

GENE DISCOVERY AND EXPRESSION ANALYSIS IN SUGARCANE – THE STORY SO FAR

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Abstract

Knowledge about gene expression during sugarcane growth and maturation is limited. A gene discovery programme has been in progress at SASEX since 1994 to identify and analyse genes that are expressed in different sugarcane tissue types and developmental stages. A database of sugarcane gene sequences has been established through the generation of Expressed Sequence Tags (ESTs). This database, containing approximately 1400 sequences from leaf and culm tissues of varying maturity, has facilitated access to a myriad of genes not previously available for sugarcane research. Submission of a proportion of the sugarcane gene sequences to the dbEST international database represented the first sugarcane ESTs released into the public domain. ESTs have been identified and the expression patterns of sugarcane genes examined in maturing internodal tissue to identify genes that may be preferentially associated with culm maturation. Differentially expressed genes detected in the culm included those associated with general cellular metabolism, cell wall synthesis, carbohydrate metabolism, regulatory processes and stress responses. The results indicate that growth and maturation of the sugarcane culm is associated with the expression of genes for a wide variety of metabolic processes. Only one gene known to be involved with sucrose metabolism was detected, suggesting that genes encoding enzymes directly associated with sucrose accumulation may not be abundantly expressed in the culm. This paper reviews the major outcomes of research at SASEX towards the molecular analysis of sugarcane metabolism during growth and maturation.

Keywords: Expressed Sequence Tags (ESTs), differential gene expression, genomics, sugarcane culm.

Introduction

Continual improvement of sucrose yield from sugarcane is the priority of commercial sugarcane industries worldwide. To date, sugarcane improvement has relied solely on traditional breeding that can be conducted without knowledge of the specific physiological or genetic basis of plant performance. However, recent advances in technologies to study higher plants at the genetic level coupled with the ability to genetically modify plants through the introduction of cloned genes have created the opportunity to improve important cultivars. Improving plant performance through genetic manipulation is dependent on an integrated understanding of plant metabolism at physiological, biochemical and genetic levels. The availability of resources such as suitable genes and gene promoters to target the expression of cloned genes to specific cells or tissue types is also important. For sugarcane, research directed towards pinpointing important elements associated with the metabolic control of growth, maturation and sucrose accumulation will yield information that can be exploited in genetic engineering efforts to improve crop performance and sucrose yield.

The pathway of sucrose accumulation in sugarcane has been studied extensively at the physiological and biochemical levels (for review, see Moore 1995; Grof and Campbell, 2001). Although a considerable amount of information about sucrose accumulation during culm maturation has been generated, the regulation of sucrose metabolism is still not well understood. Furthermore, very little is known about how metabolism in the sugarcane culm is regulated at the gene level. Much more information is therefore required about gene expression during sugarcane culm maturation and sucrose accumulation.

Recently, advances in molecular biology have facilitated the processes of rapid gene identification and large-scale, multi-gene expression analysis. These new approaches, commonly referred to as “genomics”, offer novel opportunities for investigating how complex metabolic pathways are regulated at the gene level. Genomics-based research has been used successfully to identify and analyse genes in a wide range of organisms, including many different plant species. A sugarcane genomics programme was initiated at the South African Sugar Association Experiment Station (SASEX) in 1994 to study aspects of sugarcane culm maturation and sucrose metabolism at the molecular level. In this paper, progress made in the identification and analysis of sugarcane genes expressed in leaf and culm tissues of different developmental stages is presented and key areas for future research are highlighted.

A resource of sugarcane genes

A database of sugarcane gene sequences has been established through the generation of Expressed Sequence Tags (ESTs). Parameters for the identification of sugarcane genes by EST analysis have been described previously (Carson and Botha, 2000). Approximately 1400 gene sequences have been obtained from the leaf roll (meristematic apex), immature culm (internode 2) and maturing culm (internode 7) and have provided access to a myriad of genes not previously available for sugarcane research. A large portion of the sugarcane ESTs from the meristematic apex and maturing culm were submitted to the GenBank dbEST international database and these represent the first sugarcane ESTs to be made available in the public domain.

Sequence homology searches against known gene sequences in the GenBank non-redundant peptide and nucleotide databases have assigned putative identities to approximately 70% of the sugarcane ESTs. Analysis of the ESTs revealed homology to a broad diversity of genes in all the tissue types investigated. Included are genes encoding enzymes and other proteins associated with ubiquitous metabolic pathways, transport, regulatory processes and various stress responses. The remaining ESTs either showed sequence overlap with uncharacterised coding sequences or had no match to sequences in the public databases. These ESTs could represent novel sequences and present a useful resource of tags to new sugarcane genes.

