

BIOTECHNOLOGICAL INTERVENTION

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General introduction

There are two primary strategic areas of biotechnology research and development at SASEX – **molecular markers** and **genetic engineering**. All of the current biotechnology projects fall under these two umbrellas. There is a further area which has potential, but which is so far unresearched and is therefore of very secondary concern – **the development of alternative products**. This is a topic which has aroused considerable interest recently, and is the subject of a theoretical paper presented at the current SASTA Congress (Groenewald *et al.*, 1995).

An overview of molecular markers

The overall purpose of the marker approach is to maximise use of favourable genetic characters already present in sugarcane. This is achieved by identifying characteristic molecular indicators ('markers') of important traits which can be used to develop diagnostic tests for application in the variety selection programme. There are two important types of molecular marker being investigated and established in the SASEX Biotechnology programme: metabolic product profiles and DNA (genetic) markers.

Metabolic product profiles are phenotypic markers which represent metabolic products that are in some way related to an important characteristic expressed in the growing plant; for example, chromatographic profiles of complex secondary products in sugarcane like fibre, wax or flavonoids, substances which appear to contribute to field resistance to eldana. Figure 1 shows an example of a gas chromatographic analysis of stalk wax from two varieties, N20 and N14, which differ markedly in their response to eldana: N20 is relatively resistant and N14 susceptible. Correlated with the difference in phenotype are differences in the appearance of the profile: in N20 there is a high peak 6 which represents a C28 alcohol, and an high alcohol/aldehyde ratio among the various component peaks, while in N14 both peak 6 and the overall

alcohol/aldehyde ratio are low. These distinctive differences appear from multiple regression predictive models to be diagnostic for the eldana resistance character.

DNA (genetic) markers are small segments of the basic genetic material from the chromosomes which can be identified as characteristic for certain varieties. DNA markers are generally visualised as specific bands within a banding profile resulting from gel electrophoretic separation of a wide range of DNA fragments (loci) that have been highlighted from the genome (Figure 2). Such markers have the potential to be shown to be linked to important traits such as fibre, disease resistance, and self trashing. Once linkage has been established, the marker begins to be useful in the breeding and selection programme.

The closer one moves from gene products (phenotypic markers) to the genetic material itself (DNA markers), the greater the advantage of the marker approach. Advantages of genetic markers include (1) the fact that diagnosis is independent of environment or stage of development, and (2) the potential for accurate diagnosis of characteristics using extremely small tissue samples. These two factors combine to provide the prospect of (3) very early screening of seedlings or young plants, and the concomitant capacity for greater efficiency of selection in the preliminary stages of the breeding programme.

Genetic markers

Because of their innate advantages, it is strategically opportune to concentrate current and future efforts on the DNA based genetic markers. DNA markers can be identified using a number of techniques. At SASEX the PCR-RAPD approach is used. This employs the Polymerase Chain Reaction (PCR) to amplify and thereby highlight an extensive range of genetic loci using an array of decamer (10-base) primers of random sequence (Random Amplification of Po-

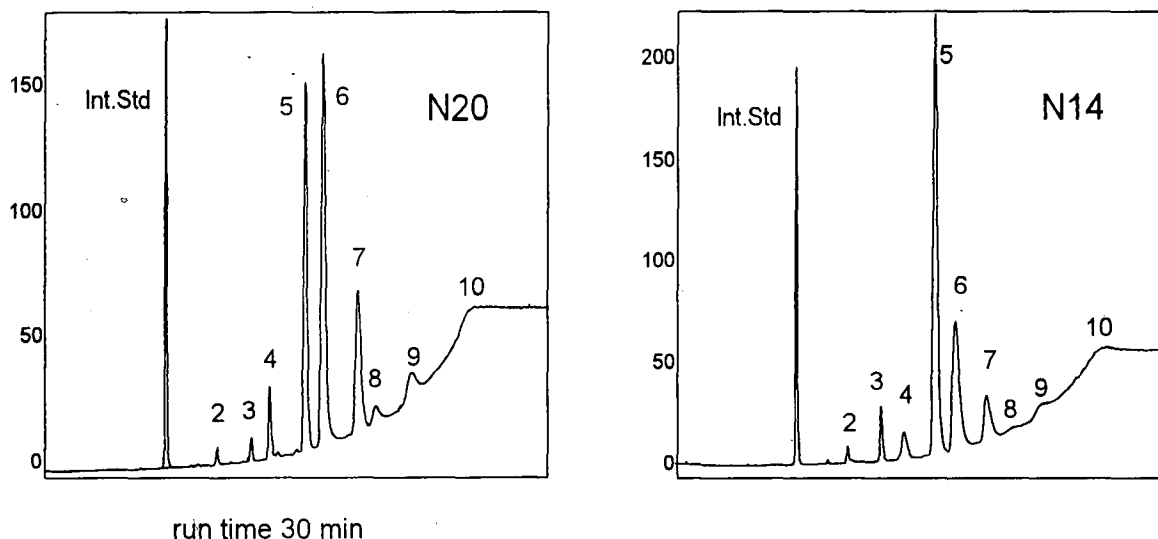


FIGURE 1 Gas chromatograms of stalk surface wax from eldana resistant (N20) and eldana susceptible (N14) commercial sugarcane clones.

