ENHANCING SUCROSE ACCUMULATION: IDENTIFYING GENE TARGETS FOR MANIPULATION

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Abstract

Between 1950 and 1970, the introduction of new sugarcane varieties and improved crop husbandry in the South African sugar industry contributed to substantial gains in sucrose yield. However, in the subsequent three decades to date, sucrose yield has remained approximately constant, despite an increased rate of variety releases. This lack of progress is a phenomenon common to many sugar industries and has been ascribed to the narrow genetic base of germplasm used in breeding programmes. Consequently, in several industries, modern molecular technologies are being used in concert with more conventional strategies in bids to improve sucrose yield traits in both sugarcane breeding stock and individual lines with commercial potential. One strategy has focused on the genetic modification of the activity of single endogenous sugarcane genes or the expression of single heterologous genes in an attempt to increase the proportion of photoassimilate accumulating as stored sucrose in the culm. This approach has met with mixed success and given that plants are adept at metabolic compensation, it is increasingly acknowledged that robust manipulation of sucrose deposition in the sugarcane stalk may depend on the co-ordinated manipulation of the activity of several genes. However, the capacity to identify and subsequently modify multiple gene targets is contingent on the availability of comprehensive knowledge of the key regulatory steps governing sucrose accumulation. This presentation reviews recent attempts at the molecular manipulation of sucrose accumulation in sugarcane and describes how genomic approaches are being applied at SASEX to enhance the potential of such endeavours.

Keywords: sucrose accumulation, genomics, expression analysis, genetic modification

Precedents and potential exist for manipulating sucrose accumulation

The level to which sugarcane sucrose yield may be enhanced through genetic manipulation is largely dependent on the physical capacity of the culm to accommodate elevated sucrose concentrations (Grof and Campbell, 2001). Predictions suggest a capacity of the stalk to accrue more than 25% sucrose on a fresh weight basis, representing approximately double current commercial yields (Moore *et al*., 1997). Such potential lends credence to the viability of modern gene transfer technology as a mechanism for enhancing sucrose accumulation in sugarcane.

Since the advent and widespread adoption of transgenesis approaches 15 years ago, much information has been obtained regarding the regulation of plant carbohydrate metabolism, primarily in model species such as *Arabidopsis thaliana* and important dicotyledonous crop species. Progress has been facilitated by the production and analysis of large numbers of transgenic lines, in which the activities of most of the individual genes encoding carbohydrate metabolism enzymes have been modulated, alone or in combination (Fernie *et al*., 2002). Due to limitations in tissue culture and transformation technology, progress has been far slower for monocotyledonous crops, a situation that is particularly acute in sugarcane (Grof and Campbell, 2001). However, regardless of these bottlenecks, the successful modification of multiple genes in dicotyledonous plants serves as a good indicator of the potential of transgenesis as a means to analyse and manipulate sucrose metabolism in sugarcane.

Gene targets currently under manipulation

In general, carbohydrate accumulation in plant storage organs is governed by various potentially rate-determining processes, the most obvious of which include photoassimilate production and partitioning within photosynthetic organs, phloem loading and transport, and sucrose transport into and compartmentation within storage parenchyma. A sizeable body of data suggests that it is this last series of processes, occurring in sink tissues, which regulate carbohydrate accumulative potential (ap Rees, 1988; Farrar, 1992; Koch, 1996; Kingston-Smith *et al*., 1999). More specifically, the modulation of sucrose catabolism within these storage tissues is considered a vital determinant of sink strength, a judgement that is consistent with the principles of long-distance transport of photosynthate (Fisher, 2000). This view has been embraced by sugarcane molecular physiologists and as a result, recent attempts to enhance sucrose accumulation in the culm have focused on the manipulation of single genes encoding sucrolytic enzymes, including invertases (Ma *et al*., 2000; Botha *et al*., 2001) and pyrophosphate-dependent phosphofructokinase (Groenewald and Botha, 2001). However, as plants are adept at compensating physiologically for small changes in their genetic or external environment (Halpin *et al*., 2001), it is likely that successful genetic manipulation of sucrose storage will depend on the coordinate modification of the activity of multiple gene targets in sucrose metabolism.

