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Manipulation of sucrose transport and yield in potato.

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PROJECT SUMMARY

Transport of photoassimilates from their sites of production in photosynthesizing leaves to sites of usage and storage is a major determinant of crop productivity and yield. Plants with reduced capacity to transport photosynthetic products (i.e. sucrose) suffer from impaired growth and development, and a decline in photosynthetic activity and yield. Evidence suggests that increasing sucrose export from leaves has the potential to enhance plant growth and yield, although this has not been tested. Increased transport of assimilates is not only predicted to enhance the development, growth and yield of harvestable sink organs (tubers), but also has the potential to increase photosynthetic capacity by limiting down-regulation of photosynthesis due to accumulation of carbohydrates in photosynthesizing cells. Decreased sugar accumulation in the leaf may also delay senescence, thus increasing net carbon fixation per leaf. A key step in the transport of sucrose, the dominant form of transported carbohydrate, is the loading of sucrose into the phloem by H^+-sucrose symporters (SUTs). The major question we aim to address in this proposal is: Does expression of a high-activity SUT in potato phloem cells lead to enhanced plant growth, yield, photosynthetic activity, and/or delayed senescence? In addition, there is some evidence that sucrose levels may have a positive impact on tuber induction (Ewing 1997).

Introduction

Improving plant productivity is a major goal for sustaining a burgeoning world population and providing a viable alternative to petroleum. A fundamental determinant of crop growth and yield is photosynthetic carbon metabolism. Strategies for enhancing crop yields include improving production of photoassimilates, improving transport of photoassimilates, and improving use of photoassimilates in sinks. We propose to test the hypothesis that enhancing a key step in the transport of photoassimilates in potato will lead to increased crop yield due to greater supply of carbon for tuber growth and development. We predict that enhanced flux of photoassimilates out of source leaves will serve to limit carbohydrate-mediated repression of photosynthesis and therefore lead to increased plant productivity and yield.

A key feature in the evolution of multicellular organisms such as plants has been the development of systems to partition resources from their sites of generation to sites of usage and storage. In plants, leaves generate much of the energy required to sustain multicellular growth through the reactions of photosynthesis. Assimilate partitioning refers to the process by which carbohydrates generated by photosynthesis are transported from "source" leaves to "sink" tissues.

Sucrose is the major photoassimilate transported in plants. Sucrose synthesized in the cytosol of mesophyll cells is transported to sink tissues via the phloem (Fig. 1). There are two recognized routes by which sucrose is loaded into the phloem. The first is symplastic transport of sucrose from mesophyll cells to the companion cell-sieve element complex through plasmodesmata (Fig. 1 a) and subsequent incorporation into a raffinose sugar. The second and more common route is the release of sucrose from mesophyll cells into the apoplast (Fig. 1 b) followed by active uptake into companion cells by H^+-coupled

sucrose symporters (Fig. 1 c). SUTs (also referred to as sucrose transporters or SUTs) couple sucrose uptake directly to the proton motive force across the plasma membrane of plant cells and transport sucrose and H^+ in a 1:1 stoichiometry (Bush, 1993). Efflux of sucrose from mesophyll cells has been characterized biochemically; however, the responsible transporters have not been isolated. Sucrose provides the osmotic driving force for the mass flow transport of solutes in the phloem. Unloading of sucrose into sink tissues can occur through plasmodesmata or via membrane transporters.

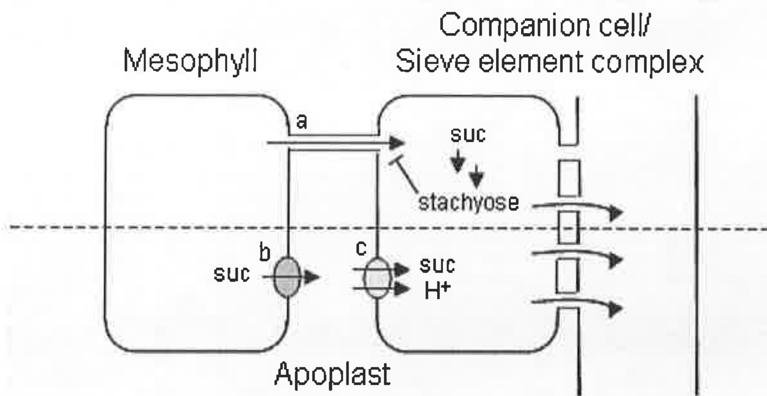

Figure 1. Schematic illustration of the 2 main routes of phloem loading in plants. Symplastic translocation of sucrose from mesophyll cytoplasm to the companion cell/sieve element complex can occur through plasmodesmata (a). Sucrose is then converted to a raffinose oligosaccharide, which is too large to diffuse back to the mesophyll cytoplasm. In apoplastic loading, sucrose efflux from mesophyll cells occurs by yet unidentified membrane transporters (b) followed by sucrose uptake from the apoplast by H^+-sucrose symporters (c).