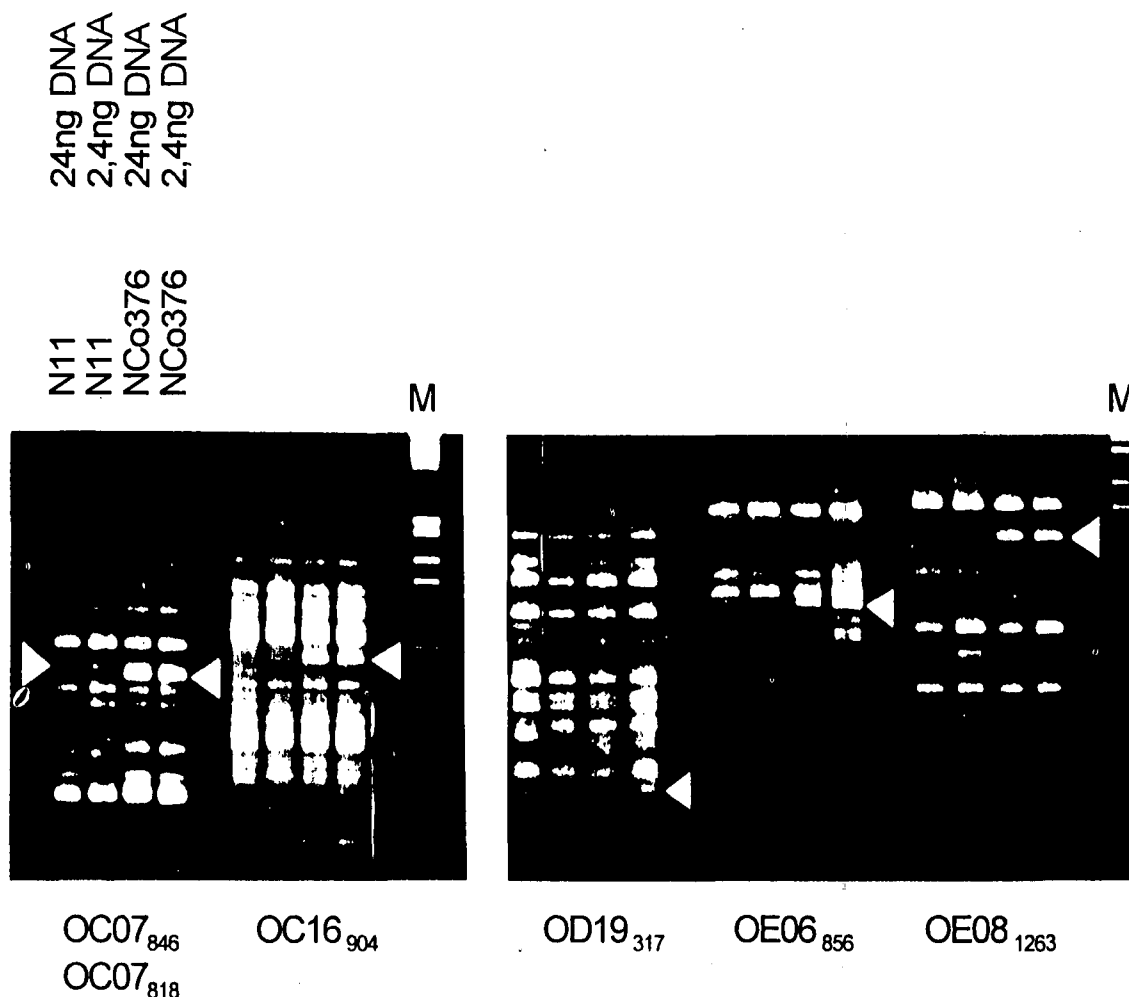


FIGURE 2 Examples of genetic loci shown by PCR-RAPD analysis to be markers (polymorphic) between sugarcane varieties N11 and NCo376.

