Lorberth et al., 1998). Interestingly, inhibition of BE1 alone does not influence the amylose-to-amylopectin ratio, indicating that at least one other BE must exist that introduces the branch points in amylopectin starch.

Because phosphate groups are a constituent of the amylopectin fraction of starch, the phosphate content should be inversely related to the amylose content, which holds true for most transgenic starches investigated so far. But the antisense-branching enzyme and antisense-R1 starches are exceptions to the rule: antisense R1 plants have a starch with a very low phosphate content despite the amylose/amylopectin ratio is unchanged. The very low phosphate level of the R1 starch indicates the function of this enzyme: it is directly involved in phosphorylation of starch. On the contrary, inhibition of BE1 strongly increases phosphate content, indicating that this enzyme could be responsible for dephosphorylation of starch. An interesting hypothesis is that this enzyme exchanges phosphate residues at the C-6 of glucose molecules against glucan chains, thereby introducing branch points instead of phosphate.

*Altering carbohydrate composition: fructan*

Oriented at improving food quality is the production of fructans in transgenic plants.

Fructans are polymers of fructose molecules, which are synthesized from sucrose as substrate. With respect to the nutritional value of potato fructans seem very interesting, because they have a positive effect on gut microflora by stimulating the growth of bifidobacteria in the colon, which reduces the production of tumor-promoting substances like ammonia and has therefore an antitumoral effect (Roberfroid and Delzenne, 1998). Besides, fructans are interesting low calorie fibers, because the -linkage of the fructose moieties cannot be cleaved by human enzymes. Bacterial fermentation and resorption of fermentation products yields an energy value of 1 kcal/g, which is about 30% of that for the free hexoses . The texture of the fiber gives a fat-like mouth feeling and therefore fructans are excellent bulking agents for low calorie foods.

Fructan synthesis is widespread in evolutionary terms: it occurs among bacteria and plants and there are also some reports of fungal fructan production.

Fructan synthesis in plants is depending on at least two enzymes, one of them producing the trisaccharide kestose, the other being a transfructosylase that uses fructans as donor and acceptor of fructosyl residues (Vijn and Smeekens, 1999).

We chose artichoke as the source for the fructosyl transferase genes, because artichoke produces the largest inulin known among the plant kingdom. The mean chain length might influence fructan yield in transgenic plants, because longer chains cause a lower osmotic load on storage organs, as fructans are in contrast to starch water soluble carbohydrates.

Transformation of Potato with the SST and FFT genes of artichoke was performed in two steps. Plants expressing the 1-SST gene only under the control of the constitutive 35S promoter of the cauliflower mosaic virus (CaMV) accumulated oligofructans in leaves and tubers (Hellwege et al., 1997). By high performance liquid chromatography (HPLC) we could show synthesis of the trisaccharide 1-kestose ($GF_2$), the tetrasaccharide 1,1-nystose ($GF_3$) and the pentasaccharide ($GF_4$) in transgenic plants, but also higher inulin homologs up to $GF_6$ could be detected in some lines. The production of molecules of a higher degree of polymerization (DP) than 3 had already been reported for purified SST, but a DP of more than 5 was never observed *in vitro*.

Whether the capability of SST to produce higher DP oligomers is of physiological relevance or just a lack of substrate specificity has yet to be demonstrated, but it seems very interesting, con-

**Table 6.** Soluble sugars in untranformed potato (Control) and a transgenic plant expressing artichoke SST and FFT (SST/FFT) in $\mu$M/g fresh weight $\pm$ standard deviation. Starch and fructan content is expressed in $\mu$M hexose equivalent / g fresh weight. Glc: glucose; Frc: fructose; Suc: sucrose; n.d.: not detectable.

| $\mu$M/ g FW | Control | SST/FFT |
|---|---|---|
| Glc | 3,73±4,4 | 7,99±5,65 |
| Frc | 0,36±0,16 | 0,4±0,2 |
| Suc | 34,2±6,5 | 28,1±6,2 |
| Starch | 836±167 | 651±110 |
| Fructan | n.d. | 41,9±7,09 |

sidering that inulin oligomers are often discussed as relevant in responses of plants to abiotic stress like cold and drought (Livingston III, 1996; Livingston III and Henson, 1998).

Production of oligo-fructans had no measurable influence on soluble sugars in leaves: no differences in glucose, fructose and sucrose content could be observed between wild type and transgenic plants. The relatively low concentration of fructan in leaves might explain this finding. Due to a high activity of invertases in leaf vacuoles, the sucrose concentration is low in this compartment, and, furthermore, the primary product 1-kestose is like sucrose subject to hydrolysis by invertases.

A different picture was obtained for tubers of the transgenic lines (Hellwege et al., 1998). In this tissue the 1-kestose was the most abundant oligosaccharide (Table 6), but again sucrose concentration was not significantly altered in the transgenic lines. In contrast to leaves, transgenic tubers contained significantly higher amounts of glucose, whereas the oligo-fructan accumulation had no influence on the starch content.