Identifying multiple potential targets by genomic approaches

Modern genomic technologies are a powerful means to identify and clone genes involved in plant carbohydrate metabolism. While these strategies have been applied with great effect to important graminaceous crop species, including maize and rice (Richmond and Somerville, 2000), their use in sugarcane has been hampered by the genomic complexity of modern commercial hybrids (Butterfield *et al*., 2001; Casu *et al*., 2001). Consequently, sugarcane molecular physiologists are faced with the prospect of adapting existing genomic and functional genomic approaches to identify genes involved sucrose synthesis and storage. Strategies developed independently within the Australian (Casu *et* al., 2001), Brazilian (Burnquist, 2001) and South African (Carson and Botha, 2000, 2002) industries have identified numerous genes associated with both sucrose metabolism and culm maturation. However, the value of these genes as potential targets for manipulation depends on further clarification of their role in sucrose accumulation.

Clarifying the role of potential targets: a novel strategy at SASEX

Over the past few years, extensive gene collections have emanated from the functional analysis of the genomes of important agricultural species, many of which are closely related to sugarcane in evolutionary terms. Researchers at SASEX have recognised that these resources, together with those from sugarcane available within the public domain, represent a valuable tool for analysis of the genetic regulation of sucrose accumulation. To this end, biochemical and physiological data have been used to select and gather a suite of 81 genes with presumed key roles in sucrose metabolism and culm maturation (Table 1). The majority of the genes (70) were obtained from the Rice DNA Bank at Tsukuba, Japan, while the remainder were from in-house EST collections. Recently developed infrastructure at SASEX is being deployed in the parallel analysis of the expression of these genes under defined developmental and environmental conditions, an approach that will provide crucial insights into the potential of these genes as targets for the manipulation of sucrose accumulation. In future work, the analytical power of these investigations will be intensified by the simultaneous analysis of the levels of numerous key metabolites and metabolic intermediates, an approach unprecedented in sugarcane research.

Selection was according to a presumed key role of the gene products in carbohydrate and cognate metabolism within the mature culm. Gene variants represent various isoforms and subunits of enzymes and several classes of sugar transporters

Table 1. Products encoded by genes selected for expression analysis.

Enzyme or other protein encoded by gene	Number of gene variants
Carbohydrate Metabolism	
Aldolase	2
Cellulose synthase	3
Enolase	3
Fructose 1,6-bisphosphatase	1
Fructokinase	1
β-Glucanase	2
UDP-Glucose dehydrogenase	2
UDP-Glucose glucosyltransferase	1
Glucose-6-phosphate isomerase	1
ADP-Glucose pyrophosphorylase	4
UDP-Glucose pyrophosphorylase	1
Hexokinase	4
Invertase	9
6-Phosphofructokinase	2
Phosphoglucokinase	1
Phosphoglucomutase	1
Pyrophosphate-fructose 6-phosphate 1-phosphotransferase	2

Enzyme or other protein encoded by gene	Number of gene variants
Sucrose-phosphate phosphatase	1
Sucrose-phosphate synthase	1
Sucrose synthase	4
Sugar transporters	10
Trehalose-phosphate phosphatase	3
Trehalose-phosphate synthase	3
Triose-phosphate isomerase	2
Cognate Metabolism	
Aconitate hydratase	1
Alcohol dehydrogenase	4
Citrate lyase	1
Citrate synthase	1
Glyceraldehyde-3-P dehydrogenase	2
Malate dehydrogenase	3
Phospho*enol*pyruvate carboxylase	3
Pyruvate kinase	2
Pyruvate carboxylase	1
Succinate dehydrogenase	2

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