Each set of four lanes represents two N11 analyses adjacent to two NCo376 analyses. For each variety, two PCR-RAPD reactions were conducted at genomic DNA concentrations differing by an order of magnitude (24 and 2.4ng DNA per reaction respectively). Loci were considered to be markers (polymorphic) only when a fragment was present at both template concentrations in the one variety and absent at both concentrations in the other variety. Markers were given designations using a convention in which the main symbols (e.g. OC16) indicate the specific Operon random decamer primer used to generate the fingerprint profile and the subscript indicates the size (bp) of the polymorphic fragment. M = Lambda - *Eco* RI + *Hind* III molecular weight standards. All DNA fragment sizes were determined using WinCam 2.1 gel image analysis software (Cybertech).

lymorphic DNA; RAPD). Each primer sequence is unique and amplifies a unique, reproducible set of DNA fragments which may be analysed by gel electrophoresis as a 'fingerprint'; fingerprints may be compared between varieties and the characteristic marker fragments (polymorphisms) identified (Figure 2). One of the positive features of the RAPD method is that it can be applied to an unmapped, relatively unsequenced genome like that of sugarcane.

There are a number of limiting factors in the development of genetic markers in sugarcane, some of which can be addressed by devising appropriate experimental strategies (Table 1). Of prime importance is the current need for a suitable population of offspring from a single cross segregating for several important traits like smut and mosaic resistance which can be used for the establishment of linkage between DNA markers and phenotype. This requirement has led to the decision to develop the SASEX AA40 population: a population of 150 offspring from the cross N18 × CP57/614 in which each individual is to be taken to variety trial status at Mount Edgecombe. The vision here is that the Biotechnology department will do the genetic analysis leading to the isolation of potential markers, while other departments such as Pathology, Entomology and Agronomy

will undertake rigorous assessments of phenotypic character which would allow linkage to be determined accurately. As in many biotechnological endeavours, in this particular exercise the strategy must be multidisciplinary. The genetic effort without the phenotype characterisation – or vice-versa – would be ineffectual.

Looking beyond the research itself, towards the development and future practical application of molecular markers in the production of new varieties for the sugar industry, there is the need to prepare for the impact of routine screening of seedlings and/or parent varieties by planning with Plant Breeding for technology transfer to a service department.

Genetic engineering

The overall purpose of the second major area of Biotechnology effort at SASEX, that of genetic engineering, is to introduce favourable novel genetic characters into sugarcane. The state of gene transfer technology is such that the novel genes involved can be from almost any biological source – plant, animal or microbial. The advantages of the approach are that (1) there is evidence from all over the

Table 1

Appropriate research strategies to address current limitations of the genetic marker approach

| Limitations | Relevant strategies |
|--|--|
| Poor knowledge of the origin of traits and basic sugarcane genetics e.g. whether genes are single or multiple, dominant or recessive, and the nature of QTLs. | Encouragement and support of fundamental work on sugarcane genetics. |
| Many RAPD markers identified appear to be single dose (only one copy exists in the whole genome) and these are possibly limited in their usefulness in screening the offspring of a polyploid like sugarcane. | Estimation of gene copy number wherever possible so as to build up a better picture of the gene dosage phenomenon in sugarcane. |
| Appears to be low genetic variation in the <i>S. officinarum</i> component of the genome, signifying that genes from the minor <i>S. spontaneum</i> component might be the only ones that can be exploited. | Routine use of <i>S. officinarum</i> and <i>S. spontaneum</i> DNA samples as references in all genetic analyses. |
| Some uncertainty expressed in the literature concerning the stability of DNA-RAPD markers outside of the immediate genetic environment from which they have been isolated. | Conversion of RAPDs to SCARS (Sequence Characterised Amplified Regions) i.e. sequence analysis of DNA-RAPD markers and the design of longer primers for specific PCR amplification of the locus in a stable manner in a wide range of germplasm. |
| Lack of suitable experimental populations for establishing linkage between DNA markers and phenotypic characters (suitable or "good" population is one consisting of many widely differing, well characterised offspring from a single cross). | Development of the SASEX AA40 population: 150 individual offspring from the cross N18 × CP57/614 in which each individual is to be taken to variety trial status. International collaboration with sugarcane research groups that already have appropriately characterised populations. |

Table 2

Limitations and strategic needs in sugarcane genetic engineering

| Limitations | Relevant strategies in relation to [Current status of research] |
|---|--|
| There is a limited number of varieties transformable at high efficiency. NCo310 is the best variety, with NCo376 being a close second. It is possible to transform any variety, but unless the efficiency is high, the exercise is unproductive in the long term. | Continue studies designed to extend the range of transformable varieties. [One current project concentrates on ancestral relatives and offspring of "good" variety NCo310.] |
| Limited number of currently available, useful genes. | Put more resources into studies based on search for novel antipest/ antifungal/ antibacterial agents and, by implication, their genes. [Only one current project in this area.] |
| Lack of promoters (gene switches) from sugarcane to target expression to particular parts of the plant. | Initiate project on Expressed Sequence Tags (ESTs) and introduce new sequencing technologies to provide more rapid access to expressed DNA sequences. [One current project is committed to the isolation of a sugarcane stem-specific promoter via differential display of cDNA.] |
| Dynamic state of pathogen-host interactions means that knowledge of pathogens and the host genes that control them will always be imperfect. | Reinforce collaboration with Pathology department. [Interactive projects already exist] |
| Biochemistry of sugarcane is not well characterised; hence it is not easy to pinpoint the genes controlling important metabolic processes such as sucrose accumulation. | Foster fundamental research in this area, particularly by communication and collaboration with international groups working on sugarcane biochemistry and sucrose metabolism. [Current projects on PFP and neutral invertase characterisation respectively.] |

