

# CURRENT APPROACHES TO DEVELOPMENTAL-STAGE SPECIFIC AND ABIOTIC-STRESS RESPONSIVE GENE EXPRESSION PROFILING: PROGRESS AND PROSPECTS IN SUGARCANE

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## Abstract

The development of many molecular crop improvement strategies is expedited by the isolation of genes that participate in delivering the desired phenotype. Consequently, great effort has been devoted worldwide to the identification of developmental-stage specific and stress responsive genes in important crop and model plant species. The substantial rate of progress reported for many of those endeavours can be ascribed to the massive scale of the gene expression profiling initiatives that have emanated from animal and plant genome sequencing projects. However, the adoption of such approaches is costly and generally restricted to plant species for which large expressed sequence tag (EST) databases exist and are available within the public domain. For other plant species, including sugarcane, alternative approaches to identifying differentially expressed genes must be sought. Recent research at SASEX has demonstrated that a combination of cDNA subtractive hybridisation and simple macro-array technology are effective in the identification of developmental-stage specific and abiotic-stress responsive genes. To date, this approach has yielded four genic fragments specifically associated with culm maturation. Also, preliminary findings have revealed that a myriad of signalling and signal transduction events, similar to those involved in pathogenesis-related responses, are elicited in sugarcane roots challenged by phytotoxic levels of aluminium. This presentation outlines the scope of such gene expression profiling strategies and their potential application to sugarcane improvement programmes.

## Molecular approaches to the analysis of crop productivity

Yield productivity within the South African sugar industry has remained approximately constant over the past three decades (Anon, 1997), despite the increased rate of cultivar releases. As a result, the exploration and implementation of strategies to overcome this apparent productivity ceiling have become priorities of many research departments at SASEX. Improvements in agronomic practice have and will undoubtedly continue to enhance production efficiency, particularly with the continual introduction of more sophisticated crop protection chemicals and decision support systems to aid crop management. However, genetic improvement of sugarcane is the area that offers the potential for major breakthroughs in increasing productivity. In this regard, progress in recombinant DNA technology has provided the capacity for the molecular analysis of crop productivity, which may be deployed at three levels, viz: (1) by

generating complete sequences of the plant genome; (2) by genetic analysis of phenotypes using genetic marker technology or (3) by metabolic analysis (Miflin, 2000). Although aspects of all three approaches form part of current strategy at SASEX, this presentation will focus on the first. To this end, some of the methods commonly used in the search for genes involved in important metabolic and developmental processes are reviewed and assessed in terms of their applicability to the research priorities of SASEX.

## Starting at the beginning: Genomics

The term 'genomics' has been coined to describe the modern discipline of mapping, sequencing and analysing genomes, the genes and chromosomes of an organism (Hieter and Boguski, 1997). Over the past decade, great international effort has been devoted to sequencing the genomes of a variety of organisms. The most widely publicised of these was the human genome project, out of which recently emanated the complete human genome sequence (International Human Genome Sequencing Consortium, 2001). In the botanical realm, only the genome of *Arabidopsis thaliana* has been completely sequenced (Ausubel, 2000), although substantial progress has been reported for rice and maize (Richmond and Somerville, 2000). The success of such enormously expensive projects can be attributed to their collaborative nature in that, in all cases, the resources of a number of major laboratories were pooled. For species such as sugarcane, which are not global priority crops or important model systems and have complex polyploid genomes, complete genome sequencing is not currently practical.

## Mining genomic data: Functional genomics

Although genome sequence data provide important clues as to genomic structure and organisation, their full value is only realised once functions have been assigned to the genes that they represent. Such functional analysis of genomic data is arguably the most challenging aspect of genomics and has become a major field of research, particularly for important crop (e.g. maize and rice) and model (e.g. *A. thaliana*) plant species (Ausubel, 2000). Sequence analysis with a view to assigning putative gene functions, or functional genomics as it referred to in the scientific parlance (Hieter and Boguski, 1997), may be approached in various ways.

