

# USE OF THE POLYMERASE CHAIN REACTION (PCR) AND RANDOM AMPLIFICATION OF POLYMORPHIC DNAs (RAPDs) FOR THE DETERMINATION OF GENETIC DISTANCES BETWEEN 21 SUGARCANE VARIETIES

M HARVEY<sup>1</sup>, BI HUCKETT<sup>1</sup> AND FC BOTHA<sup>1,2</sup>

<sup>1</sup> South African Sugar Association Experiment Station, Mount Edgecombe

<sup>2</sup> University of Natal

## Abstract

DNA was extracted from 20 interspecific sugarcane hybrids and one *Saccharum spontaneum* variety and analysed with the PCR-RAPD methodology. A total of 41 random 10-mer primers were used in the amplification reactions and these generated 356 loci. A subset of these (160 loci) was used to calculate genetic distances with the computer program NTSYS-pc and a formula devised by Nei and Li (1979). Similarity between the hybrid varieties ranged from 71,82 to 89,73%. This suggests that there is very limited genetic diversity within this group, especially as the calculated values were skewed towards maximum variation. When the same calculations were carried out between one of the hybrids (84F3097) and *S. spontaneum* variety US66-5615 the similarity was 30,4%, confirming that a much greater diversity exists between sugarcane species than within the hybrid varieties grown commercially.

## Introduction

In South Africa, as in other parts of the world, most of the sugarcane varieties grown commercially are descended from early crosses between *Saccharum officinarum*, *S. spontaneum* and *S. barberi*. Subsequently, there have been about six generations of in-breeding between the hybrids to produce the varieties grown today. Many of these are susceptible to a range of pests and diseases such as SCMV (sugarcane mosaic virus) (Bailey and Fox, 1980; 1987), RSD (ratoon stunting disease) (Steindl, 1961) and the lepidopteran borer *Eldana saccharina* (Nuss *et al.*, 1986). These factors, together with adverse environmental conditions, seriously hamper cane growth and reduce sucrose yield. Therefore, the need exists to introduce new sugarcane varieties which are able to thrive under a range of growing conditions and withstand a wide range of pests and diseases.

New plant varieties are usually bred by crossing parents which are genetically dissimilar. This introduces heterosis into the breeding population and allows selection of progeny with improved phenotypic characteristics. However, problems have been encountered in sugarcane breeding in the identification of suitable parent material. Little is known about the structure of the sugarcane genome (Roach and Daniels, 1987) and as a result, genetic distances between varieties cannot be calculated by comparing the nucleotide sequences of conserved gene regions. However, a recently developed methodology known as Random Amplification of Polymorphic DNAs (RAPDs) may be carried out with no prior knowledge of the genome (Welsh and McClelland, 1990; Williams *et al.*, 1990). This technique is based on the Polymerase Chain Reaction (PCR) amplification of random DNA segments in the presence of arbitrary primer sequences, and has been used to identify plant cultivars (Yang

and Quiros, 1993), determine paternity (Harada *et al.*, 1993), characterise genetic markers for disease resistance (Miklas *et al.*, 1993) and measure the degree of difference/similarity between varieties (Huff *et al.*, 1993). In fact, RAPDs have facilitated the direct calculation of genetic distances between many plant varieties and cultivars, for example, of apple (Harada *et al.*, 1993), buffalograss (Huff *et al.*, 1993), aquatic fern (Van Coppenolle *et al.*, 1993) and tomato (Williams and St. Clair, 1993).

This paper describes preliminary investigations which were carried out into the use of the PCR-RAPD technology for the detection of genetic diversity in sugarcane.

## Materials and methods

### Plant material

Twenty sugarcane varieties were used in this study (Table 1). All are interspecific hybrids and all but one are current or former released varieties or advanced in the selection programme. These were selected on the basis of phenotypic characteristics considered to be important for sugarcane breeding in South Africa. DNA analysis was carried out also with *S. spontaneum* variety US66-5615.