world that commercial benefits can accrue from recombination of genes in this way, and (2) the techniques for producing transgenic plants are well established in our own laboratories using gene delivery to sugarcane callus cells by particle bombardment followed by regeneration of transformed somatic embryos under genetically controlled selection conditions.

The main strategic difficulties associated with genetic engineering are outlined in Table 2. They focus on the medium- to long-term need for a continuous supply of new genetic resources: transformable germplasm as well as genes and promoters (gene switches) of value in sugarcane. Factors which exacerbate these problems include (1) the fact that

knowledge of pathogens and the host genes that control them will always be incomplete, because of the dynamic evolutionary state of pathogenic interactions, and (2) the impossibility of pinpointing the genes controlling important metabolic functions such as sucrose accumulation when the relevant enzymatic pathways in sugarcane are not well characterised.

Currently, the SASEX research programme makes use of the *ubi* and *emu* promoter constructs, particularly the former, which are based largely on sequences from maize, and genes from bacterial sources such as *nptII* (for antibiotic resistance selection in culture), *bar* and *pat* (for resistance to ammonium glufosinate herbicides). These have been ob-

tained from outside sources. In addition there is the locally isolated and characterised bacterial toxin gene denoted ER1 (for eldana resistance). In the development stage at SASEX are a modified (synthetic) ER1 heterologue (ER2), designed for increased efficiency of toxin expression in sugarcane, an antisense PFP gene (with the potential for the enhancement of sucrose accumulation), and the coat protein gene of sugarcane mosaic virus (for the induction of mosaic resistance).

In order to ensure continued provision of genetic resources for the future, the acceleration of the in-house search for sugarcane genes and promoters is essential. It is recognised that a major key to accomplishing this is the capacity to sequence DNA quickly and accurately, particularly DNA that is expressed. For this reason, new sequencing technologies are being investigated and a new project initiated which will develop a database of sugarcane Expressed Sequence Tags (ESTs) derived from messenger RNA sequences. Identification of these sequences as parts of known genes, homologous sequences or novel genes will then be possible by comparison against international database information.

Alternative products

The concept of using biotechnology to develop marketable non-sucrose products in sugarcane is attractive, since there is a significant amount of sugarcane biomass which goes to waste at the present time and an apparent potential for creating additional sources of income for growers and millers. However, the limitations are enormous: lack of knowledge of sugarcane biochemistry and metabolism is a huge barrier to intelligent choice of specific products to be researched and developed; in addition, regardless of site of storage in the plant, new products are likely to be synthesised at the expense of sucrose and, furthermore, coordinated multidisciplinary effort and considerable venture capital would be required (Groenewald *et al.*, 1995). For these reasons it is not yet appropriate to consider specific strategies in this area. However, it is clear that alternative product development would be inadvisable without long term value being put on achieving a better understanding of sugarcane chemistry and genetics.

Potential benefit of national programme on basic sugarcane biology

The need for fundamental studies in genetics and biochemistry which will lead to improved knowledge of the crop is an important common element in strategic planning for all of the SASEX Biotechnology programmes: those involving molecular markers (Table 1) and genetic engineering (Table 2) as well the theoretical one of alternative product development. Hence, some kind of broad solution to this problem would appear to be required. Possible strategies include (1) more collaborative work with Universities and Technikons and (2) an approach which puts pressure on the FRD or other government agencies to create, sponsor and administer a national programme on sugarcane research and development beyond the bounds of SASA.

Summary and conclusions

The strategies that have been devised to direct the contribution of Biotechnology to future sugar research are all channelled towards the core activity at the Station: the production of new varieties. The in-house Biotechnology programme itself is designed to provide new technologies (marker-assisted selection) and novel germplasm (transgenic sugarcane) to the Plant Breeders in this central role, and in doing so it requires the collaboration of a range of expertise in other departments. Within a broader context, Biotechnology does have access to expertise from various international sources, and is involved in regular global information exchange, but as yet there is no directly relevant national research programme. In conclusion, it must be stated that, for all of these strategies to be focused appropriately and precisely, clear analyses of industry needs and goals from both the millers and growers are essential.

REFERENCES

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