

**AN INVESTIGATION INTO THE HERITABILITY
OF COMMERCIALLY IMPORTANT TRAITS
IN A SUGARCANE POPULATION
UNDER DRYLAND CONDITIONS**

by

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PREFACE

This study was completed at the South African Sugar Association Experiment Station Plant Breeding Department. The author was registered with the Genetics Department at the University of Natal, Pietermaritzburg from February 1994 - February 1996. Doctors Paul Shanahan and Trevor Hohls supervised this work.

DECLARATION

This thesis represents the original work by the author and has not been submitted for degree purposes to any other University. Where use was made of the work of others, it has been duly acknowledged in the text.

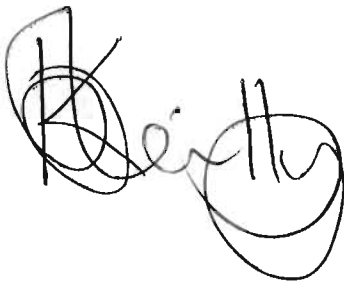
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ABSTRACT

Inheritance studies have previously been undertaken at the South African Sugar Association Experiment Station (SASEX) under irrigated conditions. Since most sugarcane is grown in South Africa under dryland (raingrown) conditions, heritability estimates were calculated under these conditions in this study and compared to those previously obtained under irrigated conditions. A sugarcane population consisting of 12 crosses, 32 offspring in each cross, and their parents were planted in the first two selection stages of the SASEX selection programme to ascertain which stage provided the most useful information when selecting parent cultivars. Data collected from Stage 2 was more reliable than data collected from Stage 1. Variance components, narrow and broad sense heritabilities, correlations among traits, and clonal repeatabilities between seasons were determined for 11 sugarcane traits at Stages 1 and 2. These traits studied included: stalk population; stalk diameter; stalk height; cane mass; dry matter % cane; fibre % cane; brix % cane; brix % dry matter; purity; pol % cane; and ers % cane. Narrow sense heritabilities of the sugarcane traits were estimated by mid-parent offspring regression. Alternative heritability estimates were obtained through restricted maximum likelihood (REML) analysis of the unbalanced North Carolina design II at Stage 2. Although narrow sense heritabilities determined by mid-parent-offspring regression were comparable with those previously determined at SASEX and by other workers, REML was more efficient than regression. Use of REML enabled additive and non-additive genetic variance components to be estimated by allocating degrees of freedom to treatments and the interactions between the different treatments. Heritability estimates varied for different traits and compared favourably with those obtained under irrigated conditions and by other workers. Additive genetic variance was more important than non-additive genetic variance for some characters, but not for stalk population, cane mass, and dry matter % cane, for which both variances were important. Selection of parent cultivars for all sucrose-related traits, fibre % cane, and stalk diameter should be as successful under raingrown as under irrigated conditions, provided that the environmental variation is determined efficiently under raingrown conditions. Environmental correlations were observed between some traits, particularly between the yield-related traits, and may have influenced heritability estimates for those traits determined by mid-parent offspring regression. Stalk diameter, fibre % cane, and brix % dry matter were

the most repeatable traits between seasons. Cane mass was the least repeatable trait between Stages 1 and 2 but was highly repeatable between plant (-P) and ratoon (-R) crops of Stage 2.

Stalk diameter was positively correlated with brix % dry matter (0.457-P and 0.623-R) and strongly negatively correlated with stalk population (-0.790-P and -0.711-R) and fibre % cane (-0.628-P and -0.651-R). Cane mass was strongly positively correlated with brix % dry matter (0.638-P and 0.679-R). By selecting for brix % dry matter and stalk diameter, indirect selection for cane mass would be possible. Brix % dry matter was determined as the most reliable trait on which to base parental and commercial cultivar selection because it was highly heritable, highly repeatable and highly positively correlated with stalk diameter and cane mass.

LIST OF ABBREVIATIONS AND SYMBOLS

The following abbreviations were frequently used in this document:

SASEX	=	South African Sugar Association Experiment Station
FAS	=	Field advisory service located at SASEX
ML	=	Maximum likelihood
REML	=	Restricted maximum likelihood
-P	=	plant crop
-R	=	ratoon crop
SS	=	single stool stage
SL	=	single line stage
Diam	=	stalk diameter
	=	average stalk diameter (cm^2) based on the assumption that the stalk is cylindrical
Pop	=	stalk population
	=	number of millable stalks per stool (SS) or m^{-1} (SL)
Ht	=	stalk height or length (cm) measured from the base of the stalk (at ground level) to the meristematic tissue at the growing point
Stool mass	=	mass of one stool of sugarcane
Cane mass	=	cane yield (kg (5 m row)^{-1})
Dm%	=	dry matter expressed as a percentage of fresh mass of cane
Fibre%	=	mass of insoluble dry matter expressed as a percentage of fresh mass of cane
Brix%	=	mass of soluble matter expressed as a percentage of fresh mass of cane
Brix%dm	=	brix expressed as a percentage of dry matter
Purity	=	ratio of pol% to brix%, expressed as a percentage
Pol%	=	mass of sucrose expressed as a percentage of fresh mass of cane
Ers%	=	mass of estimated recoverable sugar expressed as a percentage of fresh mass of cane

The following symbols were frequently used in this document:

h_a^2	=	narrow sense heritability
h_b^2	=	broad sense heritability
y_{ij}	=	the j^{th} observation on the character y in a group for which the observed character x is x_i , x 's are assumed to be measured without error
μ	=	effect common to all the x 's
μ_y	=	effect common to all the y 's
β	=	regression of the y 's on the x 's
e_{ij}	=	residual effect peculiar to the j^{th} y in the i^{th} group
b	=	regression coefficient of offspring on parents
Cov_{PO}	=	covariance of parent-offspring
F	=	inbreeding coefficient
z	=	mid-parent value
SE_b	=	standard error of regression coefficient
y_{ijkl}	=	plot mean of the l^{th} full sib progeny in a plot in the k^{th} replication of the i^{th} male parent and the j^{th} female parent
b_k	=	effect of the k^{th} replication
m_i	=	effect of the i^{th} male parent
f_j	=	effect of the j^{th} female parent
$(mf)_{ij}$	=	interaction between the male and female parents
e_{ijkl}	=	environmental effect and the remainder of the genetic effect between full sibs in the same plot
MS	=	mean squares
M_1	=	within plot mean square
M_2	=	error mean square
M_3	=	mean square of interaction of f female parents with m male parents
M_4	=	mean square of half sib family means
M_5	=	mean square of half sib family means i.e. mean square of the progeny from different females
σ_{we}^2	=	environmental variance
σ_{wg}^2	=	within plot genetic variance
σ_m^2	=	σ_f^2 = component of variance due to differences between half sibs
σ_{mf}^2	=	component of variance due to differences among full sibs within half sibs

σ^2	=	residual error which is confounded with a full component of full sib variance
σ_p^2	=	plot error variance
σ_P^2	=	phenotypic variance
σ_G^2	=	total genetic variance
σ_A^2	=	additive genetic variance
σ_D^2	=	non-additive genetic variance in the form of dominance
GCV%	=	genetic coefficient of variation (expressed as a percentage)
$\text{Cov}_{x,y}$	=	covariance of two traits, X and Y
V_P	=	phenotypic variance
V_G	=	genetic variance
V_E	=	environmental variance
V_A	=	additive genetic variance
V_D	=	dominance genetic variance
V_I	=	interaction / epistatic variance
V_x	=	variance of trait X
V_y	=	variance of trait Y
V_{x+y}	=	variance of two traits added together, X + Y
V_{cx+cy}	=	cross variance component for the sum of trait X and Y
V_{cy}	=	cross variance component for trait Y
V_{cx}	=	cross variance component for trait X
V_{ex+ey}	=	error variance component for the sum of trait X and Y
V_{ey}	=	error variance component for trait Y
V_{ex}	=	error variance component for trait X
r_p	=	phenotypic correlation between trait X and Y
r_g	=	genetic correlation between trait X and Y
r_e	=	environmental correlation between trait X and Y
F-value	=	variance ratio
CV%	=	coefficient of variation (expressed as a percentage)

INTRODUCTION

About 20 Mt of sugarcane (*Saccharum* spp hybrid) is crushed annually in South Africa, from which about 2 Mt of sugar is produced. The economic success of the South African sugarcane industry in this country is dependent on the breeding of new commercial cultivars. One of the main functions of the South African Sugar Association Experiment Station (SASEX) is the breeding and commercial release of cultivars and advising farmers in the cultivation of sugarcane. The cultivars bred must have high potential sucrose yields per hectare of cane grown. They must also be resistant to diseases and pests and adapted to the environmental stresses in which the cane will be commercially grown. An outline of the breeding and selection programme carried out at SASEX is provided in Appendix 1, while an outline of the planting, harvesting and milling of sugarcane is provided in Appendix 2.

Inheritance studies have already been undertaken at SASEX on sugarcane (Blose, 1992 and Bond and Van der Merwe, 1992). These studies were conducted on sugarcane grown under irrigated conditions where the environmental variation is less than that found in dryland areas (hereafter to be referred to as raingrown areas). Most of the commercial sugarcane in South Africa is located in the raingrown areas of the country. This investigation was carried out at Mount Edgecombe which receives an average of 994.6 mm rainfall per annum. Appendix 3 provides rainfall and temperature data recorded at Mount Edgecombe for the duration of this study. The main objective of this study was to determine the nature of heritability estimates obtained under raingrown conditions and compare these with those obtained from irrigated conditions and those of other workers.

A knowledge of the heritability of commercially important traits can contribute to the success of a sugarcane breeding programme and the correlation of these traits with the primary breeding objectives. One of the advantages of this study, over the other inheritance studies of sugarcane carried out in South Africa, is that the parents and their offspring were planted in the same trial. Data collected from such a trial are less confounded with environmental influences and information from the parent cultivars may be used to determine heritability estimates. Eleven traits were investigated: stalk diameter (cm²); stalk population (number of

stalks stool⁻¹ and number of stalks m⁻¹ in Stages 1 and 2, respectively); stalk height (cm); cane mass (kg stool⁻¹ in Stage 1 and kg (5 m row)⁻¹ in Stage 2); brix % cane; dry matter % cane; fibre % cane; brix % dry matter; purity (%); pol % cane; and ers % cane. Mid-parent offspring regression was used to determine narrow sense heritabilities of these traits. Despite an unbalanced cross-classification of the genotypes, Residual Maximum Likelihood (REML) was used to obtain a different set of estimates to those obtained from regression. REML does not require parental data. Heritability estimates obtained from these two methods of analyses were compared.

The performance of offspring can be used to predict the success of particular parental combinations when the heritabilities of the traits concerned are high. The accuracy of the predictions is improved with an increase in the number of progeny available. The first few stages of the selection programme carried out at SASEX consist of a relatively large number of offspring from each parental combination, with only a few being carried forward after three or four stages of selection. Therefore, only the first two selection stages were investigated. A constant number of offspring was studied in both stages and not a reduced number, the latter being characteristic of the normal selection programme. The number of genotypes decreases in the normal selection programme because selection takes place from one stage to the next. Heritability estimates obtained from Stage 1 were compared with those obtained from Stage 2, plant and ratoon crops, to ascertain which selection stage gave more reliable heritability estimates to be used for the selection of parent cultivars.

Correlations between the traits identified the relationships between the traits of commercial importance as these affect the selection of parent cultivars. Clonal repeatabilities were calculated to determine which traits are more reliable when selecting commercial sugarcane cultivars. Heritabilities, correlations and clonal repeatabilities obtained from Stages 1 and 2 under raingrown conditions were then compared with those obtained from trials grown under irrigated and wetland conditions.

This investigation is presented in four chapters. Chapter 1 provides a detailed literature review on quantitative genetics applied to sugarcane and possible mating designs that can and have

been used by other workers to determine heritabilities from sugarcane populations. The materials and methods of this study are divided into experimental and statistical procedures and are discussed in Chapter 2. Field adjustments were necessary for reliable comparisons to be meaningful between the heritability estimates obtained at different stages and are explained in the same chapter (Section 2.2.4). The results obtained in the study are presented and discussed in Chapter 3. Differences were observed between the two selection stages (Stages 1 and 2) and between plant (-P) and ratoon (-R) crops of Stage 2. Chapter 3 provides the heritability estimates of the 11 sugarcane traits obtained by mid-parent offspring regression and REML analysis of an unbalanced North Carolina mating design II. Comparisons between these results are discussed in Section 3.1. Phenotypic, genetic, and environmental correlations among sugarcane traits (Section 3.2) and clonal repeatabilities across seasons (Section 3.3) are also presented in Chapter 3. An overview and final conclusions are presented in Chapter 4. The results from this investigation offered a possible alternative trait for sugarcane breeders to consider when selecting parent cultivars for raingrown areas as well as a method of analysis that can efficiently estimate variance components from an unbalanced mating design.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Commercially grown sugarcane cultivars have originated from a number of different species of *Saccharum*. They are mostly higher polyploids, while some are aneuploids. Their chromosome numbers vary from 100 to 120 and can be even higher. Most cultivars grown around the world today are complex hybrids from the genus *Saccharum* belonging to the family Poaceae, in the tribe Andropogonae (Daniels and Roach, 1987).

1.2 Quantitative genetic analysis

Most commercially important traits in sugarcane are polygenic and follow a continuous distribution. The phenotypic variation of quantitative characters can be partitioned into many components attributable to different causes (Falconer, 1989):

$$V_P = V_G + V_E \quad (1.1)$$

$$V_P = V_A + V_D + V_I + V_E \quad (1.2)$$

where:

- V_P = total phenotypic variance observed;
- V_G = genotypic variance;
- V_E = environmental variance;
- V_A = additive genetic variance;
- V_D = non-additive genetic variance in the form of dominance variance;
- V_I = interaction / epistatic variance.