### DNA extraction

DNA was extracted from sugarcane using a protocol modified from Honeycutt *et al.* (1992). Approximately 3-4 g of leaf roll material was homogenised with an ultra turrax in 20 ml of ice-cold buffer (50 mM Trishydroxymethylamino-methane hydrochloride (Tris-HCl), pH 8,0, 5 mM ethylenediaminetetraacetic acid (EDTA), 0,5 mM spermidine, 1% (w/v) polyethyleneglycol (8 000), 0,1% (v/v) 2-mercaptoethanol and 0,35 M sucrose). After tissue disruption the extract was filtered through two layers of mutton cloth and centrifuged at 5 000 g for 20 min. The supernatant was then discarded, the pellet resuspended in 5 ml of wash buffer (50 mM Tris-HCl, pH 8,0, 25 mM EDTA, 0,5 mM spermidine, 0,1% (v/v) 2-mercaptoethanol and 0,35 M sucrose), and placed on ice. NaCl was added to the samples at a final concentration of 0,8 M. After further additions of sodium-dodecyl sulphate (SDS) (final concentration 0,7% (w/v)) and cetyltrimethylammonium bromide (CTAB) (final concentration 0,9% (w/v)), extracts were incubated at 60°C for 30-40 min and then allowed to cool at room temperature for 15 min. Protein was removed by partitioning in chloroform:isoamyl alcohol (24:1) and centrifugation at 3 500 g for 10 min. This was repeated before the DNA was precipitated from the aqueous phase by the addition of an equal volume of isopropanol. DNA strands were then spooled out of the solution with a glass hook and dissolved in 1 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7,5). DNA stocks of 3 ng/ $\mu$ l were made up for subsequent use in the PCR reactions.

Table 1

Phenotypic profiles of the 20 sugarcane hybrids selected for use in this study. Selection was based on the ratings obtained for various characteristics considered to be important in sugarcane breeding. Scores range from 1 (very favourable) to 9 (unfavourable)

Variety	Sucrose yield	Sucrose % cane	Smut	Mosaic	Leaf scald	Rust	Eldana
CB38-22	6	4	2	2	2	2	2
CB40-35	5	4	2	2	1	8	8
Co331	6	6	5	5	2	2	2
NCo310	6	2	9	5	1	1	5
NCo376	5	5	8	9	1	2	5
N12	4	4	5	5	2	2	3
N14	4	4	2	5	2	5	8
N16	4	3	8	5	2	1	7
N17	5	4	1	5	1	2	3
N19	4	3	2	8	1	2	5
N21	5	4	2	2	5	2	1
N24	3	1	2	2	2	2	6
74L775	5	3	5	1	2	5	5
75E247	3	2	2	9	8	5	3
77F790	3	2	5	2	2	1	6
77L1143	4	3	5	2	2	5	8
78F1025	4	3	5	2	5	5	5
79M955	5	2	8	2	2	1	6
80L432	5	3	2	2	2	2	8
84F3097	3	4	5	5	2	1	5

*DNA quantification and purity determination*

DNA concentrations and purities were measured spectrophotometrically at 260 and 280 nm. Aliquots of all DNA extracts were electrophoresed in 1% (w/v) agarose gels at 5,6 V/cm in TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA, pH 8,0) and the gels stained with ethidium bromide (0,5 µg/ml). After destaining, the banding intensity of genomic DNA fragments was compared to that of known standards in order to confirm the DNA concentration. These gels also provided a visual measure of the purity and integrity of the DNA.

*PCR-RAPD techniques*

Samples of DNA, extracted from each of the sugarcane varieties, were PCR amplified in the presence of random 10-mer primers with a G-C content of 60-70% (Operon Technologies). Each RAPD reaction mixture contained 10 mM Tris-HCl (pH 8,3), 10 mM KCl, 4 mM MgCl<sub>2</sub>, 0,1 mM each of dATP, dCTP, dTTP and dGTP, 0,2 µM primer, 15-24 ng template DNA and 1 U Taq polymerase Stoffel fragment. The Hybaid Omnigene thermal cycler was programmed for 1 cycle of 3 min at 94°C, 1 min at 35°C and 2 min at 72°C with a 2,4°C/sec ramp, followed by 40 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C with a 2,4°C/sec ramp, and a last cycle of 72°C for 7 min.