This result is astonishing for two reasons. First, it is in contrast to the results of Sevenier et al., who reported a dramatic reduction of sucrose concentration in SST-expressing sugar beet (Sevenier et al., 1998). In tap roots the sucrose level dropped down to about 5%. Second, as sucrose is unchanged in the potato tubers and fructan oligomers accumulate, the total amount of soluble sugars is elevated more than three-fold without any adverse effect on the phenotype of the plants. For potato plants that showed a strong increase in soluble sugar concentration of tubers because of a repression of starch synthesis (Muller-Rober et al., 1992), a strong reduction of tuber size was reported that coincided with an increased tuber number and an overall loss of biomass, which was not observed for the fructan potato.

The next and very important question is, whether additional expression of FFT in the SST lines would lead to the production of the full set of inulin molecules naturally occurring in the plant that was taken as a source for the fructosyltransferase genes.

By comparing the FFT genes of Jerusalem artichoke (*Helianthus tuberosus*) and artichoke (*Cynara scolymus*) in a transient expression system (tobacco protoplasts), we obtained evidence that substrate preferences of this enzyme might define the chain length distribution of the inulin synthesized (Hellwege et al., 1998). The 1-FFT of artichoke has a low affinity to 1-kestose as fructosyl acceptor *in vitro* and prefers oligomers of higher DP, whereas the *Helianthus tuberosus* enzyme produces a set of inulin oligomers of a DP of up to 10, more or less irrespective of the substrate offered.

Expression of SST and FFT genes of artichoke (Cynara scolymus) in transgenic potato yielded results that strongly support the concept of SST and FFT being the sole enzymes involved in inulin synthesis. Analysis of the fructan composition of transgenic tubers by HPLC revealed a pattern that was undistinguishable from that of artichoke (Figure 3) and gel permeation chromatography (GPC) of extracts taken from artichoke root, wild type potato tubers and transgenic tubers confirmed that the inulin pattern of artichoke and transgenic potato is principally the same with inulin molecules of a maximum size of more than 100 units (data not shown).

The inulin content is about 3-fold higher than sucrose, which seems high regarding the inulin as ad-

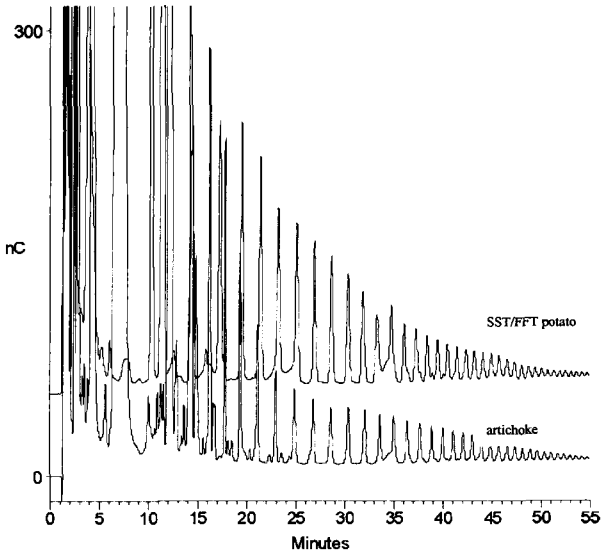


**Figure 3.** Inulin isolated from artichoke and transgenic potato plants expressing artichoke SST and FFT. The inulin preparation was analyzed by high pressure anion exchange chromatography (HPAEC) with pulsed amperometric detection.

ditional soluble sugar, but it is low considering starch, which is 20-fold higher concentrated. The transgenic potato lines display a reduction in starch content that lies within natural variation but is consistently found among transgenic lines. Therefore we conclude that fructan synthesis does not lead to an increase in total carbohydrate content of the tubers. The inulin does not establish an additional, but an alternative sink for storage carbohydrates.

The inulin potatoes can be regarded as "pre-biotic food" that should have a positive effect in human nutrition because of a stimulation of "pro-biotic" bacterial species in the colon. Inulin containing diets selectively stimulate bifidobacteria and make them the predominant bacterial species in the large intestine (Gibson and Wang, 1994). Bifidobacteria are classified "pro-biotic", because their presence in the colon cause an increase in concentration of short-chain fatty acids and a decrease of the activity of reductive enzymes and of tumor promoting substances like ammonia (Reddy et al., 1997; Gallaher et al., 1996; Rowland et al., 1998; Gallaher et al., 1996; Rowland et al., 1998). Fructans are natural food ingredients being present in wheat, onion, garlic, bananas, asparagus, and others (for Review, see Vanloo et al., 1995). The average daily consumption is estimated to be 1-4 g in the United States and 3-11 g in Europe (Roberfroid and Delzenne, 1998). In order to increase the daily intake up to the physiologically relevant threshold of 10 to 15 g/day, it would be useful to transfer the fructan synthesis capacity to other crop plants like potato or vegetables.

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