Quantitative genetic analyses enable the planning of efficient breeding methods that are suited to sugarcane. Interpretation of the variance components depends on assumptions that are made when estimating variance components. Procedures have been proposed for determining variance components (Falconer, 1989), with the following assumptions in their application to

plant breeding:

- i) diploid inheritance;
- ii) no linkages;
- iii) no epistasis;
- iv) random choice of parents from a random mating population; and
- v) no differences between reciprocal crosses.

Sugarcane deviates from these assumptions to some extent (Cockerham, 1963; Price, 1963; Stevenson, 1965; Brown, Daniels and Latter, 1968; Hogarth, 1968b; 1977; 1987; Hogarth, Wu and Heinz, 1981). It is important for sugarcane breeders to be aware of these deviations when calculating genetic parameters:

- i. Modern sugarcane cultivars are hybrids of many different species of sugarcane. Their chromosome numbers vary and many have high aneuploid chromosome numbers. Evidence has been provided to suggest that the inheritance pattern of sugarcane approximates diploidy (Price, 1963 and Brown *et al.*, 1968). Variable chromosome numbers of sugarcane may also affect the interpretations made from a detailed genetic investigation. The influence of chromosome variability on the assumptions is unknown (Hogarth, 1968b).
- ii. Presence or absence of linkage has been difficult to prove with sugarcane (Hogarth, 1968b). Cockerham (1963) has, however, explained that linkage affects the coefficients of V_I .
- iii. Coefficients of V_I are increased by an amount that is dependant on recombination values. The consequences of linkage are therefore dependant on the importance of epistasis. Epistasis exists in crops, e.g. maize (*Zea mays* L.)(Stuber and Moll, 1969; 1971) and tomato (*Lycopersicum esculentum*)(Stoner and Thompson, 1966). Although epistasis is difficult to measure in sugarcane, it has been found to be important for certain traits. Epistasis was found for mass per stalk in the ratoon crop (Hogarth, 1977). Coefficients of V_I are relatively small in comparison to the V_A and V_D (Cockerham, 1963).
- iv. Random choice of parent cultivars cannot be assumed because parents are normally chosen from a highly selected population of cultivars. Gene frequencies for cane yield and sucrose content are likely to be skewed for these characteristics that have already

been selected for.

- v. It is not always possible to make reciprocal crosses between sugarcane cultivars because some sugarcane cultivars are male sterile. When reciprocal crosses were made in the past, few differences were found between the crosses (Natarajan, Krishnamurthi and Rao, 1967; Hogarth, 1980). Maternal effects were assumed to be responsible for these differences (Hogarth, 1968b). The interpretation of genetic variances would be affected if maternal effects are important.

With all these departures from the assumptions, sugarcane breeders need to be aware that interpretations of the quantitative genetic analyses may be biased. When departures are known to occur, the interpretations must be appropriately modified.

1.3 Estimation of heritability

Heritability estimates allow the identification of traits for which selection will be effective. Breeders need to be confident that the chosen parent cultivars will produce commercially successful offspring. Cognisance should also be taken that heritability estimates are specific to the population and environment used. These estimates can vary for different populations, environments and even seasons.

There are two types of heritability, broad and narrow sense heritability. Broad sense heritability can be used to predict the genetic gain from one clonal selection stage to the next. Narrow sense heritability is of greater use to breeders in that it helps predict gains from selection of parents for a new cycle of crossing.

Broad sense heritability (h_b^2) estimates the degree to which an individual's phenotype is determined by its genotype (V_G / V_P). This heritability is more of theoretical interest than of practical importance to breeders. Many sugarcane breeders have calculated broad sense heritabilities (Table A4.1; where A refers to the Appendices).

Narrow sense heritability (h_a^2) is practically important since it expresses the extent to which

an individual's phenotype is determined by the genes transmitted by its parents. This is expressed as the proportion of additive genetic variance, which is the variance of the breeding values, relative to the phenotypic variance (V_A / V_P). When a population of parent cultivars and their offspring is investigated, the progeny often tend to resemble their parents to some extent. The degree of resemblance between parents and their offspring can be used to estimate heritability. If the parents have high values for a specific character, the progeny will tend to be high for the same character although they will diverge from their parents as a result of genetic segregation and environmental effects on both generations. This would suggest a large genetic effect and a small environmental one. When environmental effects are large and genetic effects are insignificant, there would be no or very little similarity between parents and their offspring. Resemblance between relatives will vary depending on the character being investigated. The degree of resemblance between relatives is also known as the intraclass correlation. This indicates that as individuals become more similar within a particular group, the differences between the groups tend to become greater. Narrow sense heritabilities have been determined by many sugarcane breeders over the years (Table A4.2).

Heritability estimates are specific to the material being investigated and design of the experiment. Parents that do not differ much in respect of the trait being examined cannot generate large V_A or V_G in their progeny. These parents may come from populations that have already been selected for these characters. Additive genetic and genotypic variances are dependent on parental differences as well as the experimental design that is used to separate V_A and V_G from each other and from interactions (Simmonds, 1979). Phenotypic variance can be reduced by an experimental design that reduces V_E . When V_A is higher than V_D and V_E , then the overall performance of crosses can be predicted from the performance of their parent cultivars. When V_G is mostly non-additive then progeny performance cannot be reliably predicted from the parental performance, and this would then suggest that progeny testing should be used. This progeny testing is extremely costly and undesirable.

Parent-offspring regression is dependent on the magnitude of the genetic differences between parents and upon the control of error. Larger plots, larger families, more replications and repeated measurements on individuals will increase the covariance of the regression coefficient

and reduce the parental variance (Simmonds, 1979).

Heritability estimates for different traits are most useful when compared to one another rather than viewed in isolation. Many studies of different sugarcane populations show that heritability of cane yielding ability is low, approaching zero. Since heritability of traits affects the response to selection, selection for high yielding ability will be very difficult and time consuming.

To determine heritabilities from variance components, mating designs are required. Many designs have been used in sugarcane breeding but there are some that are more efficient in measuring the genetic variation of traits than others. The number of parents available and the crosses made determine the design used.

1.3.1 Regression analysis

Regression analysis enables estimation of h_a^2 . This method allows for the analysis of unbalanced data sets. A general model for regression of one variable (y) on another variable (x) has been proposed by Kempthorne and Tandon (1953):

$$y_{ij} = \mu_y + \beta (x_i - \mu) + e_{ij} \quad (1.3)$$

where:

- y_{ij} = the j^{th} observation on the character y in a group for which the observed character x is x_i , x's are assumed to be measured without error;
- μ = effect common to all the x's;
- μ_y = effect common to all the y's;
- β = regression of the y's on the x's; and
- e_{ij} = residual effect peculiar to the j^{th} y in the i^{th} group.

The regression coefficient (b) of offspring (y) on one parent (x) is calculated as follows (Snedecor, 1946; Hallauer and Miranda, 1988):

$$b = \frac{\sum_i xy}{\sum_i x^2} = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sum_i (x_i - \bar{x})^2} = \frac{\sigma_{xy}}{\sigma_x^2} \quad (1.4)$$

The σ_{xy} consists of the following genetic component (covariance of parent-offspring, Cov_{PO}):

$$Cov_{PO} = \sum_i \frac{1}{2}(1+F) \cdot \sigma_A^2 \quad (1.5)$$

where: F = inbreeding coefficient. When there is no inbreeding (F=0),

$$Cov_{PO} = \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_{AA}^2 + \frac{1}{8}\sigma_{AAA}^2 + \dots \quad (1.6)$$

The total variation of the parental measurements was σ_x^2 , so assuming that there is no epistasis,

$$b = \frac{1}{2} \frac{\sigma_A^2}{\sigma_x^2} \quad (1.7)$$

To determine narrow sense heritability estimates (h_a^2) of these traits from parent-offspring regression,

$$h_a^2 = 2b = \frac{\sigma_A^2}{\sigma_x^2} \quad (1.8)$$

When evaluating progeny from a cross between two individuals, which is the case in this trial, the offspring are regressed on the mean of the pair of parents. Measurements of the traits are taken from the progeny (y) and both of the parents (x_1 and x_2 , respectively). The mid-parent value (z) is determined by the arithmetic mean of the parental measurements:

$$z = \frac{x_1 + x_2}{2} \quad (1.9)$$

The mid-parent offspring regression becomes:

$$b = \frac{\sigma_{xy}}{\sigma_z^2} \quad (1.10)$$

Assuming $\sigma_{x1}^2 = \sigma_{x2}^2$, and that x_1 and x_2 are uncorrelated,

$$\sigma_z^2 = \frac{1}{4}\sigma_{x1}^2 + \frac{1}{4}\sigma_{x2}^2 = \frac{1}{2}\sigma_x^2 \quad (1.11)$$

Therefore:

$$b = \frac{\frac{1}{2}\sigma_A^2}{\frac{1}{2}\sigma_x^2} = \frac{\sigma_A^2}{\sigma_x^2} = h_a^2 \quad (1.12)$$

Once the regression coefficient has been calculated, it is necessary to determine the goodness of fit of the line to the data. Deviations from the regression measure the failure of the line to fit the data. By calculating the sum of squares of the deviations an estimate of the error in fitting the line is determined. The mean square deviation from regression (σ^2) is calculated by dividing the sum of squares of deviations by the degrees of freedom, $n-2$, where n is the number of crosses (12 in this study) used to determine the regression and the 2 is because there were two averages used to calculate the deviations (one parent or mid-parent value and offspring means) (Snedecor, 1946).

The precision of an estimate of heritability is obtained from the standard error of the regression coefficient. The standard error (SE) of the regression coefficient is calculated as the square root of the mean square deviation from regression divided by the square root of the sum of squares:

$$SE_b = \frac{\sqrt{\sigma^2}}{\sqrt{\sum x_i^2}} \quad (1.13)$$

$$SE_{h_a^2} = 2SE_b \quad \text{for one parent; and} \quad (1.14)$$

$$SE_{h_a^2} = SE_b \quad \text{for the mean of both parents.} \quad (1.15)$$

Maternal effects are presumed absent in sugarcane, so the mid-parent offspring regression is still one of the most reliable ways to estimate heritability when considering the degree of resemblance between relatives. An advantage in using this method for determining heritabilities is that a mating design is not actually required to obtain an estimate of narrow sense heritability. Although the estimates may be biased upwards, they do give an indication of which traits tend to be more heritable than others. Unfortunately this method does not take the variation within crosses into account.

Hogarth (1977), Hogarth *et al.* (1981), and Tai, Miller and Dean (1981) have conducted trials using parent-offspring regression. Both genetic and environmental sources contribute to the phenotypic covariance of relatives, the covariance of phenotypic values being the sum of genetic and environmental covariances. Additive genetic variance can be determined by calculating the genetic covariance of offspring on parents. An estimate of heritability is obtained from the regression coefficient, calculated by dividing the covariance of offspring on parents by the variance of the parents. The most reliable estimate of heritability by regression analysis is obtained from regression of offspring means on the mean of the two parents (mid-parent value). The advantage of using parent-offspring regression is that the resemblance between relatives is measured directly and is not dependent on the genetic assumptions required for partitioning variance components (Simmonds, 1979). The regression coefficient does, however, rely on the separation of V_A from the other variance components.

Gilbert (1973) preferred parent-offspring regression to variance component partitioning for the estimation of h_a^2 . He believed that with all the violations of the assumptions made when determining variance components in a sugarcane population, parent-offspring regression may be a better approach to the estimation of h_a^2 . Parent-offspring regression is, however, also based on a number of assumptions (Cockerham, 1963; Dudley and Moll, 1969), some of which are identical to those required for determining variance components:

- i) diploid inheritance;
- ii) no environmental correlations between relatives;
- iii) population in linkage equilibrium or no linkage among genes controlling the traits studied;
- iv) the relatives are non-inbred;
- v) randomly mating population.

Parent-offspring regression is often used to estimate heritabilities because estimates obtained are valid when the parents have been selected for some reason and they are chosen at random from a particular population. It has been shown, however, that environmental covariances inflate heritability estimates if the assumption of no environmental correlations between relatives has been violated (Vogel, Haskins, and Gorz, 1980).

Environmental correlation occurs when parent cultivars and their offspring are planted in similar or the same environments. There is an interaction between the sugarcane genotypes and the environment and this may cause bias when using this method of analysis. The error covariances between the parents and their offspring may also be a possible source of bias. Casler (1982) recommended covariance analysis to overcome this bias. This analysis involves the planting of parent cultivars with their offspring in a range of different environments, to determine the extent of environmental correlation between relatives.

1.3.2 Estimation of variance components to determine genetic parameters

The factorial mating design (North Carolina design II, Comstock and Robinson, 1948) is suitable for sugarcane (Roach, 1968; Hogarth, 1971b; 1977; Hogarth *et al.*, 1981; Tai *et al.*, 1981; Hogarth and Kingston, 1984) and has been used in this study. Different sets of parents are used as male and female cultivars. The statistical model for the factorial mating design is:

$$y_{ijkl} = \mu + b_k + m_i + f_j + (mf)_{ij} + e_{ijkl} \quad (1.16)$$

where:

- y_{ijkl} = plot mean of the l^{th} full sib progeny in a plot in the k^{th} replication of the i^{th} male parent and the j^{th} female parent;
- μ = mean common to all observations;
- b_k = effect of the k^{th} replication;
- m_i = effect of the i^{th} male parent;
- f_j = effect of the j^{th} female parent;
- $(mf)_{ij}$ = interaction between the male and female parents;
- e_{ijkl} = environmental effect and the remainder of the genetic effect between full sibs in the same plot (Comstock and Robinson, 1948; Becker, 1984).