On completion of the amplification cycles, the PCR products were electrophoresed in 2% (w/v) agarose gels at 5,6 V/cm in TBE buffer. After staining in ethidium bromide (1 µg/ml) the gels were scored for presence (1) or absence (0) of intensely stained DNA bands. This scoring was carried out manually. The data were then analysed with the computer software program NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, written by FJ Rohlf, 1993), for the construction of a phenogram according to the UPGMA method (unweighted pair group method with an arithmetic mean). Genetic distances were calculated with a formula modified from Nei and Li (1979):

$$\%S AB = (2 (N AB) / N A + N B) \times 100$$

where %S AB is the degree of similarity between individuals A and B (expressed as a percentage), N A and N B are the number of fragments scored for individuals A and B respectively, and N AB is the number of fragments shared by A and B.

**Results and Discussion**

*Template DNA preparation*

For PCR-RAPD analysis to be carried out, a supply of genomic DNA must be extracted from the material to be analysed. One of the main advantages of using this methodology is that minute amounts of template DNA are required and that these need not be ultra pure in terms of protein contamination (Caetano-Anolles *et al.*, 1991a; 1991b and Hadrys *et al.*, 1992). As a result, a simplified extraction protocol was used to extract sugarcane DNA in this study, which produced yields between 0,050 and 0,168 mg/g leaf roll. These yields compared favourably with those of Honeycutt *et al.* (1992), who extracted between 0,070 and 0,280 mg DNA/g sugarcane leaf tissue. Those authors carried out a final DNA precipitation with 95% (v/v) ethanol and 0,2 M NaCl, a step which was omitted in this study as DNA was simply spooled out of suspension after the addition of isopropanol. This reduced the time required for the preparation of DNA stock solutions. The DNA extracts were reasonably free of contamination, as shown by A<sub>260</sub>/A<sub>280</sub> ratios between 1,60 and 1,78. These values are close to 1,8 which would indicate a pure DNA fraction (Sambrook *et al.*, 1989).

During early PCR-RAPD experiments, polymorphisms were detected in the fragment profiles of individual sugarcane plants belonging to variety N19 (results not shown). Therefore, it was necessary to extract DNA from bulked samples (Michelmore *et al.*, 1991). Preliminary investigations revealed that bulk DNA isolation from stalks obtained from three stools per variety was sufficient to eliminate individual-specific differences in the banding profiles (results not shown).

*Genetic variation between sugarcane varieties*

To assess the genetic distances between the 20 sugarcane varieties selected for use in this study, PCR-RAPD amplification was carried out in the presence of 41 individual random 10-mer primers. A total of 356 loci were resolved; that is, an average of 8,68 loci were detected per primer. From these data a set of 160 loci, generated with 15 primers, was selected for scoring and calculation of genetic distances (Table 2). This group of primers was used as it produced the

most clear and unambiguous banding profiles, which showed at least one polymorphism per primer across the twenty varieties (Williams and St. Clair, 1993). Many other authors report similar selection of a subset of suitable primers for data analysis (Huff *et al.*, 1993; Van Coppenolle *et al.*, 1993; Williams and St. Clair, 1993), although it is important to note that this may exaggerate the magnitude of calculated genetic distances.

Individual primers, within the set of 15 used in this study, resolved varying degrees of polymorphism between the sug-

Table 2

Primers used in the calculation of genetic distances between the 20 hybrid sugarcane varieties

Primer	Number of loci scored	Number of polymorphic loci
OPA-04	14	12
OPA-07	10	6
OPA-08	8	5
OPA-10	10	5
OPA-12	8	8
OPA-16	12	5
OPB-04	11	6
OPB-06	11	7
OPB-08	11	5
OPB-10	8	4
OPB-11	15	11
OPB-15	9	7
OPB-19	12	9
OPB-20	11	8
OPC-04	10	3
TOTAL	160	101

arcane hybrids. For example, OPA-08 generated eight bands and all of these were polymorphic across the 20 varieties tested, whereas the primer OPA-12 resolved a total of 12 loci, but only five of these were polymorphic (Table 2). This variability has been noted also by other authors who carried out RAPD analysis in the presence of random primers (Huff *et al.*, 1993; Prince *et al.*, 1993).

