

POSTER SUMMARY

PURSUIING HERBICIDE TOLERANCE IN SUGARCANE: SCREENING GERMPLASM AND INDUCTION THROUGH MUTAGENESISKOCH AC^{1,2}, RAMGAREEB S¹, SNYMAN SJ^{1,2}, WATT MP² and RUTHERFORD RS¹¹South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300, South Africa²School of Biological and Conservational Sciences, University of KwaZulu-Natal, Durban, South Africaaimee.koch@sugar.org.za sumita.ramgareeb@sugar.org.za sandy.snyman@sugar.org.za
wattm@ukzn.ac.za stuart.rutherford@sugar.org.za**Abstract**

Herbicide tolerance is highly desirable in commercial sugarcane. This study explores two strategies for the production of sugarcane tolerant to imazapyr, *viz.* (i) screening populations from breeding crosses for naturally occurring tolerant genotypes, and (ii) producing tolerant genotypes through *in vitro* cell mutagenesis. In the first, over 11 000 seedlings were sprayed with 0.1-1.5 L/ha Arsenal (250 g/L active ingredient - imazapyr), after which 1.25 L/ha Arsenal was selected to test 12 000 seedlings. The second approach exploited the regeneration of herbicide tolerant plants through induced somaclonal variation. Somatic embryogenesis calli of N12 were screened for somaclonal variant tolerance to imazapyr, which may have resulted from 2,4-dichlorophenoxyacetic acid (2,4-D) in the culture medium. In addition, embryogenic calli were exposed to the mutagen ethyl methanesulfonate (EMS; 8 - 96.6 mM for 4 hours). Ongoing work includes regenerating potentially tolerant cells into plants on selection medium (0.042 μ M and 0.08 μ M imazapyr) after exposure to EMS (8 mM and 16 mM). The surviving plants will be acclimatised in the greenhouse and sprayed with Arsenal to confirm tolerance.

Keywords: sugarcane, herbicide tolerance, imazapyr, chemical mutagen, ethyl methanesulfonate, somaclonal variation

Introduction

The sugar industry is constantly driven by the need for increased output; however, many biotic and abiotic factors contribute to a reduction in crop yield (Dissanayake *et al.*, 1998). Regarding weed control, the major problem is that many of the broad spectrum herbicides that are effective in the control of grasses and broadleaf weeds (Mulwa and Mwanza, 2006) also inhibit growth of sugarcane (personal communication¹).

The aim of this study was to identify and induce sugarcane lines tolerant to the herbicide imazapyr for use by the South African Sugarcane Research Institute (SASRI) Plant Breeding programme. The first objective was to screen existing sugarcane germplasm by spraying three month old seedlings with a range of Arsenal (imazapyr 250 g/L) concentrations, followed by identification of putative tolerant individuals. The second objective was based on the knowledge that a single target-site mutation in the acetolactate synthase (ALS) gene confers tolerance to ALS-inhibiting herbicides and that certain *in vitro* culture conditions (e.g. 2,4-D)

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increase the potential for small mutations and mitotic mistakes. This results in somaclonal variant cells, which can then be regenerated into variant plants (Larkin and Scowcroft, 1981). Examples in sugarcane include the generation of cell lines in medium containing 0.8 mM glyphosate, which then yielded plants with a five-fold tolerance to the herbicide (Zambrano *et al.*, 2003). This approach can be further enhanced by mutagenic agents (e.g. ethyl methanesulfonate), which increase the chance of somaclonal variation (Van Harten, 1998).

Approach and Findings

Screening of existing germplasm for herbicide tolerance to imazapyr

Approximately 11 000 three month-old seedlings from 64 parental crosses were sprayed with 0-1.5 L/ha Arsenal. At concentrations above 0.8 L/ha, Arsenal caused a significant reduction in above-ground dry plant mass compared with unsprayed controls (Figure 1). A 90% reduction of the seedling population (LD₉₀) was observed at 1.25 L/ha, the treatment selected for the ongoing testing of seedlings from 96 crosses. Any potentially tolerant seedlings will be further assessed (screening the ALS region and ALS enzyme assays) to determine if naturally occurring imazapyr tolerance is associated with particular parental crosses.