All effects are random, normal, and independent with the expectations equal to zero. Incompatibility of certain parent cultivars in sugarcane can be a problem when making certain cross combinations. This problem can be overcome by making different sets of crosses using only a few parent cultivars. This design enables the estimation of additive and non-additive genetic variance since both half and full-sib relationships exist. Epistatic effects can be estimated when the contributions from the males and females are sufficient to explain the variation between the crosses. This is dependent on the number of parent cultivars used. The

components of variance can be estimated from an analysis of variance table (Table 1.1).

Table 1.1 Skeleton analysis of variance table for the North Carolina II mating design in one environment (Hallauer and Miranda, 1988).

Source	Degrees of Freedom	MS	Expected Mean squares	
			Components of Variance	Covariance of Relatives
Replications	r-1			
Males	m-1	M_5	$\sigma^2 + r\sigma_{mf}^2 + rf\sigma_m^2$	$\sigma^2 + r(\text{Cov}_{FS} - \text{Cov}_{HSm} - \text{Cov}_{HSf}) + rf\text{Cov}_{HSm}$
Females	f-1	M_4	$\sigma^2 + r\sigma_{mf}^2 + rm\sigma_f^2$	$\sigma^2 + r(\text{Cov}_{FS} - \text{Cov}_{HSm} - \text{Cov}_{HSf}) + rm\text{Cov}_{HSf}$
Males x Females	(m-1)(f-1)	M_3	$\sigma^2 + r\sigma_{mf}^2$	$\sigma^2 + r(\text{Cov}_{FS} - \text{Cov}_{HSm} - \text{Cov}_{HSf})$
Error	(r-1)(mf-1)	M_2	σ^2	σ^2
Total	rmf-1			
Within plot	rmf(k-1)	M_1		

where:

r,m,f,k = number of replications, males, females, and plants per plot, respectively;

MS = mean squares;

M_1 = within plot mean square = $\sigma_w^2 = (\sigma_{wc}^2 + \sigma_{wg}^2) = [\sigma_{wc}^2 + (\sigma_G^2 - \text{Cov}_{FS})]$;

M_2 = error mean square = $\sigma^2 = [\sigma_{wc}^2 + (\sigma_G^2 - \text{Cov}_{FS})] / k + \sigma_p^2$;

M_3 = mean square of interaction of f female parents with m male parents;

M_4 = mean square of half sib family means, i.e. mean square of the progeny from different males;

M_5 = mean square of half sib family means;

i.e. mean square of the progeny from different females;

σ_{wc}^2 = environmental variance;

σ_{wg}^2 = within plot genetic variance;

σ_w^2 = $\frac{1}{2}\sigma_A^2 + \frac{3}{4}\sigma_D^2$;

σ_m^2 = component of variance due to differences between half sibs;

= $\sigma_f^2 = \text{Cov}_{HS} = \frac{1}{4}\sigma_A^2$ (if $F = 0$);

σ_{mf}^2 = component of variance due to differences among full sibs within half sibs;

= $\text{Cov}_{FS} - \text{Cov}_{HSm} - \text{Cov}_{HSf} = \frac{1}{4}\sigma_D^2$ (if $F = 0$);

σ^2 = residual error which is confounded with a full component of full sib variance;

= $V_p - \text{Cov}_{FS}$;

σ_p^2 = plot error variance;

V_p = phenotypic variance;

σ_G^2 = total genetic variance;

σ_A^2 = additive genetic variance; and

σ_D^2 = non-additive genetic variance in the form of dominance.

When epistatic components of variance are ignored, estimates of σ_A^2 and σ_D^2 can be obtained from σ_m^2 , σ_f^2 and σ_{fm}^2 , and an estimate of the total genetic variance (σ_G^2) can be obtained from $\sigma_A^2 + \sigma_D^2$:

where:

$$\begin{aligned}\sigma_A^2 &= 2 (\sigma_f^2 + \sigma_m^2) \\ &= 2 (1/4 \sigma_A^2 + 1/4 \sigma_A^2)\end{aligned}\quad (1.17)$$

$$\begin{aligned}\sigma_G^2 &= 2 (\sigma_f^2 + \sigma_m^2) + 4 (\sigma_{fm}^2) \\ &= 2 (1/4 \sigma_A^2 + 1/4 \sigma_A^2) + 4 (1/4 \sigma_D^2)\end{aligned}\quad (1.18)$$

$$\begin{aligned}\sigma_P^2 &= \sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2 + \sigma_w^2 + \sigma_e^2 \\ &= \sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2 + \sigma_w^2 + \sigma_e^2 \\ &= 1/4 \sigma_A^2 + 1/4 \sigma_A^2 + 1/4 \sigma_D^2 + 1/2 \sigma_A^2 + 3/4 \sigma_D^2 + \sigma_e^2 \\ &= \sigma_A^2 + \sigma_D^2 + \sigma_e^2\end{aligned}\quad (1.19)$$

$$\begin{aligned}h_a^2 &= (\sigma_f^2 + \sigma_m^2) / (\sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2 + \sigma_w^2 + \sigma_e^2) \\ &= 2 (1/4 \sigma_A^2 + 1/4 \sigma_A^2) / (1/4 \sigma_A^2 + 1/4 \sigma_A^2 + 1/4 \sigma_D^2 + 1/2 \sigma_A^2 + 3/4 \sigma_D^2 + \sigma_e^2) \\ &= \sigma_A^2 / \sigma_P^2\end{aligned}\quad (1.20)$$

$$\begin{aligned}h_b^2 &= (\sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2) / (\sigma_e^2 + \sigma_A^2 + \sigma_D^2) \\ &= 2 (1/4 \sigma_A^2 + 1/4 \sigma_A^2) + 4 (1/4 \sigma_D^2) / \sigma_P^2 \\ &= (\sigma_A^2 + \sigma_D^2) / \sigma_P^2 \\ &= \sigma_G^2 / \sigma_P^2\end{aligned}\quad (1.21)$$

$$\begin{aligned}\text{GCV \%} &= \text{genetic coefficient of variation} \\ &= 100\sigma_G / \text{general mean}\end{aligned}\quad (1.22)$$

The biparental progenies mating design was introduced by Mather in 1949. This design is conceptually simple and has been used extensively in sugarcane breeding (Brown *et al.*, 1968; Wu, Heinz, Meyer and Ladd, 1980). The parents are mated in pairs at random. Each parent is used only once. This design allows the estimation of variation within and between families. It only allows for partitioning of the V_G and V_E and no information concerning the components of genetic variation is available. Additive genetic variance can be estimated if V_D is presumed absent. Genotypic variance can also be partitioned using the covariance of the family means

on the parental values. Parents and offspring need to be grown at the same time for this to be effective. This design could be suitable for sugarcane if used in conjunction with parent-offspring regression.

With the nested (or hierarchical) mating design, also known as the North Carolina design I (Comstock and Robinson, 1948), each male parent cultivar is crossed with several different female cultivars. Each female cultivar is only used in one cross. These crosses produce several offspring, which are then planted into plots within blocks. Parent cultivars must be selected at random. Some sugarcane cultivars are incompatible with others, which indicates that this design is not appropriate for this crop. This design was modified by Morley and Heinrichs (1960), allowing for an unequal number of female cultivars to be crossed with more than one male cultivar. The problem of incompatibility of sugarcane could then be overcome (Hogarth, 1971a; 1971b). Unfortunately this design cannot provide an estimate of V_1 .

With the diallel mating design, each parent cultivar is crossed with every other parent cultivar in the parental population. However, some sugarcane cultivars are incompatible with others and other cultivars may be male sterile. This design is often unsuitable for sugarcane (Miller, 1977; Hogarth, 1980; Rao and Ethirajan, 1983). These problems can be overcome by using the partial diallel where only a sample of the crosses is analysed. The partial diallel can be used to partition the V_p into V_G and V_E , and then V_G can further be divided into the V_A and V_D (Kempthorne and Curnow, 1961; Hallauer and Miranda, 1988).

1.3.3 Estimation of variance components by maximum and residual maximum likelihood techniques

Analysis of variance (ANOVA) has been widely used to estimate variance components for fixed, mixed and random linear models. A problem associated with the use of mixed and random models has been the difficulty in estimating the variance components of the random effects. With balanced data, variance components are estimated by equating the mean squares in the ANOVA table to their expectations. Variance components cannot be estimated by ordinary ANOVA when the data is unbalanced.

Experimental error can arise from many different sources and it is important to be able to identify those sources. Variance component methodology enables the breeder to identify the error variation from a single source or cause, which contributes to the total error variation. Understanding the nature and extent of variation in experimental data will lead to the better design of future experiments and more efficient estimation of experimental effects. Often in a sugarcane breeding programme information is required on a particular population available at a particular time. This can result in the data being unbalanced as was the case in this study.

The maximum likelihood (ML) technique can be used to estimate variance components. It does not require a balanced design, nor is there any limitation on the structure of genetic relationships contained in the data (Robinson, 1987), as mentioned in the previous paragraph. The ML technique can account for more than one source of variation in the data. When many characters are being studied and the sample sizes are unequal this method of analysis is preferred to ordinary ANOVA (Hartl and Clark, 1989).

The ML technique has not been widely used to estimate variance components. Estimation of variance components by ML was complicated before the introduction of electronic computers. Estimates of variance components obtained when using ML are unfortunately usually biased because estimates of variance are not allowed to be negative and the genetic correlations cannot fall out of the range $[-1, +1]$ (Patterson and Thompson, 1975; Harville, 1977). Also, ML does not take account of the loss of degrees of freedom for estimation of fixed effects in the model, as does ANOVA. Patterson and Thompson (1971) developed residual maximum likelihood (REML) to overcome the bias caused by ML. The REML technique can estimate both treatment effects and variance components of linear mixed models (Genstat 5 reference manual, 1993). In this study, experimental design terms and genotypes were considered as random terms in the model. All the information relevant to estimating variance components is contained in a set of linearly independent error contrasts. Error contrasts are linear combinations of the observations with zero expectations. REML is equivalent to performing ML on data that has been standardised to zero. Balanced and unbalanced data is measured in the same way when using REML. REML can combine information from similar trials at different sites or at different times (Robinson, 1987). In this study variance components were

estimated using REML analysis of an unbalanced factorial (North Carolina design II) experiment.

1.4 Correlations among traits

Phenotypic, genetic and environmental correlations can be estimated from quantitative genetic experiments. Estimation of these correlations is based on components of variances and covariances that are estimated from analyses of variance and covariance, respectively. Correlations are useful in plant breeding programmes since they measure the degree of association (genetic or non-genetic) between two or more traits (Hallauer and Miranda, 1988). If genetic association exists then selection for one trait will cause changes in other traits, known as a correlated response. Correlations between characters may be environmental, which is usually found within genetically uniform individuals, or phenotypic, which is a combination of environmental and genetic correlations. Phenotypic correlation is the association between traits that can be directly observed. Genetic correlations result from pleiotropism and/or linkage disequilibrium. They have large errors and as a result precision of the estimate is dependent on the number of estimates available (Hallauer and Miranda, 1988). Environmental correlations are caused by two traits being influenced by the same environmental conditions. Environmental and genetic correlations may be very different in size. Correlations are calculated from components of variances and covariances for different mating designs (Falconer, 1989). Correlations are defined as:

$$r = \frac{Cov_{x,y}}{\sqrt{V_x V_y}} \quad (1.23)$$

where:

$Cov_{x,y}$ = covariance of two traits, X and Y;
 V_x = variance of trait X;
 V_y = variance of trait Y.

The covariance of two traits can be calculated from the following equation:

$$V_{x+y} = V_x + V_y + 2Cov_{xy} \quad (1.24)$$

where:

V_{x+y} = variance of two traits added together, X + Y.

Phenotypic, genetic and environmental correlations are calculated from equations 1.23 and 1.24:

$$r_p = \frac{1/2(V_{cx+cy} - V_{cy} - V_{cx}) + 1/2(V_{ex+ey} - V_{ey} - V_{ex})}{\sqrt{V_{cx} + V_{ex}} \cdot \sqrt{V_{cy} + V_{ey}}} \quad (1.25)$$

$$r_g = \frac{1/2(V_{cx+cy} - V_{cy} - V_{cx})}{\sqrt{V_{cx}} \cdot \sqrt{V_{cy}}} \quad (1.26)$$

$$r_e = \frac{1/2(V_{ex+ey} - V_{ey} - V_{ex})}{\sqrt{V_{ex}} \cdot \sqrt{V_{ey}}} \quad (1.27)$$

where:

- r_p = phenotypic correlation between trait X and Y;
- r_g = genetic correlation between trait X and Y;
- r_e = environmental correlation between trait X and Y;
- V_{cx+cy} = cross variance component for the sum of trait X and Y;
- V_{cy} = cross variance component for trait Y;
- V_{cx} = cross variance component for trait X;
- V_{ex+ey} = error variance component for the sum of trait X and Y;
- V_{ey} = error variance component for trait Y; and
- V_{ex} = error variance component for trait X.