Once all of the 160 loci resolved with the 15 primers had been scored (Table 2), and the data entered into the computer program NTSYS-pc, a phenogram was generated (Figure 1). The relative distances between varieties shown on the phenogram correlated well with the numerical measures of genetic distance listed in Table 3. These values were calculated from the formula of Nei and Li (1979) and multiplied by 100 to reflect per cent similarity. As can be seen in Figure 1 and Table 3, 79M955 and 80L432 were the most genetically similar, with similarity calculated as 89,73%. It is interesting to note that N12 and N16 from the same parental cross (NCo376 × Co331) were less similar to one another (81,28%) than 79M955 and 80L432 (Table 3). The varieties shown to be most distant from each other were CB38-22 and N16 (71,82% similarity) and these were the most outlying varieties on the phenogram (Figure 1).

As relatively little is known about the sequence structure of the sugarcane genome (Roach and Daniels, 1987), or the genetic complement of the varieties used in this study, no explanation can be put forward for the differences in genetic diversity calculated between the varieties. However, a general lack of genetic diversity was immediately apparent from both the phenogram (Figure 1) and Table 3. As mentioned previously, the most genetically dissimilar varieties showed a similarity of 71,82% (Table 3); that is, 28,18% of the genome was polymorphic between CB38-22 and N16. This lack