*Expressed Sequence Tags.* A common method is to search for DNA homology of fragments of expressed genes to known genes, which often leads to the tentative assignment of the

sequence to a class of genes. Once putative identities have been found for the sequences, they are referred to as Expressed Sequence Tags (ESTs). Although informative, a major drawback of this strategy is that a significant number of the open reading frames under examination may not have homology to genes of known function. For example, of the 28 000 genes identified in the *A. thaliana* genome sequencing project, 11 200 (40%) do not correspond to any known genes (White *et al.*, 2000). Similarly, in the SASEX EST programme, which is on a far more modest scale than the *A. thaliana* project, 60 (24%) of the 250 leaf roll genic fragments sequenced to date remain anonymous (Carson and Botha, 2000). Nevertheless, the potential of this approach is illustrated by the successful functional classification of 250 genes expressed in the apical meristematic region of the sugarcane culm (Carson and Botha, 2000).

**DNA hybridisation arrays.** Another approach to understanding the function of specific sequences is to examine their expression under a range of conditions, for example during development, disease or environmental challenge. Fundamental to this approach has been the development of DNA hybridisation array technology. During array production, gene-specific sequences (probes) are immobilised on a solid-state matrix, which may be nylon membrane, glass microscope slides or silicon/ceramic chips (Freeman *et al.*, 2000). The sequences are then queried with labelled copies of nucleic acids derived from samples of interest (targets). The principle upon which this approach is based is that the greater the expression of the gene, the greater the amount of labelled target and hence, the greater the output signal.

Generally, three categories of experiments, each with quite distinct goals, can be undertaken using array technology: (1) to identify highly specific 'marker' genes that display significant changes in expression during development or in response to an environmental stimulus; (2) to assess the expression of all genes, thereby providing an integrative view of the responses of a plant to a treatment; and (3) to assign putative functions to novel, uncharacterised genes, when and where such genes display altered expression (Richmond and Somerville, 2000). Of the three approaches, the first is particularly useful as it is conducive to the identification of genes differentially regulated during development and in response to different stresses, thereby giving clues as to which biochemical pathways and defence mechanisms operate under such circumstances (Sävenstrand *et al.*, 2000). The other two approaches are conducted on an extremely large scale and hence, are generally restricted to plant species for which large DNA sequence databases are available. Although an extensive expressed gene fragment sequencing project for sugarcane (SUCEST; <http://sucest.lbi.dcc.unicamp.br>) is currently under way as a joint venture of a number of laboratories in São Paulo State, Brazil, issues surrounding access to the data by external parties remain unclear. Furthermore, as the resources required by such initiatives exceed those available in smaller sugarcane research institutes such as SASEX, alternative approaches are being sought to identify genes expressed in sugarcane under conditions relevant to the South Africa industry.

**Subtractive hybridisation.** Over the past decade, methods for DNA subtractive hybridisation have been developed to select specifically for genes for which levels of expression vary under different conditions (e.g. Patel and Sive, 1995). This targeted approach to gene identification has found a niche in laboratories that work on species that do not have large DNA sequence databases and/or lack the facilities to conduct extensive gene profiling studies. A further advantage of this approach over traditional sequencing projects is that it allows for the identification of rare transcripts and transcripts of genes that are induced only under the conditions of interest. Hence, it was this approach that was selected for the current study to identify genes expressed in sugarcane in two particular situations: culm maturation and in root tips when challenged by a phytotoxic level of aluminium.

#### **Identification of sugarcane genes by subtractive hybridisation.**

Identification of genes associated with sucrose accumulation during culm maturation and aluminium tolerance in sugarcane may be of great practical value, in that once appropriately characterised, such genes have the potential to be developed as transgenes for genetic engineering strategies or molecular markers for marker-assisted selection programmes. It is anticipated that the successful application of such strategies may lead to increased sugarcane productivity. In addition, the development of the technologies serves also to evaluate approaches that may be used in future to identify genes involved in delivering other economically important phenotypes.