Rationale and Significance

Efforts to improve photosynthetic capacity per unit leaf area of plants have met with only limited success and none have targeted improving the transport of photoassimilates. Increasing the loading of sucrose into the phloem has the potential to enhance photosynthetic activity by drawing sucrose out of mesophyll cells more efficiently. This hypothesis is supported by evidence that phloem loading stimulates sucrose efflux from mesophyll cells. Therefore increased phloem loading activity is predicted to lead to increased sucrose efflux activity from photosynthetic cells, which should have a direct effect on lowering cytosolic carbohydrate pools in the mesophyll that, at high concentrations, limit photosynthetic gene repression. Moreover, because leaf senescence is also linked to hexokinase-mediated signaling, reducing sucrose levels in leaves may keep leaves photosynthetically active for longer periods before senescence and thereby contribute to further improvement of crop yield.

Experimental design and Methods

A hyper-active sucrose transporter we previously described (Lu and Bush 1998) we be expressed in the companion cells of potato under the control of the galactinol synthase

(GS) gene promoter. This sucrose transporter is 10-flod more active than the wild type transporter. We'll use the GS companion cell specific promoter rather than the sucorse transporter promoter because expression of the sucrose transporter promoter is highly regulated by sucorse abundance in the companion cell (Chiou and Bush 1998, Vaughn and Bush 2002, Ransom at al. 2003). In other work in the Bush lab, we have discovered a sucrose-sensing signal transduction pathway that controls phloem loading by regulating expression of the sucorse symporter as a function of sucrose abundance in the leaf companion cell. We hypothesis this is part of a global regulatory system that keeps photosynthesis and sink utilization of assimilate in balance. Since we may affect sucrose levels in the companion cell, we are using the GS promoter that is companion cell specific, but not regulated by sucrose. The hyperactive sucrose transporter has already been cloned into an expression cassette driven by the GS promoter (Deavours and Bush, unpublished results). The Bush lab has direct experience with every facet of this proposal and has recently successfully transformed potato using cultivar Désirée (*Solanum tuberosum* L. cv. Désirée) via *Agrobacterium tumefaciens*-mediated transformation of leaf and petiole explants using standard protocols (Rocha-Sosa et al., 1989).

Analysis

Experimental analysis of transgenic potato will include:

1) Molecular analysis: Confirm transgenic expression of hyperactive sucrose transporter under the regulation of the galactinol synthetase gene promoter. This places the symporter in the companion cell and uncouples its expression from a sucrose-sensing signal transduction pathway that tightly regulates the expression and abundance of the native sucorse transporter.

2) Transport activity: Purify plasma membrane vesicles and measure transport activity of the symporter. These methods were pioneered by the Bush lab.

3) During growth:

- Measure photosynthetic rates of leaves in control and transformed plants throughout development to determine if hyper active sucrose loading stimulates photosynthetic rates.
- Measure leaf surface area to determine if hyper active sucrose transport alters leaf size or number.
- Measure stages of leaf development through senescence to determine if hyperactive transport delays leaf senescence, thus giving more days of maximal rates of photosynthesis per leaf.
- Measure levels of key metabolites (sucorse, glucose, fructose, triosephosphates) in leaves throughout development to determine if hyper-active sucrose transporter alters pool sizes.

4) Do a complete analysis of carbon distribution at harvest between shoot and tubers. This will give total carbon fixed per plant and also determine if sucrose yield was increased because of the hyper-active transporter.

Relationship to potato industry

Successful manipulation of sucrose allocation to tuber growth and yield could have a significant impact on the industry. Moreover, the concepts developed here could be applied to amino acid allocation as well. Taken together, both yield an nutritional value could be positively enhanced.

REFERENCES TO PROJECT DESCRIPTION

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FACILITIES AND EQUIPMENT

The Bush laboratory is located in the Biology Department at Colorado State University. The laboratory is approximately 1200 ft^2 and well equipped for all molecular, biochemical and physiological experiments proposed here. Relevant equipment includes: a Baker laminar flow hood dedicated for tissue culture work, a 30 ft^3 Percival controlled

environment chamber for plant cell culture, four 60 ft^3 Percival controlled environment plant growth chambers, a Polytron plant tissue homogenizer, 2 MJR Research PCR machines, nucleic acid electrophoresis equipment, three microcentrifuges, water and dry bath incubators, a Robbins hybridization oven, 30°C and 37°C incubators, a Savant Speed-Vac concentrator, a UV-VIS spectrophotometer, a Bio-Rad electroporator, preparative and ultra-centrifuges, a scintillation counter, a rotary evaporator, two -80°C freezers, and standard laboratory items. A LI-COR 6400 infrared gas analyzer and a LI-COR 3100 leaf area meter will be provided by Dr. Alan Knapp, CSU Biology Department. We also have access to space in the university greenhouse approved for transgenic plants. Additionally, the university provides an extensive array of subsidized research support, including oligonucleotide synthesis and DNA sequencing.

Budget for sucrose-proton symporter over-expression (Year 1).

50% time postdoc (with fringe)	$22,000
hourly help ($10 hr/ 15 hr week)	$7,500
material and supplies	$5,000
Total	= $34,500

Budget justification and Time Line:

The project will take approximately 50% effort for a postdoctoral researcher for a total of 24 months (a third year may be required for necessary replication of yield experiments). In the first year we'll generate the trangenic plants (we have already started) and set up growth experiments. The second year will focus on growth and yield analysis, as outlined above. A comprehensive analysis is planned to best understand the plants response to altered assimilate partitioning. This work will have periods of heavy activity and other less involved. Thus, I am asking for 50% salary. The postdoc will also work on other projects in the lab. If we demonstrate the expected enhancement in yield, we can take these data to the USDA-NRI for federal grant support.