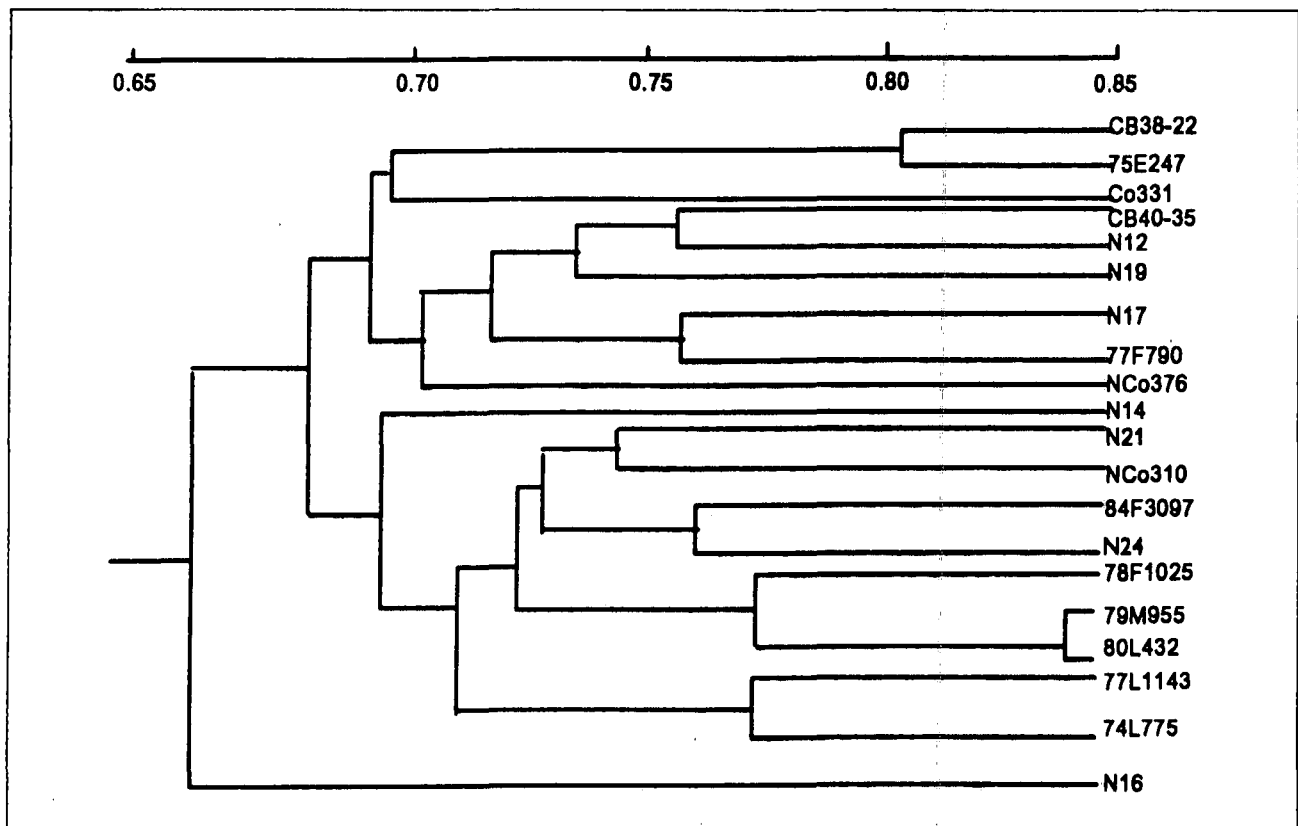


FIGURE 1 Phenogram showing the genetic distances between 20 interspecific sugarcane hybrids. The data scored from 160 RAPD loci (Table 2) were entered into the computer programme NTSYS-pc and the phenogram generated using the UPGMA method of analysis.

Table 3

Genetic distances calculated between the 20 sugarcane hybrids. Values were determined using a modified formula of Nei and Li (1979) (see text for details)

Variety	CB38-22	CB40-35	Co331	N12	N14	N16	N17	N19	N21	NCo376	75E247	77F790	78F1025	79M955	80L432	84F3097	N24	NCo310	74L775	77L1143
CB38-22	100	84.39	78.61	79.31	78.21	71.82	79.07	79.07	83.53	80.00	86.39	79.56	76.14	79.31	75.98	82.02	82.42	77.01	75.86	76.74
CB40-35		100	82.02	83.80	80.43	80.65	79.10	81.36	81.14	81.18	80.46	83.87	82.87	80.45	79.35	76.50	79.14	78.21	78.21	80.22
Co331			100	81.56	79.35	79.57	74.58	74.58	76.57	76.47	79.31	83.87	79.56	80.45	76.09	76.50	77.01	73.74	73.74	73.44
N12				100	84.32	81.28	79.78	83.15	81.81	78.36	76.57	80.21	79.12	78.89	77.84	78.26	77.66	81.11	76.67	80.90
N14					100	79.17	79.78	76.50	81.77	77.27	76.67	80.21	81.28	81.08	83.16	80.42	76.68	82.16	82.16	80.87
N16						100	76.76	77.84	77.60	74.16	72.53	81.44	80.42	81.28	82.29	78.53	76.92	80.21	74.87	76.76
N17							100	81.81	80.46	76.92	75.14	84.320	82.22	82.02	79.78	82.41	81.72	82.02	79.78	82.95
N19								100	80.46	80.47	76.30	83.24	78.89	77.53	76.50	80.22	78.49	77.53	77.52	79.55
N21									100	80.24	79.53	79.78	77.52	82.95	79.56	81.11	80.43	82.95	77.27	78.16
NCo376										100	74.70	79.78	79.77	77.19	73.86	77.71	79.33	80.70	79.53	78.11
75E247											100	81.32	76.84	76.57	75.98	78.69	75.43	74.29	75.14	
77F790												100	82.54	83.42	81.25	80.63	81.03	80.21	77.01	81.08
78F1025													100	86.81	85.56	82.80	83.16	82.42	83.52	81.11
79M955														100	89.73	84.78	80.85	83.33	82.22	82.02
80L432															100	82.54	79.79	82.16	81.08	84.15
84F3097																100	85.42	82.61	83.70	82.42
N24																	100	84.04	84.04	81.72
NCo310																		100	83.33	85.39
74L775																			100	87.64
77L1143																				100

of diversity echoes the findings of other authors, who have reported no cytoplasmic variation and limited polymorphism in chloroplast genome structure between 'high sucrose' producing sugarcane varieties (Sobral *et al.*, 1994).

