SHORT COMMUNICATION

STEM-SPECIFIC PROMOTERS FROM SORGHUM AND MAIZE FOR USE IN SUGARCANE

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Abstract

Genetic engineering of sugarcane (*Saccharum* spp. hybrids) to enhance sucrose content and borer resistance in the region of the stem requires appropriate transgenes and promoters. A promoter is essential to drive gene transcription and is therefore critical to the success of the transgenic approach. There are no suitable stem-active promoters available in the public domain. The goal of this work was to identify a gene showing stem-specific gene expression in sugarcane, maize and sorghum, isolate its promoter from maize and/or sorghum and evaluate promoter activity and specificity in sugarcane using a transgenic test system. Maize and sorghum are relatives of sugarcane in the family *Gramineae* but have much simpler genomes. A previously described stem-specific RNA transcript from sugarcane was used as the starting point. Classical molecular techniques were used to (i) demonstrate that this gene candidate had suitable characteristics across the three plant species, (ii) extract upstream promoter sequences from genomic libraries, (iii) construct test vectors for sugarcane transformation and (iv) obtain transgenic plants for tissue-based expression assays. Assessment of promoter-driven transgene expression in sugarcane has revealed a high level of silencing. Novel strategies to address this problem are being devised and implemented.

Keywords: sugarcane, *Gramineae,* genetic engineering, promoters, gene expression, gene regulation

Introduction

Many of the crucial biological processes residing in the stalk of sugarcane are directly related to crop productivity. Sucrose is accumulated as the stalk matures, for example Moore (1995). The stalk is also the site of entry of the sugarcane stem borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Leslie, 1993; Kvedaras *et al.*, 2007), and measures are needed to reduce insect entry in this region of the plant. Tactics for the genetic manipulation of sugarcane to enhance phenotypes such as sugar levels and borer resistance require not only appropriate gene sequences, but also promoters that switch on in this target zone. A promoter is an essential DNA-based genomic element adjacent to a gene sequence that drives transcription in response to developmental or other signals. It is critical to the success of the transgenic approach. There are no suitable stem-specific promoters available in the public domain. The goal of this work was to obtain a novel promoter that would be useful in sugarcane. Functional exploitation of the polyploid sugarcane genome is impeded by its extreme complexity and the common presence of inactive genes and pseudogenes. Closely related members of the *Gramineae* family such as sorghum (*Sorghum bicolor*) and maize (*Zea mays*) have comparatively simpler genomes, hence their use as primary sources of promoter sequences in this work.

Experimental Strategy

Figure 1 shows the flow of information and research activity used in this study to (i) identify a low copy number gene showing stem-specific expression in three related grass genera (sugarcane, maize and sorghum), (ii) recover its cognate promoter region from sorghum or maize genomic DNA and (iii) evaluate the functionality of the isolated promoter *in vivo* using a transgenic sugarcane test system. A previously described stem-specific RNA transcript from sugarcane (sequence '51') was used as the starting point (Potier and Birch, 2001). Specificity of expression and copy number in sugarcane, maize and sorghum were investigated by Northern and Southern analysis respectively. Methods used to isolate the promoter region included inverse PCR and probing of Lambda bacteriophage and Bacterial Artificial Chromosome (BAC) libraries. Identification of the precise location of the promoter within larger segments of genomic DNA was achieved by a combination of hybridisation and sequencing methods. Between one and four kilobases upstream of the transcript template sequence was used to generate test vectors through fusion to a 'reporter' gene. In this case, the β-glucuronidase (*GUS*) gene was used, as its expression can be traced easily by colour assay. The constructs containing the promoter-*GUS* sequence were introduced into embryonic sugarcane cells by conventional transformation technology and whole transgenic plants regenerated for tissue-based testing of *GUS* expression.

Results and Discussion

Despite the function of the gene product of '51' being unknown, this transcript was seen as a good candidate for the isolation of the corresponding promoter region in maize and sorghum. Northern analyses (Figure 2) confirmed the stem-specificity of the transcript in sorghum, maize and the two hybrid varieties of sugarcane, N19 and 88H0019. In the ancestral varieties Coimbatore (*Saccharum spontaneum*) and Black Cheribon (*Saccharum officinarum*) the expression patterns were slightly different. Gene '51' was expressed in the stem and roots in Coimbatore, whereas its expression was restricted to the top part of the stem in Black Cheribon. Southern analyses (data not shown) revealed a relatively low gene copy number in sorghum and maize. This was an important criterion for proceeding with '51' since the lower the copy number the greater the chance of retrieving a functional gene. It was found impossible to design primers for inverse PCR because the base composition of the transcript showed unusually high levels of G and C. Library screening was therefore used as an alternative strategy for accessing the relevant portion of the genome. A maize Lambda clone and two sorghum BAC clones containing sequences homologous to clone '51' were isolated. From these clones, a maize promoter sequence was recovered (2.5 kb), sequenced and fused to the *GUS* reporter gene in constructs designed for sugarcane transformation. Similarly, two sorghum promoter sequences (2.2 kb and 3 kb) were used to produce *GUS* constructs. Transgenic lines have been regenerated and these are currently undergoing molecular analysis to confirm constructs integration. For each test construct, about 15 plants containing the desired transgenes will be grown to maturity, and the different tissues assayed for *GUS* expression to evaluate localisation of promoter activity.