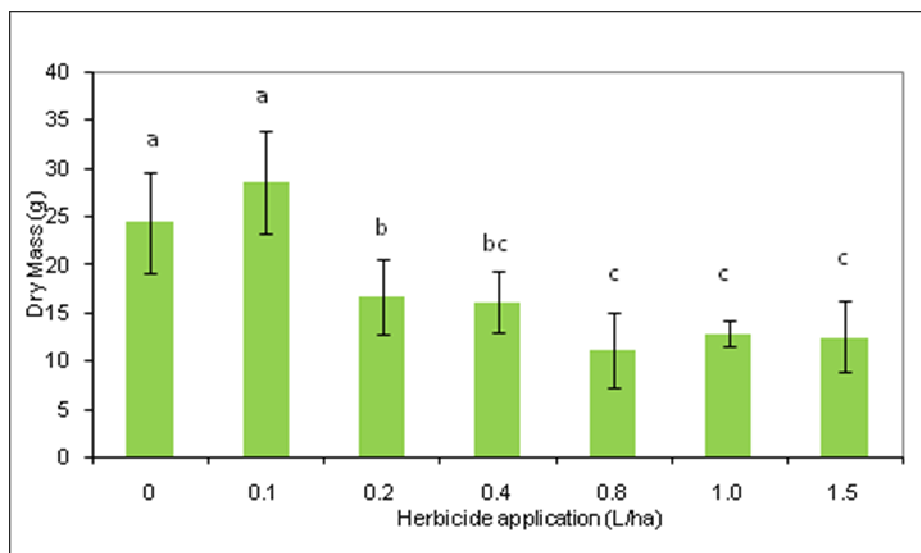


Figure 1. The effect of Arsenal on above-ground dry mass of sugarcane seedlings, 47 days after the spraying treatment.

Bar graph with standard error bars (n=7-8 seedling trays, each tray contains approximately 200 seedlings). Dissimilar alphabet characters denote a statistical significance ($p < 0.05$).

Somaclonal variation for induction of herbicide tolerance in N12

The strategy employed in this study is to induce somaclonal variation in embryogenic callus cells of N12 (Snyman, 2004) by EMS exposure, followed by selection of imazapyr tolerant cells and then selection of plants regenerated from them. Preliminary studies included:

- (1) Callus cells were exposed to 0-96.6 mM EMS for four hours (0 reflecting somaclonal variation expected from 2,4-D) and placed on medium without imazapyr. Based on the percentage of regenerated plants that were green and healthy (high), albino (low) and

chimeric (low), 8 mM and 16 mM EMS were the concentrations chosen for further studies.

- (2) Callus cells, not treated with EMS, were exposed to 0-0.12 μM imazapyr, with 0.042 μM imazapyr resulting in 50% inhibition of plantlet regeneration (LD_{50}) (Figure 2).

The extent of mutations caused by treatments (1) and (2) alone is being assessed by amplified fragment length polymorphism analyses. This aims to link the herbicide tolerant phenotype with the desired genotypic single base pair mutation. The combination of treatments (1) and (2) is being tested for variant plant production, followed by acclimatisation of the plantlets that survive, and herbicide application in the greenhouse.

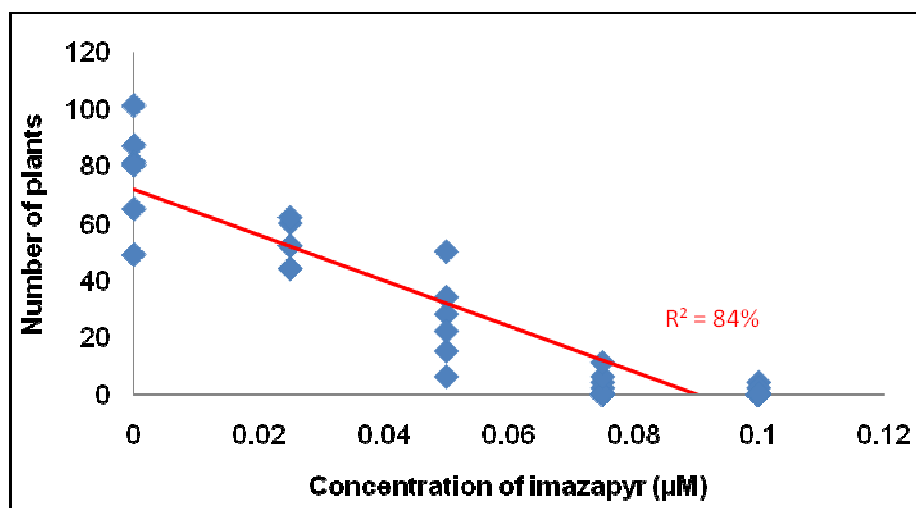


Figure 2. The influence of increasing concentrations of imazapyr on plant regeneration during *in vitro* culture of sugarcane.

Plants were counted 12 weeks after embryogenic callus was placed on germination medium. Regression analysis revealed that within-treatment variation was low and that the concentration range used was appropriate to calculate a $\text{LD}_{50} = 0.042 \mu\text{M}$ ($R^2=84\%$).

Concluding remarks

Although herbicide tolerant plants have not yet been identified, parameters were established for (i) herbicide application to seedlings, (ii) EMS dosage treatment for induced mutagenesis, and (iii) *in vitro* imazapyr selection of mutated cells. Results indicate that 2,4-D in the medium does not induce much somaclonal variation; treatment with EMS appears essential to generate large numbers of mutants for screening.

The final outcome of this study may identify herbicide tolerant genotypes of use to the breeding programme. Further, such an approach could be adapted to obtain other useful variants (e.g. rust tolerance) in sugarcane.

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