Selection of individuals will be influenced by correlations among traits. A positive correlation between commercially important characters is favourable for breeders as this will facilitate combined selection for both traits. A negative correlation will hinder the recovery of recombinants that are high in both characters and may not allow the expression of both characters at a high level. Correlation between characters may allow selection for another character, other than the one of commercial interest, that may be easier to measure. This trait

needs to be highly heritable and highly correlated to the trait of interest to be beneficial to the selection process. Correlations can indicate whether simultaneous selection for two traits, such as cane yield and sucrose percentage, is likely to be successful. Genetic correlations among sugarcane traits have been determined by other workers (Table A4.3)

1.5 Clonal repeatability

Sugarcane is propagated vegetatively. In a selection programme sugarcane cultivars are planted in a number of different selection stages before they are considered for release to sugarcane growers. Breeders select sugarcane cultivars based on their trial results and the more superior ones are planted to the next selection stage. Cane setts are cut from the stalks of these more superior cultivars and planted into the following stage. Seasonal and environmental variations influence the phenotypic expression of different traits of these clones. Clonal repeatabilities of traits need to be determined to assist breeders in their selection for these superior sugarcane cultivars. When a trait has a low repeatability between selection stages breeders should use family selection rather than individual selection for that particular trait to be more effective (Skinner, 1971).

Plant breeders use repeatability to relate to the h_b^2 of characters among a group of clones or pure lines. Repeatability is used to compare characters rather than improve estimates of heritability. Many breeders have determined clonal repeatabilities of sugarcane traits between plant and ratoon crops (Table A4.4).

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental procedures

The sugarcane population used in this investigation was randomly selected from the 1991 crossing season at SASEX, Mount Edgecombe. The parent cultivars were planted in the glasshouse and photoperiod house in September 1990. Crosses between these parents were made in the glasshouse between May and August 1991. The seed was sown in seed boxes in the glasshouse in January 1992. After germinating they were planted in pots on terraces outside the glasshouse. Cane setts, approximately 300 mm in length, were cut from the terrace seedlings and planted in the Single stool stage (stage 1) field trial in September 1992. In November of the following year, the population being studied was planted in the Single line stage (stage 2, plant) field trial, and allowed to ratoon once (stage 2, ratoon). These were the first two of five selection stages in the breeding and selection programme at SASEX (Appendix 1) but this investigation stops after the first ratoon crop of stage 2. The planting, ratooning, sampling and harvesting dates of all three crops are presented in Table A5.1.

2.1.1 Stage 1 - Single Stool Stage

One hundred and eighty crosses were planted in the single stool field in September 1992. Some of these crosses were replicated twice. From these crosses 15 were randomly selected to be used for this investigation (Table 2.1). The parents of the selected crosses were planted in the same trial as their offspring. Three of these crosses (cross numbers 13, 14, and 15) were excluded from the analyses because the origin of one of the parents used in these crosses was unknown (cultivar name = Co281). Data was collected from these crosses, however, as it was used to make field adjustments in both stages of the investigation and for the determination of clonal repeatabilities.

Table 2.1 Parents and crosses from a sugarcane population planted in Stages 1 and 2 under raingrown conditions, at Mount Edgecombe, with numbers assigned to simplify handling of data and drawing up of maps.

Cross number	Cross name	Female parent name	Female parent number	Male parent name	Male parent number
1	BB1156	NCo376	P3	80L0432	P5
2	BB0740	81E0313	P14	84E1334	P12
3	BB0336	78L0960	P13	84E1334	P12
4	BB0690	CB40/35	P6	77F3089	P7
5	BB0994	NCo376	P3	R570	P4
6	BB0250	NCo376	P3	84E1334	P12
7	BB1710	MZC74/275	P1	71E0280	P2
8	A239/1	Co419	P17	US1694	P18
9	W1267	76M1566	P8	CB40/35	P6
10	BB1316	MZC74/275	P1	83F2019	P10
11	BB0941	NCo376	P3	US82-23	P19
12	BB0897	75E0247	P9	R570	P4
13	BB0794	Co281	P15	NCo376	P3
14	BB0057	Co281	P15	US66/56/15	P16
15	AA0079	Co281	P15	Co312	P20

The single stools were planted from cane setts in a 'snakelike' fashion across six fields (Figure A6.1). The selected crosses used in this trial were planted amongst the crosses used for the normal plant breeding programme. Each cross was replicated twice, with each plant (stool) representing a different genotype. Thirty-two offspring were randomly selected from each of the crosses, 16 from each replication. The parent cultivars of these crosses were planted in the same trial, each being represented by four single stools, replicated twice. The parents were planted together in two groups (replications), separate from their offspring.

Individual measurements were taken from all the progeny and parents being investigated. The traits to be recorded were stalk population (number of stalks per stool), stalk diameter (cm²), stalk height (cm), estimated stool mass (kg per stool), dry matter % cane, fibre % cane, brix % cane, brix % dry matter, purity (%), pol % cane, and ers % cane. The number of

stalks used to determine these traits and the methods of determination are explained in Appendix 5.

2.1.2 Stage 2 - Single Line Stage

Stage 2 was planted towards the end of October 1993. Approximately 10 stalks were taken from each of the offspring and parental stools in Stage 1 (from the selected population of 15 crosses) and planted into a 5 m single line at Stage 2. The lines were spaced 1.2 m apart and planted in banks down the field (Figures A6.2). There were large (2 m wide) and small (1 m wide) breaks between the banks. The 32 offspring of each cross were divided into four replications of eight individuals. These replications were randomly planted in the field. The parents were replicated six times, and were randomly planted within each of the replications. A control cultivar (cultivar NCo376) was planted before and after every replication of eight progeny and parental group to enable the measurement of field variation. This control cultivar is a commercially grown cultivar. Border rows of the same control cultivar were planted around the trial.

Field records were taken when the cane was approximately 12 months old. Details of number of stalks and methods used for taking these measurements are provided in Appendix 5. Stalk diameter (cm^2) and population (number of stalks per 5 m line) were measured first from each of the single lines. Then stalk height (cm) was measured, when sucrose samples were being collected for the millroom. Cane mass (kg (5 m row)^{-1}) was recorded for each of the single lines at harvest. Final cane yield (kg (5 m row)^{-1}) was determined by adding the mass of the 12 stalk sample sent to the millroom to the mass of each of the lines at harvest.

The harvested field was ratooned for a second crop. In this way seasonal differences could be determined. The cane was 11 months old when field measurements were taken. The same traits were measured in the ratoon crop as in the plant crop and Stage 1. Similar methods and procedures used for the plant crop were used to measure the traits in the ratoon crop.

2.2 Field adjustments and statistical procedures

The parent cultivars were planted in the same trial as their offspring. In this way information from both the parents and offspring could be collected and regression analyses used to determine the relationship between the offspring and their parents. REML analysis required that only the offspring data be collected, although knowledge of the parents used to make the crosses was still necessary. The results obtained from the regression analysis were compared with those obtained from the REML analysis. Correlations between traits and clonal repeatabilities were also determined in this investigation. Mid-parent-offspring regression and field adjustments were determined using a computer spreadsheet programme (Lotus 1-2-3), and the other results obtained from REML analysis were produced using a statistical computer package (Genstat 5, Release 3).

2.2.1 Field adjustments

Prior to calculating heritabilities, correlations and repeatabilities, field adjustments were made at both the selection stages. This enabled removal of environmental variation within and between the fields used for trials. Results could then be compared.

2.2.1.1 Stage 1 - Single Stool Stage

The data was adjusted to account for field variation. The trial was planted in six different fields (Appendix 6) resulting in within as well as between field variation. Cross means for the 11 traits were determined as follows:

- i) the mean of the offspring in each replication (r_1 - r_2) for each of the 15 crosses (c_1 - c_{15}) and eleven traits (t_1 - t_{11}) was determined, e.g. r_1, c_1, t_1 = mean of replication 1 for cross 1 and trait 1, r_2, c_1, t_1 = mean of replication 2 for cross 1 and trait 1;
- ii) means of the crosses were determined by summing the means of the two replications and dividing them by two, e.g. mean of cross 1 for trait 1 = $(r_1, c_1, t_1 + r_2, c_1, t_1) / 2$.

These cross means were then used in the regression analysis. It was also necessary to adjust

individual data to be able to determine clonal repeatabilities. Offspring from each cross were adjusted by multiplying each observation to the cross mean divided by the mean of r1 or r2, depending on the replication it was located in.

Parent cultivars were not adjusted in the same way as the crosses. All parents were planted together in two different fields. Each parent cultivar was represented by four single stools in each of the two fields. The area planted to parent cultivars was relatively small and uniform for each replication and little variation was observed between the two replications. The parent values to be used in the analyses were then determined by calculating the mean of the eight stools for each of the parent cultivars for each of the traits.

2.2.1.2 Stage 2 - Single Line Stage

The field plan in Stage 2 allowed the data to be adjusted more efficiently than Stage 1. There were a number of reasons for this:

- i) the trial was planted into one field in Stage 2, and not over six as in the Stage 1;
- ii) a control cultivar (NCo376) was planted at regular intervals throughout the Stage 2 field to enable measurement of field variation;
- iii) families were divided into four groups of eight cultivars to allow for more efficient family adjustments in Stage 2; and
- iv) each offspring was represented by a 5 m line with a varying number of stools in each line in Stage 2, while there was only one stool representing each cultivar in Stage 1.

The control cultivars were initially used to make field adjustments. However, the control cultivars seemed to be extremely variable and they could not be reliably used in the analysis. Parents and offspring were planted in rows and banks along the field (Figure A6.2). It was possible to use the rows and banks to help adjust the data. Field variation was observed down the banks and along the rows. Each of the single lines (plots) was allocated a bank and row number depending on its position in the field. There were 13 banks across the field and a maximum of 84 rows down the field. The parents and controls were removed from the adjustments so as not to bias any of the bank or row adjustments. The cross information was

used when determining correlations and clonal repeatabilities. A bias would be caused by the parents as the parent groups were located in some banks and not in others, and they were generally superior to the offspring. The first row of each bank was planted to the control cultivar which would also have biased the adjustments for the first few rows. This first row was, therefore, excluded.

The data were initially adjusted for the banks as follows:

- i) the mean for each of the traits was determined (T);
- ii) the mean of each of the banks was then calculated (B), the last two banks 12 and 13 being combined because there was only one cross in bank 13; and
- iii) to calculate the adjustment for each bank the following calculation was then used (Butterfield, 1995; personal communication):

$$\frac{\left(\frac{3T}{B}\right) + 1}{4} = \text{bank adjustment; and} \quad (2.1)$$

- iv) each of these adjustment values was then multiplied by each individual value depending on the bank it was located in.

The bank adjusted data were then adjusted for the rows:

- i) the field means for the traits, adjusted for the banks, were calculated (F);
- ii) the means of all the rows were then calculated (R);
- iii) to get a more representative sample of the rows the 84 rows were divided into 5 rows each, e.g. Rows 1 - 5, 6 - 10, 11 - 15, etc. The rows got shorter down the field and had fewer individuals in them. To obtain comparative samples with the previous rows, rows 56 - 63 (8 rows) were grouped together and rows 64 - 78 (15 rows) were also grouped together. Row numbers 1, and 79 - 84 were excluded from the adjustments as they only contained parent and control cultivars.
- iv) to calculate the adjustment for rows (Butterfield, 1995; personal communication):

$$\frac{\left(\frac{3F}{R}\right) + 1}{4} = \text{row adjustment; and} \quad (2.2)$$

- v) this adjustment value was then multiplied to the bank adjusted data to get the final field adjustments for the offspring.

The effectiveness of these adjustments had to be ascertained. Variance ratios (F-values) of the crosses before and after the adjustments were determined to give an indication of this effectiveness (Programme A7.1). Adjustments must remove the field variation (mainly replication differences) without removing variation due to cross differences. F-values and coefficients of variation (CV%) were presented in Table A7.1. Figures A7.1 - A7.4 showed field trends before and after adjustments were made. It could be seen from the figures that field variation was reduced. F-values decreased initially when bank effects were removed and then increased again when row effects were removed without significantly affecting differences between crosses. The CV% decreased slightly with all traits, while F-values seemed to either increase or decrease slightly depending on the trait. Therefore, field variation was reduced without variation between the crosses being removed, and this indicates that adjustments were successful. These adjusted offspring values were used in the regression and REML analyses.

It was decided that parent cultivars need not be adjusted in the same way as the offspring. Parents were sufficiently represented in each of the six replications. Parent means for traits were determined by calculating the mean of the six replications for each parent cultivar. These adjusted parental values were used in the regression analysis.

2.2.2 Statistical procedures

2.2.2.1 Heritability estimates

The parental combinations were selected at random from a selected population to be used in the normal breeding and selection programme carried out at SASEX. Some parent cultivars were used more than once in some of the crosses. Due to the unbalanced nature of this study there were only two possible methods of analysis: regression and REML. Both methods of analysis required certain assumptions to be made. Unfortunately the sugarcane crop violates these assumptions to some extent and breeders need to be aware of these violations when determining variance components. These assumptions and violations were discussed in detail in Section 1.2 (REML analysis of an unbalanced North Carolina design II) and Section 1.3.1 (mid-parent offspring regression).

Mid-parent offspring regression was used to determine h_a^2 at Stage 1 and Stage 2-P and 2-R (Section 1.3.1). The mean of the offspring of the 12 crosses selected at random for this investigation were regressed against their mid-parent values (an example of this regression was presented in Tables A7.2 and A7.3). Regression coefficients determined for the traits at the different stages were equal to the narrow sense heritabilities of the traits.

Although parents were selected at random for this investigation, the parents of the single crosses were classified in such a way that the data obtained from Stage 2 could also be analysed as an unbalanced North Carolina design II (Section 1.3.2 and 1.3.3). Due to the unbalanced nature of the cross-classification, REML was used to determine the variance components. This enabled a comparison of heritability estimates obtained by REML with those obtained by regression. Using the North Carolina mating design II, m males ($m=9$) were crossed with f females ($f=8$) and evaluated in r replications ($r=4$) (Programme A7.2) (Hohls, 1994; personal communication). All treatment effects were random for this investigation. There were no fixed effects. Female, male and female \times male interaction terms were used to determine additive and non-additive genetic variance components (Equations 1.17-1.19 in Section 1.3.2). Additive, non-additive and residual variance components were then used to calculate h_a^2 and h_b^2 (Equations 1.20 and 1.21).