To put the results from the intrahybrid comparisons (Table 3) into perspective, a genetic distance calculation was carried out between one of the hybrids (84F3097) and a variety of *S. spontaneum* (US66-5615). This yielded a similarity value of 30,4%; that is, 69,6% of the genomes of these plants were dissimilar, indicating a much greater genetic diversity. A possible explanation for this is the dilution of the *S. spontaneum* genome in the interspecific hybrids as a result of unequal chromosome transmission (2n + n) (Sobral *et al.*, 1994). Therefore, the comparison carried out here between 84F3097 and US66-5615 reflects interspecific genetic diversity, which apparently is much greater than that measured between the hybrid commercial sugarcane varieties.

*Identification of sugarcane varieties*

In addition to being used as markers for genetic distance calculations, RAPDs have proved useful also in the identification and characterisation of plant materials. For example, polymorphisms in RAPD profiles have been used to discriminate between varieties of rice (Welsh and McClelland, 1990), soybean (Caetano-Anolles *et al.* 1991, 1991a; 1991b), barley (Weining and Langridge, 1991) and apple (Mulcahy *et al.*, 1993; Harada *et al.*, 1993). Thus another potential application of the data set generated in this study would be to identify sugarcane varieties. At present these varieties are characterised on the basis of leaf, stalk and bud morphologies (Anon., 1986). However, phenotypic traits are often unstable as their expression is dependent on the environment in which the plant is grown (Wrigley *et al.*, 1987). Genetic characters, such as the RAPD loci scored in this study, would be more reliable as varietal markers.

The RAPD products of the 15 primers scored here could be used to resolve fingerprints, or patterns of DNA fragments, specific for each hybrid. These could be used directly in varietal identification, as has been carried out by Weining and Langridge (1991). However, varieties may also be identified by the presence or absence of one particular band, known as a 'variety or cultivar-specific marker' (Yang and Quiros, 1993). In this study, primers have been characterised that appear to be able to identify eight varieties (Table 4).

However, these findings are preliminary, and certain experiments need to be repeated to determine the reproducibility of the results.

Table 4

Primers which resolve putative varietal-specific markers. Single bands detected with each of these primers appear to be specific to the variety indicated

Primer	Variety for which primer appears to generate a marker
OPA-04	78F1025
OPA-07	N21
OPA-12	N12
OPA-16	79M955
OPB-04	83F2019
OPB-06	CB40-35
OPB-11	80L432
OPB-11	N24

**Conclusions**

The phenogram (Figure 1) and calculated values of genetic distance (Table 3) suggest that there is limited genetic diversity within the sugarcane genome, particularly within the interspecific hybrid varieties currently grown commercially. This may have serious implications for sugarcane breeding, as breeders are attempting to introgress new traits into existing varieties from a very limited background of genetic diversity. To introduce new traits, for example resistance to viral diseases, it would be necessary to cross genetically dissimilar individuals to introduce maximum heterosis into the breeding population. In the light of findings that much greater genetic diversity exists between sugarcane species than within the group of interspecific hybrids studied, it is suggested that the interspecific hybrids be crossed with varieties of other sugarcane species, such as *S. spontaneum*. This may improve genetic heterosis and result in the selection of new commercial varieties which are more genetically distinct and perhaps more economically successful.

### Acknowledgements

We wish to thank the staff of the Plant Breeding department at the Experiment Station for their assistance in the selection, identification and collection of clones.