Several groups have undertaken assessment of promoter functionality in transgenic sugarcane. Their work has led to the realisation that sugarcane exhibits a high occurrence of transgene silencing (promoter silencing), with the notable exception of the ubiquitin promoters that express constitutively (Christensen and Quail, 1996; Yew *et al.*, 2001). Recent research efforts, mostly on *Arabidopsis*, have started to elucidate the mechanisms of transcriptional gene silencing (TGS) (Huettel *et al.*, 2007). One of the key features of that phenomenon to be established early on was hypermethylation of DNA. Several enzymes and protein complexes are involved in this epigenetic DNA modification. Although it is yet not known whether TGS

and PTGS (post-transcriptional gene silencing) mechanisms are similar in sugarcane and *Arabidopsis*, we have initiated work on possible approaches to counteract gene silencing (see SASTA 2008 poster, 'Strategies for the alleviation of promoter silencing in sugarcane'). On the other hand, gene silencing may be used as a positive tool to down-regulate specific genes or metabolic pathways within a plant. Despite a high level of variability in sugarcane genomes (polyploidy and aneuploidy), gene expression seems to be tightly regulated. Overcoming gene silencing is part of the future challenge in obtaining functional, tissue-specific, inducible and even cell-type specific promoters for use in sugarcane.

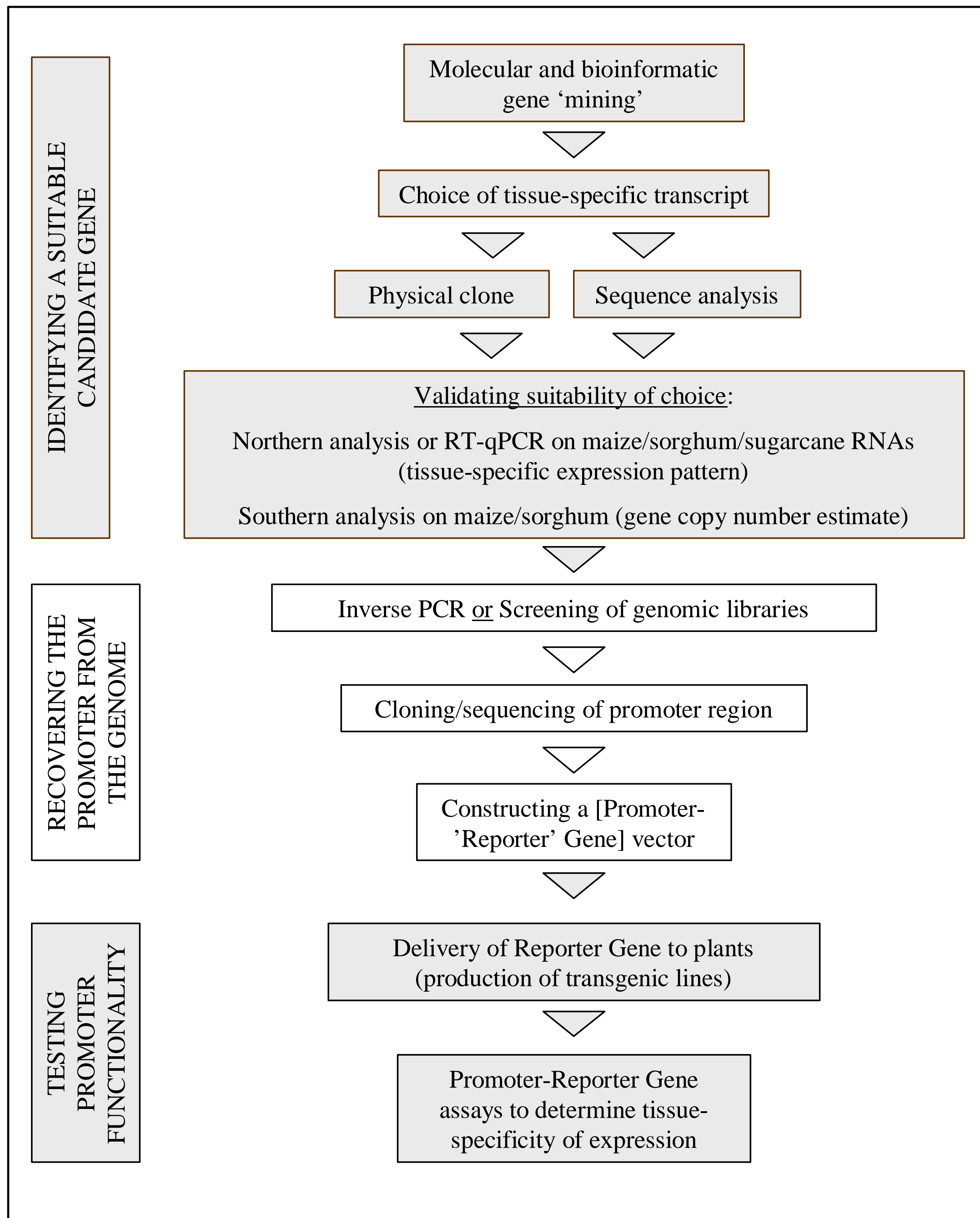

Figure 1. Strategy for the identification, isolation and testing of a novel promoter.