**Developmental stage-specific gene fragments (ESTs).** A method of reciprocal subtraction (Patel and Sive, 1995) between immature (internode 2) and maturing (internode 7) internodal regions of the sugarcane culm was used to isolate genic fragments associated with maturation. To confirm the specificity of the fragments isolated, 400 were subjected to expression analysis using membrane-based DNA hybridisation arrays. The results revealed that 37% and 31% of the fragments were associated with genes expressed preferentially in immature and maturing regions of the culm, respectively (results not shown). Further examination, via Northern hybridisation analysis, provided conclusive evidence that two of the fragments corresponded to genes expressed exclusively in the immature region of the culm, with another pair specific to the maturing culm (Table 1). Sequence homology searches against international databases revealed a putative stress-responsive function for three fragments identified. The specificity and consistency of these expression patterns are currently being validated under a variety of conditions.

**Aluminium elicited gene fragments (ESTs).** Soil acidification in the South African sugar industry has received considerable attention due to the role it plays in the recruitment of phytotoxic aluminium species into the rhizosphere (e.g. Schumann *et al.*, 1999). Consequently, a project was initiated to investigate the molecular response of sugarcane roots to aluminium challenge. For this investigation, a suppression subtractive hybridisation technique (Diatchenko *et al.*, 1996) was selected due its capacity to detect low abundance gene transcripts, such as those involved in signalling and signal transduction. This

approach was necessary as a possible role of aluminium as an elicitor of pathogenesis-related signal transduction pathways has been suggested (Hamel *et al.*, 1998). Expression profiling by means of membrane-based DNA arrays revealed that the expression of at least 32% of 298 of the genic fragments isolated from sugarcane root tips may have been attributable to aluminium challenge. Results of sequence homology searches for putative functions for a small sub-set (12) of the genic fragments were consistent with the notion that aluminium acts as an elicitor of pathogenesis-related pathways (results not shown). Northern hybridisation analyses have been initiated to validate these results and, to date, the aluminium induction specificity of only one of the genes has been confirmed unequivocally (Table 1). Complementary studies are in progress to assess the root and cultivar specificity of genes with confirmed aluminium-elicited expression patterns.

### Concluding remarks and future work


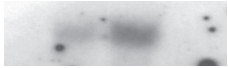
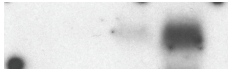


Recent technological advances in the field of functional genomics have provided plant scientists with an extensive toolkit for identifying candidate genes involved in important developmental and stress related processes. As a result, workers in the field are now faced with the challenging task of identifying approaches that are compatible with their research goals and the resources at their disposal. Experience at SASEX over

the past three years has demonstrated that a relatively modest combination of DNA subtractive hybridisation and membrane-based array technology is effective in isolating genic fragments associated with culm maturation and root responses to aluminium challenge. Analysis of the differential expression patterns of genes identified to date under a variety of conditions is a current research priority, as is expansion of the repertoire of differentially expressed sequences to include other stresses of importance to the industry. While this focus on gene identification and functional analysis is necessary for basic discovery, it alone is not sufficient for crop improvement. Hence, once suitable candidate genes have been found and fully characterised, they will feed into genetic engineering and marker development strategies.

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**Table 1. Expression specificity of culm developmental stage-specific ESTs and an aluminium-elicited genic fragment isolated by DNA subtractive hybridisation. The DNA fragments were used as probes against size fractionated total RNA from various parts of the sugarcane plant (L = leaf; LR = leaf roll; Int2 = internode 2; Int7 = internode 7) or root tips exposed (+Al) or not exposed (-Al) to 250 µM Al<sup>3+</sup> for 24 h. The intensity of the bands is directly proportional to the level of expression of the gene to which the probe corresponds.**

Expression profile	Putative identity	Putative function
ESTs expressed only in the immature culm		
L LR Int2 Int7		
	Putative senescence-associated protein	regulation/signal transduction
	Latex-abundant protein	stress response
ESTs expressed only in the maturing culm		
L LR Int2 Int7		
	Jacalin	stress response
	GOS9 protein	stress response
Genic sequence expressed in root tips in response to aluminium challenge		
+ Al - Al		
	ND*	ND

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