### REFERENCES

- Anon. (1986). Sugarcane varieties grown in South Africa. Bulletin No. 4, SA Sugar Association Experiment Station, Mount Edgecombe.
- Bailey, RA and Fox, PH (1980). The susceptibility of varieties to mosaic and the effect of planting date on mosaic incidence in South Africa. *Proc S Afr Sug Technol Ass* 54: 161-167.
- Bailey, RA and Fox, PH (1987). A preliminary report on the effect of sugarcane mosaic virus on the yield of sugarcane varieties NCo376 and N12. *Proc S Afr Sug Technol Ass* 61: 1-4.
- Caetano-Anolles, G, Bassam, BJ and Gresshoff, PM (1991a). DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnology* 9: 553-556.
- Caetano-Anolles, G, Bassam, BJ and Gresshoff, PM (1991b). DNA amplification fingerprinting: a strategy for genome analysis. *Plant Mol Biol Rep* 9: 294-307.
- Hadrys, H, Balick, M and Schierwater, B (1992). Application of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol Ecol* 1: 55-63.
- Harada, T, Matsukawa, K, Sato, T, Ishikawa, R, Niizeki, M and Saito, K (1993). DNA-RAPDs detect genetic variation and paternity in *Malus*. *Euphytica* 65: 87-91.
- Honeycutt, HJ, Sobral, BWS, Kiem, P and Irvine, JE (1992). A rapid DNA extraction method for sugarcane and its relatives. *Plant Mol Biol Rep* 10: 66-72.
- Huff, DR, Peakall, R and Smouse, PE (1993). RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. *Theor Appl Genet* 86: 927-934.
- Michelmore, RW, Paran, I and Kesseli, RV (1991). Identification of markers linked to disease-resistance genes by bulk segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci USA* 88: 9828-9832.
- Miklas, PN, Stavely, JR and Kelly, JD (1993). Identification and potential use of a molecular marker for rust resistance in common bean. *Theor Appl Genet* 85: 745-749.
- Mulcahy, DL, Cresti, M, Sansavini, S, Douglas, GC, Linskens, HF, Bergamini Mulchy, G, Vignani, R and Pancaldi, M (1993). The use of random amplified polymorphic DNAs to fingerprint apple genotypes. *Scientia Horticulturae* 54: 89-96.
- Nei, M and Li, W-H (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA* 76: 5269-5273.
- Nuss, KJ, Bond, RS and Atkinson, PR (1986). Susceptibility of sugarcane to the borer *Eldana saccharina* Walker and selection for resistance. *Proc S Afr Sug Technol Ass* 60: 153-155.
- Prince, JP, Loaiza-Figueroa, F and Tanksley, SD (1993). Restriction fragment length polymorphism and genetic distance among Mexican accessions of *Capsicum*. *Genome* 35: 726-732.
- Roach, BT and Daniels, J (1987). A review of the origin and improvement of sugarcane. In: Copersucar International Breeding Workshop. (Eds) SP Copersucar. Copersucar, Brazil. pp 1-32.
- Sambrook, J, Fritsch, EF and Maniatis, T (1989). *Molecular Cloning: a laboratory manual*. Cold Spring Harbour: Cold Spring Harbour Laboratory Press.
- Sobral, BWS, Braga, DPV, LaHood, ES and Kiem, P (1994). Phylogenetic analysis of chloroplast restriction site mutations in the *Saccharinae* (Griseb.) subtribe of the *Andropogoneae* (Dumort.) tribe. *Theor Appl Genet* (in press).
- Steindl, DRL (1961). Ratoon stunting disease. In: *Sugarcane diseases of the world*, Vol 1. (Eds) JP Martin, EV Abbott and CG Hughes. Elsevier Publishing Company, Amsterdam. p 542.
- Van Coppenolle, B, Watanabe, I, Van Hove, C and McCouch, SR (1993). Genetic diversity and phylogeny analysis of *Azolla* based on DNA amplification by arbitrary primers. *Genome* 36(4): 686-693.
- Weining, S and Langridge, P (1991). Identification and mapping in cereals based on the polymerase chain reaction in *Brassica*. *Theor Appl Genet* 82: 632-727.
- Welsh, J and McClelland, M (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nuc Acids Res* 18: 7213-7218.
- Williams, JGK, Kubelik, AR, Livak, JK, Rafalski, JA and Tingey, SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 18: 6531-6535.
- Williams, CE and St. Clair, DA (1993). Phenetic relationships and levels of variability detected by restriction fragment length polymorphisms and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. *Genome* 36: 619-629.
- Wrigley, CW, Batley, IL and Skerritt, JH (1987). Complementing traditional methods of identifying cereal varieties with novel procedures. *Seed Sci Technol* 15: 679-688.
- Yang, X and Quiros, C (1993). Identification of celery cultivars with RAPD markers. *Theor Appl Genet* 86: 205-212.