2.2.2.2 Correlations among traits

The development of selection strategies requires a knowledge of the relationship among traits considered in the sugarcane breeding programme. Information on phenotypic and genetic correlations is therefore important. Information from the 12 crosses at Stage 2, plant and ratoon crops, was used to determine correlations among traits. Variances (V_{x+y} , V_x , V_y) for all 11 sugarcane traits were obtained from the REML analysis of an unbalanced North Carolina design II (Programme A7.3) where crosses ($c=12$) and replications ($r=4$) were the treatment effects. These treatment effects were random. Covariances were determined from variance components, using the relationship expressed in equation 1.24, where:

$$Cov_{x,y} = \frac{1}{2}(V_{x+y} - V_x - V_y) \quad ; \text{ and } x \text{ and } y \text{ were two traits under consideration.}$$

Phenotypic, genetic and environmental correlations were then calculated using equations 1.25, 1.26 and 1.27, respectively.

2.2.2.3 Clonal repeatabilities between stages

Information from all 15 crosses was used to determine clonal repeatabilities between selection stages (Stage 1 and 2) and between Stage 2-P and -R. Clonal repeatabilities were determined from the F-values of the different traits determined between seasons: Stage 1 and Stage 2-P; Stage 1 and Stage 2-R; and Stage 2-P and Stage 2-R. These F-values were calculated from ANOVA tables where the variance ratios of the individuals were determined over two seasons $[(F\text{-value} - 1) / F\text{-value}]$ (Programme A7.4).

It was possible that some of the families may have biased the estimates of repeatability as there may have been differences in the variation of a trait within a particular family. To compare these differences, repeatabilities were also calculated for the individuals within each of the families.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Heritability estimates

3.1.1 Mid-parent-offspring regression

Mid-parent offspring regression was used to estimate h_a^2 of 11 sugarcane traits at Stages 1 and 2-P and -R (Table 3.1). Large differences in h_a^2 of traits within Stages 1 and 2 were observed. In Stage 1, stalk population had the highest h_a^2 , followed by brix % dry matter, stalk diameter, and fibre % cane. Purity had the lowest estimate at this stage. Standard errors obtained at Stage 1 were high and more than 30% of the h_a^2 for certain traits (Table 3.1). This may have been due to the field differences within and between the six fields, where the trial was planted.

Table 3.1 Narrow sense heritabilities of a sugarcane population under raingrown conditions for 11 traits at Stages 1 and 2, plant and ratoon crops, using mid-parent offspring regression.

Sugarcane traits	h_a^2 (Stage 1)	h_a^2 (Stage 2, plant crop)	h_a^2 (Stage 2, ratoon crop)
Stalk diameter	0.570 ± 0.311	0.612 ± 0.194	0.507 ± 0.219
Stalk population	0.853 ± 0.294	0.435 ± 0.152	0.299 ± 0.203
Stalk height	0.279 ± 0.197	0.502 ± 0.212	0.472 ± 0.198
Single stool or single line mass	0.340 ± 0.203	0.421 ± 0.187	0.175 ± 0.107
Dry matter % cane	0.342 ± 0.189	0.447 ± 0.323	0.427 ± 0.209
Fibre % cane	0.511 ± 0.212	0.740 ± 0.190	0.498 ± 0.252
Brix % cane	0.416 ± 0.168	0.802 ± 0.201	0.777 ± 0.159
Brix % dry matter	0.574 ± 0.193	0.843 ± 0.134	0.687 ± 0.223
Purity	$0.000^\dagger \pm 0.159$	0.840 ± 0.169	0.835 ± 0.072
Pol % cane	0.491 ± 0.201	0.849 ± 0.191	0.786 ± 0.117
Ers % cane	0.384 ± 0.206	0.865 ± 0.179	0.796 ± 0.110

where:

h_a^2 = narrow sense heritability; and

† = the regression coefficient was slightly below zero (-0.045) and was therefore rounded up to 0.000.

Heritabilities of traits determined at Stage 1, except for stalk population, were lower than those determined at Stage 2-P. This could possibly have been due to the lower V_E component observed in the population at Stage 2-P. The reduced V_E component at Stage 2-P was probably due to the larger plots and better representation of the genotypes at this stage compared to each genotype being represented by a single stool at Stage 1. The heritability estimates obtained at Stage 2-R were also lower than those obtained at Stage 2-P. Ratooning ability of the cultivars could have influenced the data recorded in the ratoon crop and therefore been partly responsible for the lower heritabilities obtained at Stage 2-R. The cumulative effect of environmental factors could have affected the performance of the genotypes over two seasons and therefore adversely influenced the heritabilities obtained in the ratoon crop.

The Stage 1 trial was planted over six fields and it was difficult to ascertain whether the variation observed between heritabilities was due to genotypic variation or field variation. Another possible reason for these high standard errors may have been that there was only a single stool representing each genotype and the data collected from these stools may not have been very reliable. Other studies have reported heritability estimates that compare favourably with the estimates obtained in this trial (Table A4.2), except for Gravois, Milligan and Martin (1991) whose estimates were generally much lower.

From the high h_a^2 obtained for stalk population (0.853 ± 0.294), brix % dry matter (0.574 ± 0.193), stalk diameter (0.570 ± 0.311), and fibre % cane (0.511 ± 0.212) at Stage 1 (Table 3.1), it could be concluded that most of the genetic variance was additive, rather than non-additive for these traits. Purity had the lowest h_a^2 at this stage (0.000 ± 0.159). This estimate was much lower than anticipated but could be explained. The drought of 1992/93 had a severe effect on the purities of this cane by stressing the cane. The additive genetic differences for purity were obscured by the large amount of environmental variation. The h_a^2 for purity was much lower at Stage 1 than those obtained by other workers (Table A4.2). The standard errors estimated by other workers were, however, very high which could indicate that this trait is difficult to measure. Stalk height also had a very low h_a^2 at Stage 1. This low estimate was due to the large environmental influence on the sugarcane. Competition between neighbouring stools would also have affected the h_a^2 for this trait.

Mid-parent offspring regression gave more reliable estimates of h_a^2 in Stage 2-P and -R than in Stage 1. This was possibly due to the standard errors being much lower and the environmental variation being measured more efficiently in Stage 2. All the estimates, except for stalk population, were higher than those obtained in Stage 1, the highest being the sucrose-related traits of ers % cane, pol % cane, brix % dry matter, purity, and brix % cane. Fibre % cane and stalk diameter also had high estimates of h_a^2 . Stalk population had a much lower h_a^2 at Stage 2 than was obtained at Stage 1. This could have been caused by the variable number of stools planted in each of the single lines at Stage 2. The planting method made stool counting difficult. The cane setts were overlapped when placed in the furrows, to ensure uniform germination in a single line. Boyce (1970) indicated that sugarcane compensates for decreasing stool density under favourable growing conditions in such a way that the maximum yield per unit area is achieved irrespective of the number of stools planted in a given length. However, this applies to cane grown with adequate water and nutrients. For raingrown conditions, under which this trial was grown, this compensation for decreasing stool density may not occur.

Competition existed between neighbouring single lines at Stage 2. The crosses were planted together in four replications of eight lines and the parents were also planted in one group next to one other. This competition between the neighbouring lines within these replications may have affected the expression of some of these traits, especially those associated with cane yield, e.g. stalk height and cane mass.

Cane mass at Stage 2-R is dependent on the ratoonability of cultivars. Ratoonability appears to be genetic, but cannot be shown from this investigation. Some cultivars did not ratoon well in this trial and resulted in gaps between stools within some of the lines. This is possibly why the estimate of heritability was so low for cane mass and the standard error was so high in the ratoon crop at Stage 2 when compared with the plant crop. Environmental variation was significantly higher in the ratoon crop than the plant crop.

Purity h_a^2 was significantly higher in Stage 2 than in Stage 1. The climatic stress was not as severe as that which occurred at Stage 1, and the cane was not as mature when sampled at

Stage 2. The field plan of the trial at Stage 2 allowed for more efficient field adjustment than in Stage 1.

3.1.2 REML analysis

The REML technique was used to determine h_a^2 and h_b^2 at Stage 2-P and -R. Genetic effects were initially determined using an unbalanced North Carolina design II (Table 3.2). Genetic effects of all replication interaction terms (σ_{rf}^2 , σ_{rm}^2 , and σ_{rfm}^2) were pooled with the residual error (σ_e^2) to get a combined residual error (σ_c^2) (Table 3.2). The female, male, female \times male interaction, and combined residual error genetic effects were then used to determine h_a^2 and h_b^2 . There were notable differences in the genetic effects of some of the sugarcane traits in Stage 2 -P and -R.

In Stage 2, the female \times male interaction genetic effects (σ_{fm}^2) were insignificant for most sugarcane traits, except for cane mass in -P and -R, and stalk population and dry matter % cane in -R, where this effect contributed significantly to h_a^2 and h_b^2 of these traits. The genetic effect caused by replications (σ_r^2) for stalk height (27.050) in -P, and cane mass in -P and -R (1.345 and 1.375, respectively), was large. Although this effect was not used to determine h_a^2 and h_b^2 , it was still important for selection purposes. The genetic effect of replications on these traits was not anticipated as field adjustments were made to remove any possible differences between replications, caused by the field layout. Competition between neighbouring single lines within replications was possibly the cause for this significant genetic effect. Some sugarcane genotypes germinate earlier and grow faster than others and therefore can affect the growth of neighbouring genotypes. These factors play an important role when selecting potential commercial cultivars at this selection stage. Breeders often tend to select the taller sugarcane genotypes over the shorter, more compact ones from a single line trial, as their phenotypes are more attractive. These genotypes are known, however, not to perform well at later selection stages (Thomas, 1994; personal communication). Results obtained in this investigation (Table 3.2) indicated that sugarcane breeders need to be more cautious when selecting potential commercial cultivars in Stage 2, based on cane mass and stalk height.

Table 3.2 Genetic effects of a sugarcane population under raingrown conditions for 11 traits at Stage 2, plant and ratoon crops, using REML analysis of an unbalanced North Carolina design II.

Stage	Genetic Effects	Stalk diameter	Stalk population	Stalk height	Cane mass	Dry matter %cane	Fibre%cane	Brix%cane	Brix%dry matter	Purity	Pol%cane	Ers%cane
Stage 2, plant crop:	σ_r^2	<0.001	0.044	27.050	1.345	0.184	<0.001	0.021	0.001	0.400	0.055	0.063
	σ_f^2	0.367	0.182	71.320	32.640	0.857	0.405	0.058	5.321	1.148	0.131	0.189
	σ_m^2	0.140	1.379	0.026	0.017	0.112	0.515	0.618	1.858	5.251	0.982	1.234
	σ_{rf}^2	<0.001	0.615	0.026	0.017	0.014	0.094	0.108	1.144	0.001	0.050	0.035
	σ_{rm}^2	<0.001	0.001	0.026	0.017	<0.001	0.350	0.030	2.345	1.254	<0.001	<0.001
	σ_{fm}^2	<0.001	0.078	0.026	12.310	<0.001	0.051	<0.001	0.001	0.001	<0.001	<0.001
	σ_{rfm}^2	0.106	0.001	192.100	26.110	0.385	<0.001	0.138	0.001	1.086	0.285	0.371
	σ_e^2	0.952	12.690	262.600	171.600	2.781	2.872	0.941	13.370	7.849	1.242	1.535
	σ_c^2	1.058	13.308	454.753	197.744	3.181	3.315	1.217	16.860	10.190	1.577	1.942
Stage 2, ratoon crop:	σ_r^2	<0.001	0.002	0.038	1.375	<0.001	<0.001	0.037	0.268	0.001	0.011	<0.001
	σ_f^2	0.236	0.831	113.300	5.085	0.278	0.438	0.106	3.167	1.799	0.175	0.249
	σ_m^2	0.205	0.002	42.770	0.022	<0.001	0.458	0.708	5.608	7.205	1.258	1.713
	σ_{rf}^2	<0.001	0.316	3.910	0.022	<0.001	<0.001	<0.001	0.001	0.264	<0.001	<0.001
	σ_{rm}^2	0.073	0.444	20.340	1.010	0.252	0.095	<0.001	0.001	1.253	<0.001	<0.001
	σ_{fm}^2	<0.001	2.119	0.038	22.610	0.358	<0.001	<0.001	0.001	0.001	<0.001	<0.001
	σ_{rfm}^2	<0.001	0.002	29.920	0.022	<0.001	0.479	0.455	4.689	0.612	0.577	0.718
	σ_e^2	1.070	15.230	376.600	219.500	2.442	2.516	0.997	12.990	6.010	1.276	1.564
	σ_c^2	1.143	15.992	430.770	220.554	2.695	3.089	1.451	17.682	8.139	1.853	2.283

where: REML = restricted maximum likelihood; <0.001 = genetic effects with values less than 0.001; σ_r^2 = genetic effect of replications; σ_f^2 = genetic effect of female parents; σ_m^2 = genetic effect of male parents; σ_{rf}^2 = genetic effect of interaction between replications and female parents; σ_{rm}^2 = genetic effect of interaction between replications and male parents; σ_{fm}^2 = genetic effect of interaction between female and male parents; σ_{rfm}^2 = genetic effect of interaction between replications, female and male parents; σ_e^2 = genetic effect of residual error; and σ_c^2 = genetic effect of combined residual error (replication interaction terms combined with residual error).

