POSTER SUMMARY

STRATEGIES FOR THE ALLEVIATION OF PROMOTER SILENCING IN SUGARCANE

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Abstract

Many steps are required for the successful production of genetically modified sugarcane. One of them involves the isolation of functional promoter elements that will allow for the targeted expression of transgenes. Several reports from different groups have shown that promoter silencing in sugarcane is the norm rather than the exception. The high occurrence of gene silencing is independent of transgene copy number or sites of insertions. We are trying to counteract this phenomenon by co-bombarding the usual selection plasmid and promoter-GUS test construct with a plasmid containing a viral suppressor of gene silencing. In the same manner, based on reported hypermethylation of silenced promoters, we are using a plasmid that will block the expression of an endogenous sugarcane DNA methyltransferase. In addition, we are evaluating the 'side-effects' of the viral suppressor of gene silencing when expressed on its own in sugarcane and the effects that reduced DNA methylation could have on the sugarcane plant. The results of these experiments will point towards an approach to improve targeted gene expression in sugarcane.

Keywords: sugarcane, genetic engineering, promoters, silencing, DNA methylation, viral suppressor

Introduction

The possibility of expressing a particular gene in a specific tissue at a precise time during plant development is of great appeal for the production of transgenic sugarcane with improved characteristics. Although many technical problems have been solved during the past 15 years, transgene expression in a complex genetic background, such as the one found in sugarcane, remains challenging. One of the most pressing hurdles to overcome is gene silencing. This phenomenon results in a transgene becoming rapidly non-functional after its insertion in the host genome, without alteration of its primary DNA sequence.

Epigenetic regulation of gene expression in plants has proven to be a multi-faceted, complex and versatile means of responding to environmental factors as well as controlling developmental processes. The regulation of gene expression through silencing impacts on diverse mechanisms such as plant development, virus and transposon inactivation and also transgene expression. Although methylation is not the only epigenetic mechanism to influence gene expression, it has been widely reported to occur (Paszkowski and Whitman, 2001). Both transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) appear to involve DNA hypermethylation in the promoter region for TGS, and in the coding region in the case of PTGS (Vaucheret and Fagard, 2001). Different enzymes are involved in this

phenomenon; Met1 (DNA Methyltransferase) is one of them and is present in sugarcane. Initial studies that were conducted on the methylation step showed that *Met* mutants (in *Arabidopsis*) were not able to maintain a silenced locus (Morel *et al*., 2000).

There is a clear link between PTGS, TGS and RNA interference; some of the steps are shared, although there are different mechanisms.

P1/Hc-Pro is a plant viral suppressor of RNA silencing identified in potyvirus. Original reports demonstrated that it suppresses both transgene- and viral-induced silencing (Anandalakshmi *et al*., 1998). It is however clear that it operates at a different level than methylation in the silencing pathway.

Although Met1 and P1/HcPro are differently involved in the gene silencing mechanisms, which are complex, we are investigating whether they could be used to prevent transgene silencing. On the one hand, the effects of down-regulation of the endogenous Met1 gene are being explored, and on the other hand the effects of expressing the P1/HcPro viral protein in transgenic sugarcane carrying reporter gene constructs will be assessed.

Experimental approach

The β-glucuronidase enzyme (GUS) 'reporter' system is being used to assess the two strategies for suppression of transgene silencing. This system allows for a relatively easy assay of the transgene activity. Various promoter-GUS constructs are being tested with and without additional constructs designed to impact on TGS.

The first strategy involved generating a construct that would suppress the formation of methylase transcripts in sugarcane, and therefore prevent the maintenance of DNA methylation. The construct is using the intron hairpin RNA (ihp-RNA) structure that was shown to fully knock out the production of corresponding transcripts (Wesley *et al*., 2001), and consequently inhibit protein production. The construct (pKO Met) includes the maize Ubiquitin promoter, 260bp fragment of the sugarcane Met1 sequence, a maize intron, the 260bp Met1 fragment in the reverse orientation and the Nos-terminator sequence.

The second strategy made use of pUbi P1/HcPro, a viral silencing suppression plasmid containing the sequence of the sorghum mosaic virus P1/HcPro, with the maize Ubiquitin promoter (personal communication[1]).

Sugarcane transformation

Table 1 highlights the different constructs and combinations of constructs that were used in the production of transgenic plants. Each promoter-GUS construct was co-bombarded into sugarcane with pEmuKN (selection construct), with pEmuKN and pUbi P1/HcPro, and with pEmuKN and pKO Met. Also incorporated in the experiment were controls that would assess the effects on the plants of the two plasmids pKO Met and pUbi P1/HcPro.

Molecular analysis of plants regenerated after bombardment is used to eliminate individuals that do not harbour the required transgenes. Selected plants go forward for histochemical and/or fluorometric GUS assays (enzymatic assays) to evaluate both the tissue-specificity and the strength of GUS expression.

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Table 1. Constructs used in the production of transgenic plants.

Bombard-ment series no	Promoter and specificity	GUS construct	Selection construct	pKO Met	pUbi P1/HcPro
1	S51 / STEM	✓	✓		
2		✓	✓	✓	
3		✓	✓		✓
4	E18 / STEM	✓	✓		
5		✓	✓	✓	
6		✓	✓		✓
7	G5.1 / YOUNG STEM	✓	✓		
8		✓	✓	✓	
9		✓	✓		✓
10	G5.2 / YOUNG STEM	✓	✓		
11		✓	✓	✓	
12		✓	✓		✓
13	A combo / ROOT	✓	✓		
14		✓	✓	✓	
15		✓	✓		✓
16	AS4 / ROOT	✓	✓		
17		✓	✓	✓	
18		✓	✓		✓
19	A10 / ROOT	✓	✓		
20		✓	✓	✓	
21		✓	✓		✓
22	Control HcPro		✓		✓
23	Control pKO Met		✓	✓	

Findings

Initial results will be presented and interpreted in the poster. Results will shed light on the possible mechanisms involved in gene silencing in sugarcane. More specific means of preventing this phenomenon could then be devised and applied (e.g. Kapoor *et al.*, 2005). The successful use of tissue-specific promoters is to a large degree dependent on overcoming the propensity of some plants such as sugarcane to silence transgenes.

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