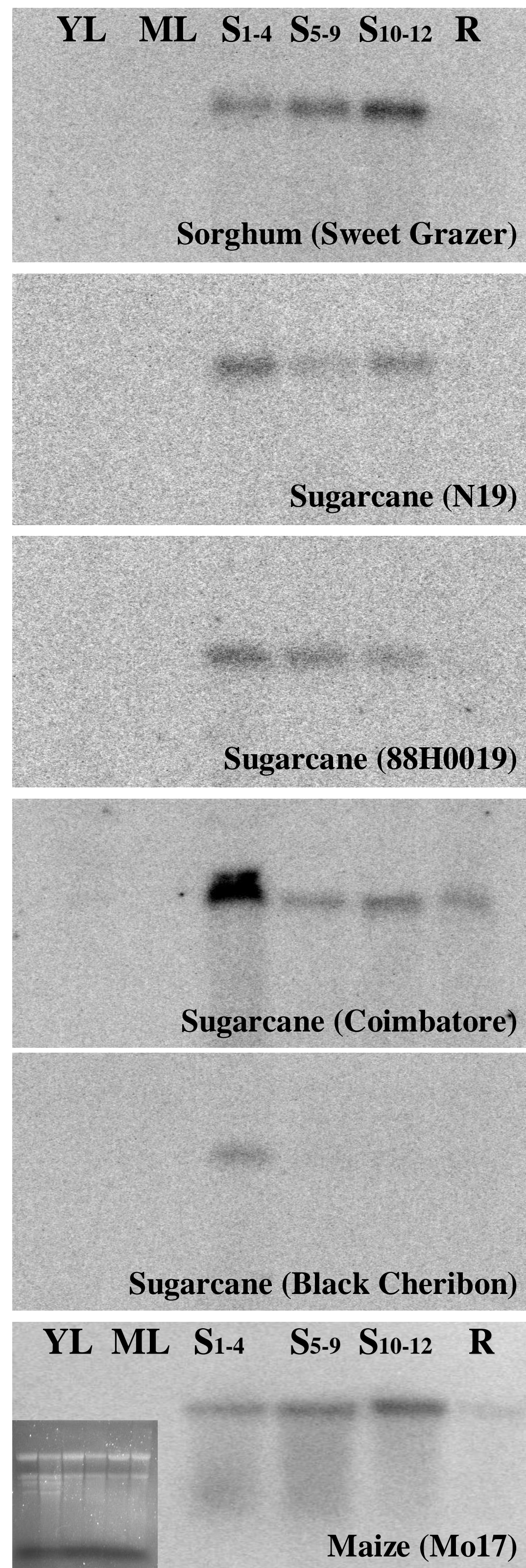

Figure 2. Northern blot analysis showing stem-specific transcription of gene '51' in sorghum, ancestral and hybrid sugarcane genotypes, and maize. YL, young leaf; ML, mature leaf; S1-4, upper (young) internodal region of stem; S5-9, middle internodal region of stem; S10-12, lower (mature) region of stem; R, root. Insert (bottom lower corner): Ethidium bromide-stained total RNAs after electrophoretic separation in an agarose gel, prior to blotting.

REFERENCES

Christensen AH and Quail PH (1996). Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants. *Transgenic Research* 5: 213-218.

Huettel B, Kanno T, Daxinger L, Bucher E, van der Winden J, Matzke AJM and Matzke M (2007). RNA-directed DNA methylation mediated by DRD1 and Pol Ivb: A versatile pathway for transcriptional gene silencing in plants. *Biochim Biophys Acta* 1769: 358-374.

Kvedaras OL, Keeping MG, Goebel FR and Byrne MJ (2007). Larval performance of the pyralid borer *Eldana saccharina* Walker and stalk damage in sugarcane: Influence of plant silicon, cultivar and feeding site. *Int J Pest Man* 53(3): 183-194.

Leslie GW (1993). Dispersal behaviour of neonate larvae of the pyralid sugarcane borer *Eldana saccharina*. *Proc S Afr Sug Technol Ass* 67: 122-126.

Moore PH (1995). Temporal and spatial regulation of sucrose accumulation in the sugarcane stem. *Australian Journal of Plant Physiology* 22: 661-679.

Potier BAM and Birch RG (2001). Sugarcane plant promoters to express heterologous nucleic acids. Patent application WO 01/18211 A1.

Yew NS, Przybylska M, Ziegler RJ, Liu D and Cheng SH (2001). High and sustained transgene expression *in vivo* from plasmid vectors containing a hybrid ubiquitin promoter. *Molecular Therapy* 4: 75-82.