The genetic effects were used to calculate variance components and h_a^2 and h_b^2 at Stage 2-P and -R (Table 3.3). The h_a^2 estimates obtained by regression were generally similar to those obtained by REML. The standard errors were lower for the estimates obtained by regression analysis. The h_a^2 and h_b^2 estimates of three traits differed for method of analysis used: stalk population; fibre % cane; and brix % dry matter. A possible reason for these differences is that all the offspring and therefore within family variance is considered when using REML, while only the mean of the offspring is considered when using regression. The sucrose-related traits (brix % cane, brix % dry matter, purity, pol % cane, and ers % cane) had very high h_a^2 . This holds true for both methods of analysis. Stalk diameter was also highly heritable at Stage 2. The standard error for stalk height at Stage 2-P exceeded the h_a^2 and h_b^2 estimates when REML was used. Stalk height is not a reliable trait at Stage 2 because it is affected by competition between neighbouring single lines. Generally, however, all the standard errors were high when REML was used. This was due to the completely unbalanced cross-classification used in this investigation and REML does not allow any variance components to be negative, applying a positive constraint on variance components.

When comparing h_a^2 and h_b^2 at Stage 2 it was observed that the estimates were very similar. This indicated that the interaction between male and female parents was very small, almost nonexistent, since very few degrees of freedom were allocated to the interaction terms. The h_a^2 and h_b^2 for cane mass were the only estimates in the plant crop that differed significantly. This indicated that the interaction between female and male parents was significant and non-additive genetic variance was therefore important for this trait. The interaction term for stalk population and fibre % cane contributed some non-additive effect but not enough to alter the heritabilities significantly.

The results obtained in the ratoon crop were very similar to those obtained in the plant crop, except for three traits. REML estimates of h_a^2 for stalk population, cane mass, and dry matter % cane were much lower than those obtained by regression. They were also significantly lower than REML estimates of h_b^2 . These three traits were therefore affected by non-additive genetic effects. Dominance genetic variance was important for stalk population by Hogarth *et al.* (1981).

Table 3.3 Variance components, heritabilities, and genetic coefficients of variation of a sugarcane population under raingrown conditions for 11 traits at Stage 2, plant and ratoon crops, using REML analysis of an unbalanced North Carolina design II.

Stage		Stalk diameter	Stalk population	Stalk height	Cane mass	Dry matter %cane	Fibre %cane	Brix %cane	Brix %dry matter	Purity	Pol %cane	Ers %cane
Stage 2, plant crop:	V_A	1.014	3.122	142.692	65.314	1.938	1.840	1.352	14.358	12.798	2.226	2.846
	V_G	1.014	3.434	142.796	114.554	1.938	2.044	1.352	14.362	12.802	2.226	2.846
	V_P	1.459	14.329	333.972	216.567	3.750	3.840	1.617	20.550	14.249	2.355	2.958
	h_a^2	0.696	0.218	0.427	0.302	0.516	0.479	0.836	0.699	0.898	0.945	0.962
	$SE(h_a^2)$	0.433	0.194	0.513	0.346	0.352	0.368	0.371	0.448	0.404	0.398	0.406
	h_b^2	0.696	0.240	0.428	0.529	0.517	0.532	0.836	0.699	0.898	0.945	0.962
	$SE(h_b^2)$	0.318	0.165	0.547	0.342	0.304	0.258	0.324	0.306	0.328	0.341	0.344
	GM	5.660	16.010	121.800	42.680	29.550	15.000	14.480	49.590	86.000	12.500	13.260
	$\sqrt{V_G}$	1.010	1.850	11.950	13.030	1.390	1.430	1.170	3.790	3.580	1.490	1.690
	GCV %	17.800	11.570	9.810	30.530	4.710	9.520	8.050	7.640	4.160	11.950	12.730
Stage 2, ratoon crop:	V_A	0.882	1.666	312.140	10.214	0.556	1.792	1.628	17.550	18.008	2.866	3.924
	V_G	0.882	10.142	312.292	100.654	1.988	1.792	1.628	17.554	18.008	2.866	3.924
	V_P	1.511	18.182	532.708	247.220	3.078	3.412	1.811	21.766	15.015	2.709	3.526
	h_a^2	0.583	0.092	0.586	0.041	0.181	0.525	0.899	0.806	*1.000	*1.000	*1.000
	$SE(h_a^2)$	0.376	0.312	0.403	0.222	0.381	0.415	0.467	0.519	0.415	0.449	0.449
	h_b^2	0.584	0.558	0.586	0.407	0.646	0.526	0.898	0.807	*1.000	*1.000	*1.000
	$SE(h_b^2)$	0.250	0.336	0.294	0.264	0.366	0.313	0.408	0.396	0.324	0.391	0.388
	GM	5.870	14.910	125.900	40.780	30.500	13.820	16.580	54.810	89.570	14.870	13.260
	$\sqrt{V_G}$	0.940	3.180	14.110	17.810	1.410	1.340	1.280	4.190	4.240	1.690	1.980
	GCV %	15.980	21.360	11.200	43.680	4.630	9.680	7.700	7.650	4.740	11.390	14.980

where:

REML = restricted maximum likelihood; V_A = additive genetic variance; V_G = genotypic variance; V_P = phenotypic variance; h_a^2 = narrow sense heritability; $SE(h_a^2)$ = standard error of narrow sense heritability; h_b^2 = broad sense heritability; $SE(h_b^2)$ = standard error of broad sense heritability; GM = general mean; $\sqrt{V_G}$ = square root of genotypic variance; GCV % = genotypic coefficient of variation; and * = heritabilities were calculated as being larger than 1.000.

These results are comparable with those obtained under irrigated conditions. Blose (1992) obtained low estimates of h_a^2 for cane mass (0.00 ± 0.00 in 1983 and 0.10 ± 0.03 in 1985), and high estimates for brix % cane (0.57 ± 0.08 in 1983 and 0.42 ± 0.07 in 1985) and ers % cane (0.85 ± 0.10 in 1983 and 0.50 ± 0.08 in 1985). Although the standard errors of the heritabilities obtained in this trial were higher than those obtained by Blose it can still be concluded from these results that selection of parent cultivars for sucrose-related traits, fibre % cane, and stalk diameter should be as successful under raingrown conditions as under irrigated or wetland conditions (Table A4.1 and A4.2). Progeny testing would be more suitable for making progress with cane mass, stalk height and population. Progress could, however, still be made when selecting parents for cane mass, stalk height and population as they do have substantial additive genetic variance but the non-additive variation is also large which indicates that predictions based on these traits are not likely to be reliable.

3.2 Correlations among traits

Phenotypic, genetic, and environmental correlations (r_p , r_g , and r_e , respectively) among the 11 traits were determined at Stage 2-P and -R to identify the relationships that exist between these traits (Tables 3.4 and 3.5).

High positive genetic correlations between traits imply that when selecting for one of the traits there will be indirect selection for the other trait. If the correlation between two traits is almost zero then selection in one direction for one trait will not have a negative effect on the other trait.

Table 3.4 Phenotypic, genetic, and environmental correlations among sugarcane traits in Stage 2, plant crop, under raingrown conditions.

Trait	r	Pop	Ht	Mass	DM %	Fib %	Brix %	Brix %dm	Pur	Pol %	Ers %
Diam	r_p	-0.400	0.206	0.260	-0.270	-0.321	0.098	0.277	-0.035	0.060	0.065
	r_g	-0.790	0.768	0.667	-0.600	-0.628	0.142	0.457	-0.138	0.061	0.060
	r_e	-0.325	0.070	0.149	-0.157	-0.195	0.073	0.184	0.022	0.060	0.068
Pop	r_p		0.121	0.441	-0.002	-0.014	0.013	0.016	0.092	0.039	0.047
	r_g		-0.546	-0.129	-0.034	-0.179	0.188	0.215	0.200	0.191	0.197
	r_e		0.203	0.522	0.003	0.018	-0.032	-0.032	0.070	-0.000	0.008
Ht	r_p			0.573	0.089	-0.015	0.157	0.090	0.096	0.143	0.133
	r_g			0.795	-0.773	-0.790	0.156	0.570	-0.175	0.059	0.061
	r_e			0.541	0.243	0.146	0.168	-0.028	0.177	0.180	0.168
Mass	r_p				-0.177	-0.171	-0.002	0.125	0.039	0.010	0.024
	r_g				-0.891	-0.894	0.157	0.638	-0.111	0.077	0.090
	r_e				-0.033	-0.001	-0.052	-0.021	0.088	-0.012	0.003
DM %	r_p					0.790	0.262	-0.438	0.355	0.304	0.257
	r_g					0.657	0.261	-0.274	0.547	0.344	0.334
	r_e					0.831	0.269	-0.504	0.292	0.301	0.237
Fib %	r_p						-0.384	-0.895	-0.130	-0.327	-0.360
	r_g						-0.556	-0.905	-0.293	-0.485	-0.498
	r_e						-0.312	-0.896	-0.060	-0.257	-0.301
Brix %	r_p							0.750	0.744	0.974	0.956
	r_g							0.857	0.973	0.999	1.000 [†]
	r_e							0.693	0.607	0.959	0.927
Brix %dm	r_p								0.456	0.699	0.715
	r_g								0.682	0.813	0.823
	r_e								0.333	0.635	0.655
Pur	r_p									0.872	0.898
	r_g									0.983	0.977
	r_e									0.802	0.849
Pol %	r_p										0.996
	r_g										1.000 [†]
	r_e										0.993

where:

Diam = stalk diameter;

Ht = stalk height;

DM% = dry matter % cane;

Brix% = brix % cane;

Pur = purity;

Ers% = ers % cane;

 r_p = phenotypic correlations; r_e = environmental correlations; and

Pop = stalk population;

Mass = cane mass of single lines;

Fib% = fibre % cane;

Brix%dm = brix % dry matter;

Pol% = pol % cane;

r = correlations;

 r_g = genetic correlations;[†] = correlation subject to high estimation standard error.

Table 3.5 Phenotypic, genetic, and environmental correlations among sugarcane traits in Stage 2, ratoon crop, under raingrown conditions.

Trait	r	Pop	Ht	Mass	DM %	Fib %	Brix %	Brix %dm	Pur	Pol %	Ers %
Diam	r_p	-0.355	0.088	0.124	-0.199	-0.308	0.166	0.297	-0.056	0.104	0.094
	r_g	-0.711	0.498	0.144	-0.307	-0.651	0.450	0.623	-0.182	0.229	0.178
	r_e	-0.264	-0.050	0.126	-0.168	-0.189	0.035	0.161	0.025	0.036	0.048
Pop	r_p		0.152	0.594	0.046	0.073	-0.034	-0.060	0.073	-0.002	0.006
	r_g		-0.264	0.369	-0.150	-0.037	-0.090	-0.001	0.194	0.014	0.049
	r_e		0.248	0.628	0.086	0.100	-0.018	-0.078	0.027	-0.007	-0.010
Ht	r_p			0.496	-0.031	-0.018	-0.020	0.009	-0.129	-0.064	-0.077
	r_g			0.721	-0.620	-0.593	0.074	0.419	-0.486	-0.145	-0.185
	r_e			0.471	0.119	0.152	-0.057	-0.135	0.091	-0.028	-0.027
Mass	r_p				-0.063	-0.088	0.044	0.090	0.049	0.048	0.053
	r_g				-0.664	-0.812	0.298	0.679	-0.010	0.194	0.188
	r_e				0.024	0.032	-0.010	-0.024	0.077	0.015	0.022
DM %	r_p					0.755	0.296	-0.434	0.346	0.346	0.304
	r_g					0.602	0.302	-0.256	0.505	0.400	0.394
	r_e					0.796	0.301	-0.496	0.303	0.342	0.285
Fib %	r_p						-0.402	-0.917	-0.069	-0.324	-0.342
	r_g						-0.579	-0.926	-0.188	-0.464	-0.452
	r_e						-0.336	-0.917	-0.005	-0.268	-0.301
Brix %	r_p							0.730	0.584	0.954	0.923
	r_g							0.844	0.740	0.963	0.943
	r_e							0.676	0.480	0.955	0.920
Brix %dm	r_p								0.305	0.652	0.652
	r_g								0.475	0.754	0.739
	r_e								0.201	0.603	0.614
Pur	r_p									0.798	0.846
	r_g									0.895	0.921
	r_e									0.715	0.777
Pol %	r_p										0.995
	r_g										0.997
	r_e										0.993

where:

Diam = stalk diameter;

Ht = stalk height;

DM% = dry matter % cane;

Brix% = brix % cane;

Pur = purity;

Ers% = ers % cane;

 r_p = phenotypic correlations; r_e = environmental correlations; and

Pop = stalk population;

Mass = cane mass of single lines;

Fib% = fibre % cane;

Brix%dm = brix % dry matter;

Pol% = pol % cane;

 r = correlations; r_g = genetic correlations; r_e = correlation subject to high estimation standard error.

The results presented in Tables 3.4 and 3.5 indicate strong relationships between some of the traits. Genetic correlations were similar for Stage 2-P and -R. Breeders can use these correlations for selection purposes. These results indicate that genetic improvement of stalk diameter would lead to strongly correlated gains in stalk height (0.768-P and 0.498-R) and brix % dry matter (0.457-P and 0.623-R). It can also be observed that stalk diameter was strongly negatively correlated to stalk population (-0.790-P and -0.711-R). This negative correlation indicates that if breeders select too strongly for either of these two traits, it will be at the other trait's expense. Selection for higher stalk population cultivars is not, however, desirable and most sugarcane producing countries, especially in the tropical countries, have thick-stalked commercial cultivars, with very low stalk populations. Stalk diameter was negatively correlated with fibre % cane (-0.628-P and -0.651-R). Therefore, there would be an advantage in selecting for high diameter, low fibre cultivars as they will also have a high brix % dry matter. High brix % dry matter is desirable as sugarcane breeders are ultimately selecting for high sucrose yielding (t ha^{-1}) commercial cultivars.

