

BUSTER RESISTANT SUGARCANE

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Abstract

Herbicide treatment of crops allows economic weed control. To be useful, herbicides must distinguish between crop plant and weed, and this often limits usage. Engineering herbicide resistance into crop plants via genetic transformation techniques is a novel approach to an age old problem. A gene construct which confers resistance to the herbicide Buster^R (active ingredient glufosinate ammonium), was obtained for research purposes from AgrEvo, Germany. Using the technique of microprojectile bombardment for DNA transfer and embryogenic callus as the recipient material, herbicide resistance was engineered into sugarcane variety NCo310. Young transgenic cane plants grown in the glasshouse were asymptomatic when sprayed with 4 L/ha of the herbicide. Nontransformed NCo310 plants showed phytotoxic symptoms within days of being sprayed and plants died after three weeks. Further evaluation at a small scale field trial showed the transgenic plants to be resistant to a rate of 7 L/ha Buster after repeated applications on a plant crop and two ratoons.

Keywords: herbicide resistance, transgenic sugarcane

Introduction

Herbicide resistant crops represent one of the first and most highly publicised applications of plant biotechnology. Of the many traits that can be altered or conferred through biotechnology, herbicide resistance was chosen for two primary reasons. First, the biochemistry and genetics of several mechanisms of herbicide resistance were already understood. Secondly, agrochemical companies, whose considerable research budgets permitted rapid advances to be made, carried out much of the research and development work because they predicted sufficient economic returns from associated herbicide sales to justify their investments. The genes responsible for resistance have been isolated, characterised and inserted into several major crops. Herbicide resistance was the first trait introduced into cane using genetic engineering at the South African Sugar Association Experiment Station (SASEX).

NCo310 was the first variety to be genetically engineered at SASEX. Successful transformation of sugarcane is dependent on white embryogenic callus as the target tissue and NCo310 produces large amounts of this type of callus in culture. It was the variety used in early sugarcane transformation experiments carried out in the USA by Gallo-Meagher and Irvine (1993; 1996).

The aim of this paper is to describe the evaluation of engineered herbicide resistance in transgenic sugarcane of variety NCo310 under field conditions.

Experimental approach

Production of transgenic sugarcane in vitro

Sugarcane variety NCo310 was transformed by microprojectile bombardment of embryogenic callus (Snyman *et al.*, 1996). The DNA vector used to transform callus contained the synthetic *pat* gene which confers resistance to the herbicide Buster (active ingredient: 200 g/L glufosinate ammonium; AgrEvo, Germany). Plants from three independent bombardment events were analysed at the DNA level according to methods described by Gallo-Meagher and Irvine (1996). One of the plants was chosen for further evaluation in the field as it was resistant to 4 L/ha herbicide when sprayed in the glasshouse.

Field evaluation of transgenic sugarcane

At present, transgenic plants require regulatory approval before small scale field tests can be initiated. The South African Committee for Genetic Experimentation (SAGENE) gave authorisation for the field trial, provided that the trial site was secure and that access was limited to certain staff members. One of the conditions was that the transgenic cane had to be destroyed by incineration after the trial. Replicate plants of transgenic individuals are contained in a glasshouse with restricted access.

Buster was applied in approximately 200 L water/ha with a hydraulically operated knapsack fitted with a Teejet nozzle (110°) operating at one bar pressure. One to seven litres of Buster/ha in increments of one liter was applied to the plant crop three months after planting. Cane was cut back and allowed to ratoon normally. The two ratoon crops received a single application of 7 L/ha herbicide resulting in some of the transgenic cane receiving a total of 21 L/ha from plant to second ratoon. Applications were made at six and four months after removing the plant and first ratoon respectively.

Results and Discussion

Preliminary DNA analysis of the transgenic plants in the laboratory indicates that the *pat* gene is present in all three of the clones produced (¹unpublished data). One transgenic

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clone was chosen for field evaluation of gene expression based on its resistance to Buster (4 L/ha) in the glasshouse.

Transgenic cane in the field appeared unharmed by three repeated herbicide applications (Figure 1), with no evidence of accumulated damage over time. This indicates that the introduced gene is stably integrated and is expressed during successive ratoons of the crop. The non-transformed plant cane displayed herbicide phytotoxicity at all rates tested, but damage was extreme beyond rates of 4 L/ha. Regeneration of the non-transformed cane in the first ratoon was complete for the range of rates applied to the plant crop. However, severe population losses in the second ratoon were evident in non-transformed cane that had initially received the high rates in the plant crop (5-7 L/ha).

Gallo-Meagher and Irvine (1996) were the first to report on the production of herbicide resistant transgenic sugarcane using a gene similar to the *pat* gene used in this study. Although their analysis of the genetically engineered plants at the DNA level was extensive, they did not present results of field testing the plants for resistance to the herbicide Ignite (active ingredient: 6 g/L glufosinate ammonium).

Although NCo310 is no longer a major commercial variety in the South African sugar industry, its successful use in this study has paved the way for similar technology to be used in existing commercially grown varieties.

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Figure 1. Transgenic sugarcane plants of variety NCo310. The herbicide Buster (AgrEvo, Germany) has no effect on genetically engineered cane, which grows vigorously and displays no phytotoxic reaction. The effect on the non-transgenic sugarcane is deleterious, particularly at rates above 4 L/ha.