A WILD-TYPE INSECTICIDAL GENE FROM A BACTERIUM IS POORLY EXPRESSED IN SUGARCANE: AN OVERVIEW.

GM MEYER[1], MB KEEPING[1], DA WATT[1], TN CASSIM[1,2], FC BOTHA[1,3] , BI HUCKETT[1,2,3]

[1] *South African Sugar Association Experiment Station, Private Bag X02, Mount Edgecombe, 4300*

[2] *School of Life and Environmental Sciences, University of Natal, King George V Avenue, Durban, 4041*

[3] *Institute for Plant Biotechnology, University of Stellenbosch, Private Bag X01, Matieland, 7602*

Abstract

Predation of sugarcane stalks by *Eldana saccharina* accounts for substantial yield losses in many cane growing areas in South Africa. Consequently, production of sugarcane able to withstand attack by this stalk borer is an important sugar industry goal. Certain genes from the bacterium *Bacillus thuringiensis* (Bt) produce toxins that specifically target lepidopteran insects, of which *E. saccharina* is one. In an attempt to confer resistance to this pest, a genetic construct containing a shortened version of a suitable wild-type Bt insecticidal gene was inserted into cultivar NCo310 embryogenic callus by particle bombardment. Molecular techniques demonstrated that the bacterial gene was integrated into the genome of 57 plants regenerated via tissue culture. However, further analysis revealed that the gene produced transcripts (mRNA) in only 80% of these transgenic plants. More striking was the observation that only a few full length transcripts, necessary for the synthesis of the insecticidal protein, were produced in all cases. Poor expression of the toxin in these plants was confirmed during eldana-resistance pot trials, where none of the lines displayed resistance to infestation by the insect. These results indicate that, while inserting native bacterial genes has the potential of conferring useful phenotypes to cane, bacterial genes often do not have the characteristics necessary for optimal expression in the molecular environment of the plant.

Introduction

Yield losses resulting from damage to sugarcane by the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) are severe in many cane growing areas in South Africa and several means of minimising the effects of this pest have been implemented. Eldana resistance was introduced as a selection criterion in the breeding programme in 1978 (Bond, 1988) and, while this approach has been successful, it does suffer from some important limitations. For example, eldana resistance in sugarcane is often positively correlated with undesirable traits such as high fibre (Rutherford et al., 1993). A further drawback is that selection for eldana-resistance occurs at late stages in the breeding programme, resulting in much valuable high-yielding germplasm being discarded (Butterfield and Thomas, 1996). In addition to breeding for eldana resistance, numerous agronomic practices, used routinely in the industry, aim to reduce losses incurred by the effects of eldana predation. Unfortunately, certain of these approaches may have negative effects on yield (e.g. early harvesting, reduced nitrogen fertilizer application). Consequently, the search for alternative ways of combating eldana is a major industry priority.

Bacillus thuringiensis (Bt) is a gram-positive bacterium that produces an insecticidal protein crystal on sporulation and the genes (*Cry* genes) responsible for producing toxin proteins have been characterised. It has been demonstrated that there are several categories of these toxin genes, with each having a relatively narrow insecticidal range e.g. *CryI* genes are active only against lepidopterans, such as eldana. As such bacterial toxin genes are relatively simple, they are amenable for genetically engineering insect resistance into higher plants. The successful implementation of such an approach in sugarcane would afford several advantages, *viz*. rescue of high-yielding clones discarded during selection, more widespread cultivation of high-yielding clones and harvesting cane at an optimum age for sucrose accumulation. Another advantage is that Bt cane would provide consistent eldana control, an important consideration given the unpredictability of eldana population dynamics.

The goals of this study were to introduce a Bt toxin gene with demonstrated activity against eldana into sugarcane and to assess the efficacy of the transgenic plants to resist predation by the insect. If successful, such an approach would serve as a valuable adjunct to current approaches used to minimise losses incurred by eldana.

Aproach and Outcomes

Transgene Constructs and Plant Transformation

A native *Bacillus thuringiensis* strain containing a *CryIA*(c) insecticidal gene was isolated from the soil of a cane field at Mount Edgecombe. The protein produced by this gene was shown to be particularly effective against *E. saccharina* (Herrera et al., 1994). Consequently, a shortened form of the gene was inserted into genetic constructs containing all the elements necessary for expression in sugarcane. A second genetic construct, containing the neomycin phosphotransferase II *(nptII)* gene conferring resistance to the antibiotic geneticin, was introduced together with the *CryIA(c)* constructs into embryogenic callus via a particle inflow gun. Subsequently, callus was subjected to selection on geneticin-containing medium and 60 plants were regenerated and transferred to the glasshouse for genetic assessment and eldana inoculation trials.

Evidence for presence of the CryIA(c) *gene*

To determine whether the *CryIA(c)* gene had been inserted into plants surviving selection on the antibiotic geneticin, DNA was extracted from glasshouse acclimated plants and subjected to polymerase chain reaction (PCR) analysis. Of the 60 plants tested, 57 were PCR positive, suggesting that the gene had been successfully inserted. Confirmation of these results was obtained by DNA hybridisation analysis, a more informative technique. Results indicated that the 57 plants contained multiple copies of the transgene, although in some cases the integration patterns were similar, suggesting that they might have originated from a single transformation event (Ingelbrecht et al., 1999).

Evidence for expression of the CryIA(c) *gene*

Evidence for the production of the *CryIA(c)* transgene mRNA via transcription was initially obtained from leaf material of the hardened off plants. Most (80%) of the transformants tested positive for transgene mRNA via the RT-PCR technique. However, this technique has limitations, such as being unable to indicate the *in vivo* size of the mRNA being amplified. Consequently, Northern blot analysis was undertaken to provide more information about the transgene transcripts. Analysis of a subset of the transformants revealed wide variation in band intensity (mRNA density). This variation may be a result of differences in transgene position in the genome, due to random integration following delivery by particle bombardment (Cassim et al., 1999). Transgenic plants also showed unusual multiple sizes of *CryIA(c)* mRNA. Although full length mRNA was present, it was clear that a large proportion of the molecules had not reached full length during transcription. Such premature termination of transcription has been reported by other researchers working with wild-type *Cry* genes (Diehn et al., 1998) and is interpreted as a manifestation of the inefficiency of bacterial gene transcription in a plant molecular environment.

Using immuno-hybridisation technology, the *CryIA(c)* protein was not detectable in any of the transgenic plants, supporting a causal link between the low levels of full-length transcript and undetectable levels of translation product. It has been shown that premature termination of wild type Bt gene transcripts is associated with mRNA instability and is a cause of poor expression in higher plant systems (Diehn et al., 1998; De Rocher et al., 1998). Poor expression of the *CryIA(c)* transgene in sugarcane was further evident from the results of eldana resistance pot trials in which none of the transgenic plants displayed significantly increased resistance to predation by the insect.

Conclusion

The results of this study show that while some bacterial genes encode products that confer useful traits to plants, they may be affected by factors that prevent optimal expression in plants. Polyadenylation signals found in bacteria, together with other AT-rich motifs and rare codons are recognised to be inhibitory to expression in the plant molecular environment (Diehn et al., 1998). Such barriers have resulted in bacterial gene sequences being altered to suit plant usage while maintaining the structure, and therefore function, of the encoded protein. An example of this is insertion of a resynthesised *Cry* gene into cotton, resulting in large increases in transgene expression in the host plant (Perlak et al., 1991). Commercial genetic engineering programmes for insect resistance now use resynthesised *Cry* genes. In keeping with this approach, the Biotechnology Department at SASEX is incorporating resynthesised genes into the current eldana programme.

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