At SASEX it is believed that ratoonability is positively correlated with stalk population, although no trials have been conducted to prove this correlation. If stalk population is positively correlated with ratoonability and it has been shown in this study that stalk population is strongly negatively correlated with stalk diameter, then breeders should be cautious of selecting for high stalk diameter cultivars. South Africa is one of the few countries that has not selected too strongly for stalk diameter. Sugarcane cultivars with extremely thick stalks have been found not to ratoon well under raingrown conditions and are therefore usually discarded in the early stages of breeding and selection programmes. South African commercial cultivars have higher stalk populations than those developed in other countries, but are well known for their good ratooning ability. Many other countries have been unable to produce good ratooning cultivars and this may be related to their higher stalk diameter and lower stalk population cultivars. Other experiments would need to be conducted to investigate the correlation between stalk population and ratoonability.

Genetic correlations between stalk population and cane mass differed for plant and ratoon crops. Correlation was negative in the plant crop and positive in the ratoon crop for these

traits. This could possibly be explained by the ratoonability of the cane. The lower population cultivars tended not to ratoon well and may have resulted in the mass of these lines being very low, lower than they would have been in the plant crop. This would have led to the positive correlation between stalk population and cane mass in the ratoon crop.

It was concluded from previous research (Table A4.1 and A4.2) and Tables 3.1, 3.2 and 3.3 that cane mass has a very low heritability value. If it was possible to find a trait with a high heritability estimate that was strongly correlated with cane mass, indirect selection for cane mass could be made. From results presented in Tables 3.4 and 3.5, cane mass was strongly positively correlated with brix % dry matter (0.638-P and 0.679-R) and genetic correlations among sucrose-related traits were all high and positive. It would be possible, therefore, to indirectly improve cane mass by selecting for brix % dry matter. Breeders can use this finding to their advantage and select for cane mass and sucrose content at the same time.

Fibre % cane was negatively correlated with the yield-related traits. Dry matter % cane was the only trait found to be highly correlated with fibre% cane in this investigation. From these results it would not be advisable for breeders to select for this trait as high sucrose levels are required in commercial cultivars. These findings agree with those obtained by Brown *et al.* (1969) but not those of Brown (1965), who concluded that selection for fibre % cane would not lead to a reduced sugar content.

Phenotypic correlations were all markedly lower than genotypic correlations, although the trends were very similar. It was only in a few cases that the genetic correlation among traits was negative and the phenotypic correlation was positive (stalk population correlated with stalk height and cane mass, and stalk height with dry matter % cane). There was one case where the genetic correlation between cane mass and brix % cane was positive and the phenotypic correlation between them was negative. When this did occur, phenotypic correlations were close to zero. Phenotypic correlation between stalk population and cane mass was quite high, although the genetic correlation was low and the environmental correlation seemed to have a strong influence on the phenotypes of the sugarcane grown in this trial.

Environmental correlations can cause resemblance between relatives. This makes it necessary to distinguish these correlations from genetic correlations. Resemblance between relatives is common if members of the same family are reared or planted together in the same environment (Falconer, 1989). It is also possible for certain environmental circumstances to increase differences between unrelated individuals. Results presented in Table 3.4 and 3.5 indicate that environmental correlations do occur between some traits. Heritability estimates obtained by regression require an assumption of no environmental correlation between relatives. The estimates obtained in this investigation by mid-parent-offspring regression could, therefore, be biased (Vogel *et al.*, 1980; Casler, 1982). For most traits investigated environmental correlations were low. Environmental correlations were, however, high between stalk population and cane mass, stalk height and cane mass, dm % cane and fibre % cane, dm % cane and brix % dry matter (negative correlation), fibre % cane and brix % dry matter, and among the sucrose-related traits. A positive environmental correlation will occur between two traits when a micro environment that favours one of the traits is also favoured by the other.

One of the main reasons for the high correlations observed among the sucrose-related traits is that these traits are dependently derived from three measurements: sample mass; a saccharimeter reading; and a refractometer reading. As a result these correlations need to be considered with caution.

3.3 Clonal repeatabilities between seasons

Breeders need to determine repeatabilities of traits selected over a number of seasons. Seasonal variation is a common phenomenon in South Africa. This variation is believed to be of greater importance to the phenotypic expression of cultivars than the variation between different environmental sites in the same season (Bond, 1994; personal communication). An aim of this investigation was to measure this seasonal variation and its effect on the expression of traits. The clonal repeatabilities were determined for all individuals in the population, for all 15 crosses, for the 11 traits (Table 3.6). Clonal repeatabilities varied for the different traits. Stalk diameter, fibre % cane and brix % dry matter were the most repeatable traits, and were consistent over the three seasons. Cane mass was the least repeatable trait observed

between Stages 1 and 2, although it was repeatable when compared between plant and ratoon crops of Stage 2. The low repeatability may have been due to the method of recording the cane mass at the different stages. Cane mass at Stage 1 was an estimation using a formula which multiplies stalk population, height and diameter of a single stool (Bond, 1979), whereas cane mass at Stage 2 was the directly measured mass of a 5 m line of cane. These were not truly comparable as they were estimated differently.

Table 3.6 Clonal repeatabilities between Stages 1 and 2, plant and ratoon crops, for a sugarcane population under raingrown conditions, for 11 traits.

Sugarcane trait	Clonal repeatabilities		
	S1 vs S2 (plant)	S1 vs S2 (ratoon)	S2 (plant) vs S2 (ratoon)
Stalk diameter	0.80	0.77	0.84
Stalk population	0.58	0.55	0.76
Stalk height	0.55	0.38	0.72
Cane mass	0.19	0.13	0.82
Dm % cane	0.65	0.69	0.80
Fibre % cane	0.76	0.76	0.89
Brix % cane	0.50	0.45	0.62
Brix % dm	0.70	0.67	0.83
Purity	0.40	0.44	0.67
Pol % cane	0.51	0.46	0.67
Ers % cane	0.52	0.47	0.69

where:

S1 = Stage 1 - Single Stool Stage; and

S2 = Stage 2 - Single Line Stage.

These results may be of benefit to sugarcane breeders when selecting commercial cultivars. Presently, at SASEX, breeders select cultivars based on ers % cane or brix % cane and cane yield (t ha^{-1}). According to the findings in this investigation brix % dry matter is highly positively correlated with cane mass (0.638-P and 0.679-R) and other sucrose-related traits. This trait may, therefore, be a more reliable trait on which to base cultivar selection.

Variation of the traits within and between the crosses is believed to have influenced and biased some of the repeatability estimates obtained. The traits were then compared for the different crosses (Table 3.7) and it was observed that some traits had very different repeatability values for the different crosses. Cross number 9 had very low repeatability estimates for all traits, except for diameter, population and height. The estimates obtained from this particular cross may have influenced the overall repeatability estimates. Ultimately, sugarcane breeders are interested in knowing the repeatability of traits generally, and not necessarily within each cross planted. This information will be used to aid the selection process for potentially successful commercial cultivars.

Table 3.7 Clonal repeatabilities between Stages 1 and 2 (plant crop) for 15 sugarcane crosses under raingrown conditions.

Cross	Diam	Pop	Ht	Mass	Dm%	Fib%	Brix %	Brix % dm	Pur	Pol%	Ers%
1 (32) [†]	0.49	0.73	0.65	0.26	0.71	0.79	0.46	0.74	0.50	0.48	0.50
2 (24)	0.72	0.73	0.85	0.17	0.67	0.61	0.43	0.43	0.34	0.50	0.51
3 (32)	0.52	0.34	0.78	0.24	0.39	0.62	0.41	0.64	0.22	0.41	0.44
4 (25)	0.41	0.41	0.71	0.16	0.59	0.70	0.75	0.77	0.05	0.67	0.63
5 (29)	0.77	0.55	0.37	0.00	0.49	0.81	0.37	0.80	0.00	0.28	0.27
6 (32)	0.69	0.48	0.68	0.28	0.84	0.87	0.44	0.73	0.43	0.53	0.55
7 (30)	0.68	0.11	0.65	0.00	0.80	0.89	0.46	0.83	0.00	0.30	0.28
8 (32)	0.70	0.65	0.58	0.35	0.51	0.61	0.59	0.71	0.02	0.46	0.42
9 (31)	0.69	0.62	0.60	0.18	0.07	0.40	0.00	0.26	0.02	0.00	0.00
10 (32)	0.64	0.47	0.59	0.42	0.76	0.71	0.46	0.53	0.49	0.49	0.49
11 (31)	0.79	0.43	0.38	0.13	0.53	0.64	0.30	0.58	0.05	0.27	0.27
12 (32)	0.88	0.47	0.55	0.22	0.71	0.90	0.62	0.84	0.63	0.65	0.68
13 (32)	0.75	0.56	0.51	0.34	0.83	0.83	0.44	0.69	0.72	0.63	0.68
14 (31)	0.83	0.58	0.59	0.22	0.71	0.73	0.43	0.58	0.17	0.32	0.26
15 (23)	0.87	0.28	0.80	0.25	0.72	0.74	0.55	0.71	0.19	0.48	0.46

where:

[†] = number of offspring per cross, e.g. 1 (32) - cross # 1 with 32 offspring

CHAPTER 4

OVERVIEW OF RESULTS AND FINAL CONCLUSIONS

Some very interesting and useful results were obtained from this investigation. The results compared favourably with those obtained under irrigated conditions and by other sugarcane workers (Table A4.1 to A4.4), although not much work has been done on dry matter % cane, fibre % cane, brix % dry matter, and purity. The similarity between results was encouraging because it was initially anticipated that the environmental effect on sugarcane under raingrown conditions would be large and difficult to account for. Although standard errors obtained in this investigation were slightly higher than those obtained by others there were still large enough differences between the heritability values of the 11 sugarcane traits to be of any benefit to breeders ie. relative values are useful. The sucrose-related traits were highly heritable at Stages 1 and 2, except for purity at Stage 1. This low heritability estimate was explained in detail in Chapter 3. Stalk diameter was highly heritable and has been selected for extensively in many breeding programmes. Cane mass was the least heritable trait recorded in this study. Sugarcane breeders are ultimately selecting for sucrose yield per hectare which stresses the importance of being able to make progress with cane mass. The low heritability value of cane mass has resulted in slow progress with this trait in the past. Breeders have been searching for a trait that is highly heritable and positively correlated to cane mass so that cane mass could be indirectly selected for. Brix % dry matter has not been considered for selection purposes in the past but from the results obtained in this study this trait should possibly be considered in the future. Although its h^2 value is slightly lower than the other sucrose-related traits, it is still highly heritable (Tables 3.1 and 3.3), extremely repeatable (Tables 3.6 and 3.7) and strongly positively correlated with cane mass (Tables 3.4 and 3.5). Brix % dry matter was observed as being highly repeatable between Stages 1 and 2 and between plant and ratoon crops of Stage 2. It must be noted that these results have been obtained from only one sugarcane population grown on a single environmental site. Other trials need to be conducted at a number of different sites to establish the reliability of these results. Once the reliability of these results has been confirmed, then brix % dry matter can be used as an alternative selection trait by breeders to make good progress in their selection for high sucrose yield per

hectare. It must be noted that dry matter % cane, fibre % cane, brix % cane, brix % dry matter, purity, pol % cane and ers % cane are dependently derived from three measurements: sample mass; a saccharimeter reading; and a refractometer reading.

A possible disadvantage for using brix % dry matter for selection purposes is that the present analysis (java ratio) performed, at SASEX, on Stage 2 genotypes from the normal breeding and selection programme does not provide information on this trait or dry matter % cane, and fibre % cane. The main reason for this is the longer time period required to analyse cane samples when the direct test is used (see Glossary for definition). A large number of samples from a Stage 2 trial sent through the millroom for this selection stage would require many days to complete the analysis. This problem could, however, be overcome by using Near Infrared (NIR) spectroscopy, although the initial expense of the equipment (NIR spectrophotometer) would be high. The progress that could be made from selecting for brix % dry matter should outweigh the cost of the equipment.

South Africa is one of the few countries that does not select for stalk diameter. The reason given for this has been the strong negative correlation between stalk diameter and stalk population. Stalk population is believed to be positively correlated with good ratoonability, which indicates that selection for high stalk diameter may indirectly result in poor ratoonability of sugarcane cultivars. South African sugarcane cultivars are well known for their good ratooning ability. The author has, however, been unable to find any published evidence to suggest that a positive correlation does exist between ratoonability and stalk population. It is suggested that an investigation be conducted determining the importance of this relationship. In this study stalk diameter was positively correlated to brix % dry matter and, as both of these traits are highly heritable, it should be possible to indirectly select for brix % dry matter by selecting for stalk diameter. This indirect selection could lead to higher sucrose yield per hectare, which further stresses the importance of the research on the relationship between stalk population and ratoonability.

Evidence was provided to suggest that data collected in Stage 1 was not as reliable as that collected in Stage 2. The standard errors in Stage 1 were more than 30% of the heritability

estimates obtained for many of the sugarcane traits. Reasons for these high standard errors included the difficulty in determining the field variation in Stage 1 and the poor representation of a genotype by a single stool of sugarcane. In Stage 2 the field plan was such that field variation could be measured more accurately and each genotype was represented by a single 5 m row of sugarcane stools. Stage 2 would, therefore, provide more reliable information on the genotypes which could be used more confidently by breeders. In the stages following Stage 2 the number of genotypes remaining in each family is too low to provide adequate information on the parental combinations. The results suggest that the plant crop of Stage 2 provides sufficient information for parental selection because the ratoon crop had very similar heritability estimates to those obtained in the plant crop. There were only three traits that differed significantly between plant and ratoon crops: stalk population; cane mass; and dry matter % cane. These traits were influenced by non-additive genetic variation. Non-additive genetic variation was observed when REML was used to analyse an unbalanced North Carolina mating design II at Stage 2. Information on non-additive genetic variance could not be obtained by mid-parent offspring regression.