Analysis of sugarcane gene transcript abundance in the leaf, leaf roll, immature culm and maturing culm has identified several differentially expressed genes exhibiting tissue and developmental stage specificity or preference (Carson *et al.*, 2002). Molecular manipulation of sugarcane is currently limited by the poor availability of genetic resources such as promoters required to drive the tissue or organ-specific expression of introduced transgenes. The tissue-preferential genes isolated at SASEX provide a valuable resource of candidate genes for future promoter isolation and characterisation.

Gene expression during sugarcane culm maturation

To explore gene expression during culm maturation, ESTs have been identified and analysed in immature (low sucrose-accumulating) and maturing (high sucrose-accumulating) tissues. ESTs were isolated from a maturing culm total cDNA library and subtracted cDNA libraries prepared by reciprocal subtractive hybridisation between the immature and maturing culm. The latter are

enriched for transcripts preferentially expressed in distinct culm developmental stages and facilitate the detection of genes up- or down-regulated during culm maturation. ESTs were identified by random selection of cDNA clones from the maturing culm total and subtracted cDNA libraries followed by analysis of transcript abundance to identify genes exhibiting culm-preferential expression patterns (Carson and Botha, 2002; Carson *et al.*, 2002).

EST data revealed that genes associated with a wide range of cellular and metabolic processes are expressed in the culm. There was evidence for the up-regulation in the culm of genes that encode proteins associated with carbohydrate metabolism, cell wall synthesis, various stress responses and the regulation of cellular metabolism. The range of specific genes detected in the immature and maturing culm was distinctly different from one another. Examples of genes abundantly expressed in the immature culm included UDP-glucose dehydrogenase, cellulose synthase, trehalose-6-phosphate synthase, and a drought-induced protein. In the maturing culm, the preferential expression of genes such as callose synthase, glycerol-3-phosphate permease and jacalin was evident. The expression profiles displayed by these genes suggest that they have important roles in sugarcane culm maturation however, further characterisation is necessary to establish specific functions for these genes.

Sugarcane genomics programmes in other sugarcane-growing countries have reported similar results to those obtained at SASEX. The analysis of 7419 and 2030 maturing culm ESTs in other programmes has indicated that genes associated with processes such as cell wall metabolism, protein expression, signal transduction, transport and stress responses are abundantly expressed (Casu *et al.*, 2001; Paterson 2002). All these studies suggest that, during growth and maturation, the sugarcane culm is actively engaged in a wide variety of metabolic processes as well as responsive to environmental conditions and stresses. The results indicate that the sugarcane culm is not simply an organ for sucrose storage but is a complex and dynamic structure.

Genetic regulation of sucrose accumulation

Biochemical studies have described changes in the activities of key sucrose-metabolising enzymes in internodes of differing maturity. However, with the exception of sucrose synthase, no genes encoding the key enzymes of sucrose metabolism were detected in the current analysis, despite enriching for transcripts preferentially expressed during culm maturation. The abundant expression of transcripts encoding sucrose synthase in immature internodes corresponded favourably with protein expression data (Buczynski *et al.*, 1993). Nonetheless, there was an apparent lack of association between the types of genes identified as preferentially expressed in the culm and available biochemical and physiological data regarding sucrose accumulation. Few sucrose metabolism-related genes have been identified in maturing internodal tissue in other sugarcane genomics programmes (Casu *et al.*, 2001; Paterson, 2002). These results suggest that genes encoding enzymes directly associated with the pathway of sucrose accumulation may not be abundantly expressed in the maturing sugarcane culm. Such findings raise new questions about how sucrose accumulation is regulated in sugarcane. It is possible that genes encoding key enzymes in the sucrose accumulation process are expressed at low levels in the culm. The complexities of the regulation of transcription during culm maturation require further investigation.

The way forward for genomics research at SASEX

Genomics research at SASEX has significantly advanced gene acquisition and knowledge of gene expression in sugarcane. It has also provided new insights into the complexities of metabolism during culm maturation. A platform has been provided from which future genomics strategies for sugarcane can be formulated. Studies are in progress to characterise the expression behaviour of sugarcane genes preferentially expressed in the culm. This will result in the detection of candidates

suitable for promoter isolation or use as transgenes in sugarcane improvement programmes. Furthermore, considerable potential exists for the exploitation of the resource of anonymous sequences to identify novel genes that may be associated with the regulation of sucrose accumulation in sugarcane.

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