Estimating h_a^2 by mid-parent-offspring regression is acceptable for breeding purposes; however, there are many disadvantages for using this particular method of analysis. Data from both parent cultivars and their offspring needs to be collected when using mid-parent-offspring regression. Although this added information could be of benefit to breeders, parent cultivars are not always available and they take up field space in a trial that could be planted to other potential commercial cultivars. Environmental correlation between parents and offspring can cause resemblance between relatives which would cause a bias in the heritability estimates obtained. In this study environmental correlation was observed between some traits which may have caused a bias in the estimates obtained by regression. Breeders should plant parent cultivars and their offspring in separate environments when using regression to overcome this environmental correlation effect (Casler, 1982). It would also be possible to plant clones of a population of parents and their offspring in a different set of environments to determine this environmental correlation. Breeders are often restricted by the limited field area available to them when conducting research and usually prefer to use a higher number of crosses or more offspring per cross in their trials rather than planting parent cultivars together with their

offspring. The variation within crosses is also not considered when using regression, and this information can be of importance to breeders. For these reasons it would be more beneficial to use other methods of analysis to determine heritabilities.

In this investigation REML analysis of an unbalanced North Carolina mating design II at Stage 2 was used as an alternative method of analysis to determine heritabilities. The use of REML was effective for analysis of trial data in Stage 2. Information is often required from a sugarcane population that has already been planted in the field. This can result in the data being unbalanced which is why REML would be effective for analysis in sugarcane. Although the results obtained by regression were very similar to those obtained by REML, REML was able to estimate genotypic, additive and non-additive genetic variance components, thereby enabling the determination of both h_a^2 and h_b^2 . Unbalanced mating designs can also be analysed when using REML. For sugarcane this may be a great advantage because there are many sugarcane cultivars that are either male sterile or incompatible with other cultivars. The cross-classification used in this investigation was unbalanced and REML was used to allocate degrees of freedom to all the treatments (factors) and the interactions between them used in the statistical model. Many factors were considered as having a possible influence on the phenotypes observed at Stage 2. The interaction between male and female parents was significant for cane mass in both plant and ratoon crops, and stalk population and dry matter % cane in the ratoon crop. The large interaction (refer to the mean squares in Tables A7.4 and A7.5) resulted in a marked difference between additive genetic variance and genotypic variance components (Table 3.3). Non-additive genetic variance was, therefore, considered important in those traits. From these results it could be concluded that stalk population, cane mass, and dry matter % cane are not reliable traits for use in parental selection. The genetic effect due to replications was large for stalk height in the plant crop (Table 3.2). This indicated that there were differences between replications for this trait, possibly caused by competition between neighbouring single lines.

The standard errors of the heritabilities obtained by REML were high. This was because of the extremely unbalanced nature of the cross-classification, the lower degrees of freedom allocated to the interaction terms by REML, and the positive constraint set on the

determination of variance components by REML. Only 12 crosses were used in this investigation and it can be concluded from the large standard errors obtained in this investigation that a larger base population (with more crosses) would reduce the standard errors markedly. It must be stressed that more carefully planned cross-classifications are required to obtain more reliable information on sugarcane populations. Trials to determine the genotype \times environment interaction ($G\times E$) should also be conducted in raingrown and irrigated areas to investigate the effect different environments have on genotypes. It is also important to know how this $G\times E$ would influence heritability estimates of different sugarcane populations.

GLOSSARY

additive genetic variance (V_A): genetic variance associated with the average effects of substituting one allele for another; variance of the breeding values, where the breeding value of an individual is judged by the mean value of its progeny.

aneuploid: having fewer or more chromosomes than an exact multiple of the haploid number.

brix % cane: sum of all the soluble matter in a sugar solution expressed as a percentage by mass, measured by a refractometer. A ray of light passed through a solution will be refracted or bent at the surface by an amount related to the dissolved solids in the solution.

broad sense heritability: see degree of genetic determination

cane yield: mass of all the sugarcane stalks in each of the sugarcane stools determined per unit area.

clone: a group of genetically identical cells or individuals derived by asexual division from a common ancestor.

correlation coefficient: a statistical measure of the extent to which variations in one variable are related to variations in another.

covariance: a statistical measure used in computing the correlation coefficient between two variables; the mean of the product of the deviations of two variables from their individual means.

degree of genetic determination (DGD): also known as broad sense heritability (h_b^2), is a heritability estimate expressed as the ratio V_G/V_P . This ratio expresses the extent to which an individual's phenotype is determined by its genotype.

diallel crossing design: design was developed for parents that range from inbred lines to broad genetic base cultivars; crosses are made in pairs for n number of parents; the same parents are used as males and females where each parent cultivar is crossed with every other parent cultivar available.

diploid: a cell having two chromosomes sets, or an individual having two chromosomes sets in each of its cells.

direct test: a millroom analysis where the sugarcane is weighed, disintegrated, and blended with the diluting water, sucrose, moisture and fibre contents are determined directly.

dominance variance (V_D): genetic variance at a single locus attributable to dominance of one allele over another.

dry matter % cane: all the solids, including fibre, sucrose and water soluble impurities, found in a sugarcane sample, expressed as a percentage of fresh mass of cane.

epistasis: a situation in which an allele of one gene obliterates the phenotypic expression of all allelic alternatives of another gene.

ers % cane: mass of estimated recoverable sucrose as a percentage of mass of fresh sugarcane.

family selection: a breeding technique of selecting parents on the basis of the average performance of their progeny.

fibre % cane: all the water insoluble matter in a sugarcane sample.

gene: the fundamental physical unit of heredity, recognised through its variant alleles, which transmits a specification(s) from one generation to the next; a segment of DNA coding for one function or several related functions.

heritability in the narrow sense: (h_n^2) determines the degree of resemblance between relatives and is the ratio V_A/V_P that expresses the extent to which an individual's phenotype is determined by the genes transmitted from its parents.

Java ratio: a millroom analysis where sugarcane is weighed, crushed in a small mill, the expressed juice analysed, and the sucrose % cane is estimated.

linkage: the tendency of certain genes to remain associated through several generations because they are situated on the same chromosome.

mid-parent value: the mean of the values of a quantitative phenotype for two specific parents.

pleiotropism: one gene simultaneously affects several physiological or biochemical pathways.

pol % cane: apparent sucrose in a sugarcane sample, given as a percentage by mass, measured by a saccharimeter or polarimeter. Polarised light is passed through a sugarcane solution. Sucrose molecules rotate the plane of polarisation by an amount that is proportional to the concentration of the sucrose.

polyploid: a cell having three or more chromosome sets, or an organism composed of such cells.

purity: the pol to brix percentage ratio, termed the refractive apparent purity.

quantitative inheritance: continuous variation; the existence of a range of phenotypes for a specific character, differing by degree rather than by distinctive qualitative differences.

ratooning: stubble regrowth following harvest of plant cane cut at or below ground level, so that part of the stalk is left underground and it is this that produces the succeeding growth of cane known as ratooning. The old root system supports the regenerating crop until new roots can take over.

sett: a vegetative propagule consisting of one or more stem nodes with buds and root primordia.

stalk height: length of a sugarcane stalk - the distance between the base of the stalk (at ground level) and the meristematic tissue at the growing point.

stalk population: number of millable stalks per stool / or per unit area.

standard deviations: deviations where variates have been standardised to have a zero mean and unit variance.

sucrose: a pure disaccharide $C_{12}H_{22}O_{11}$ also known as saccharose or cane sugar.

stool: a single sugarcane plant that is made up of many stalks/tillers.

variance: a measure of the dispersion of variability of a trait about its mean in a population.

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APPENDIX 1

THE SOUTH AFRICAN SUGAR ASSOCIATION EXPERIMENT STATION PLANT BREEDING PROGRAMME

Plant breeding programmes are divided into three main divisions: selection of parent cultivars; flowering and seed production; and field selection, cultivar evaluation and release of commercial cultivars.

A1.1 Selection of parent cultivars

Prospective parent cultivars are planted into fields at the Central Field Station, where flowering can be profuse, and into sand bins in the glasshouse and photoperiod house facilities at SASEX, Mount Edgecombe. Flowering is artificially induced in these latter facilities. Parents are selected from different environments for many desirable characters. These parents include commercial, imported, and promising cultivars that are still going through the selection programme, cultivars that are not commercially acceptable but have one or more important traits that are required in the offspring, and parents to be used for breeding purposes, including *Saccharum spontaneum*, *S. robustum*, *Erianthus* sect. *Ripidium* Henrard.

Use is made of general combining ability (additive genetic variance) where information from the offspring is used to aid in the selection of parent cultivars. Proven crosses are still occasionally made. The parents of these crosses are proven and imported cultivars.

A1.2 Flowering and seed production

Sugarcane cultivars seldom set seed under natural field conditions. The main problem with producing seed in the field is that the pollen produced is infertile. Floral development is influenced by climate, day length, and water stress. Thus flowering is seasonal and can be profuse one year and almost absent in another. Temperatures must be kept above 20°C to

ensure pollen viability, fertilization, and seed development. The relative humidities must also be maintained at high levels (Brett, 1950; Nuss, 1979; 1980). Thus a glasshouse was constructed at SASEX (Brett and Harding, 1974; Nuss and Brett, 1977). While the natural day length decline in the field is about two minutes per day, a 30 s decline is required to induce flowering. The photoperiod house controls this decline in day length, thereby artificially inducing flowering. Each parent cultivar is allocated to a particular photoperiod house and glasshouse treatment. The treatment determines whether the parent will be male or female, and the approximate time of flowering. Flowering can, therefore, be synchronised between the different parent cultivars (Nuss, 1980).

Setts of selected parent cultivars are planted into bins in the glasshouse and photoperiod house at the beginning of September. Once germination has occurred, bins are moved outside, where they remain until February. Nitrogen is applied until December. Nitrogen application is terminated since high nitrogen levels reduce pollen fertility. Photoperiod and glasshouse treatments start in February and bins are moved inside at night. Day lengths are controlled with an artificial dawn and a natural sunset. Artificial dawn is controlled by switching on lights in the early morning. The first flower initials are observed in April. Flowering occurs between May and August each year. When flower initials are seen the stalks are marcotted (air layered). Marcotting is the cutting of the flowering stalk at the base and enclosing the lower part of the stalk in a metal sleeve which contains a vermiculite and compost soil mix. This is used as a rooting medium. The severed base is placed in a water solution and SO_2 gas is bubbled through to sustain the stalk until rooting occurs. Once there is rooting, flowers can be moved around the glasshouse and crosses set up in separate compartments.

When anthers are observed, a pollen sample is taken and tested for viability. This is done three times a week. Potassium iodide is used to stain pollen grains. If more than 30% of the pollen grains are stained then those flowers are used as male parents. If there is less than 30% staining or no pollen is seen at all, then flowers are used as female parents. When the staining test is complete, parents to be used for that particular day of crossing are entered into a computer database. Information on the characteristics of each cultivar has already been entered into the database at the beginning of the crossing season. Mid-parent values are

calculated by the computer and parental combinations are rated for traits of importance. Parental combinations are then set up in separate compartments (to prevent contamination) in the glasshouse. Male tassels are placed above female tassels. Every morning male tassels are shaken to release their pollen onto the female tassels. This continues for two weeks to allow fertilization to occur. Males are then discarded and females are moved to a ripening area in the glasshouse. Once seed set has taken place the fuzz (seed) is collected and stored in a deep freeze until January the following year when the seed is sown.

A1.3 Field selection, cultivar evaluation and release of commercial cultivars

Seed is removed from the deep freeze and sown in the glasshouse. Fuzz is spread out on the surface of a sterilised mixture of compost, soil and river sand. Two flats are used per tassel. This is covered lightly with peat moss. Seedlings are given frequent light watering. Once germination has taken place the seedlings are gradually moved outside the glasshouse to harden them off. Crosses are allocated to selection sites and planted into blocks on the terraces. Seedlings are kept trimmed until they are planted into the field.

Annually there are about 135 000 seedlings planted to selection sites at SASEX. For selection purposes the industry is divided into regions (Figure A1.1). Irrigated regions in the north have a particular disease spectrum (smut - *Ustilago scitaminea*, pokkah boeng - *Giberella fujikuroi*, gumming - *Xanthomonas campestris* var *vasculorum*, and leaf scald - *Xanthomonas albilineans*). A selection farm for these conditions is located at Pongola and Stage 5 cultivars are tested at three other sites: Malelane, Mhlume, and Ubombo. The Pongola farm is divided into early and late planting cycles.

There are five selection farms for the southern raingrown areas and these have a different disease (rust - *Puccinia melanocephala*, red rot - *Glomerella tucumanensis*, sugarcane mosaic virus - SCMV) and pest (sugarcane borer - *Eldana saccharina*) spectrum. Mtunzini, Shakaskraal, Mount Edgecombe, and Central Field Station (in Umhlanga Rocks) are located along the coast and Bruyns Hill is in the Natal midlands, near Wartburg. Stage 5 cultivars are tested at four additional sites: La Mercy and Umhlatuzi Flats along the coast, and Eston and

Seven Oaks in the midlands. The southern selection programme is further divided into long (1½ to 2 years) and short cutting cycles (1 year).

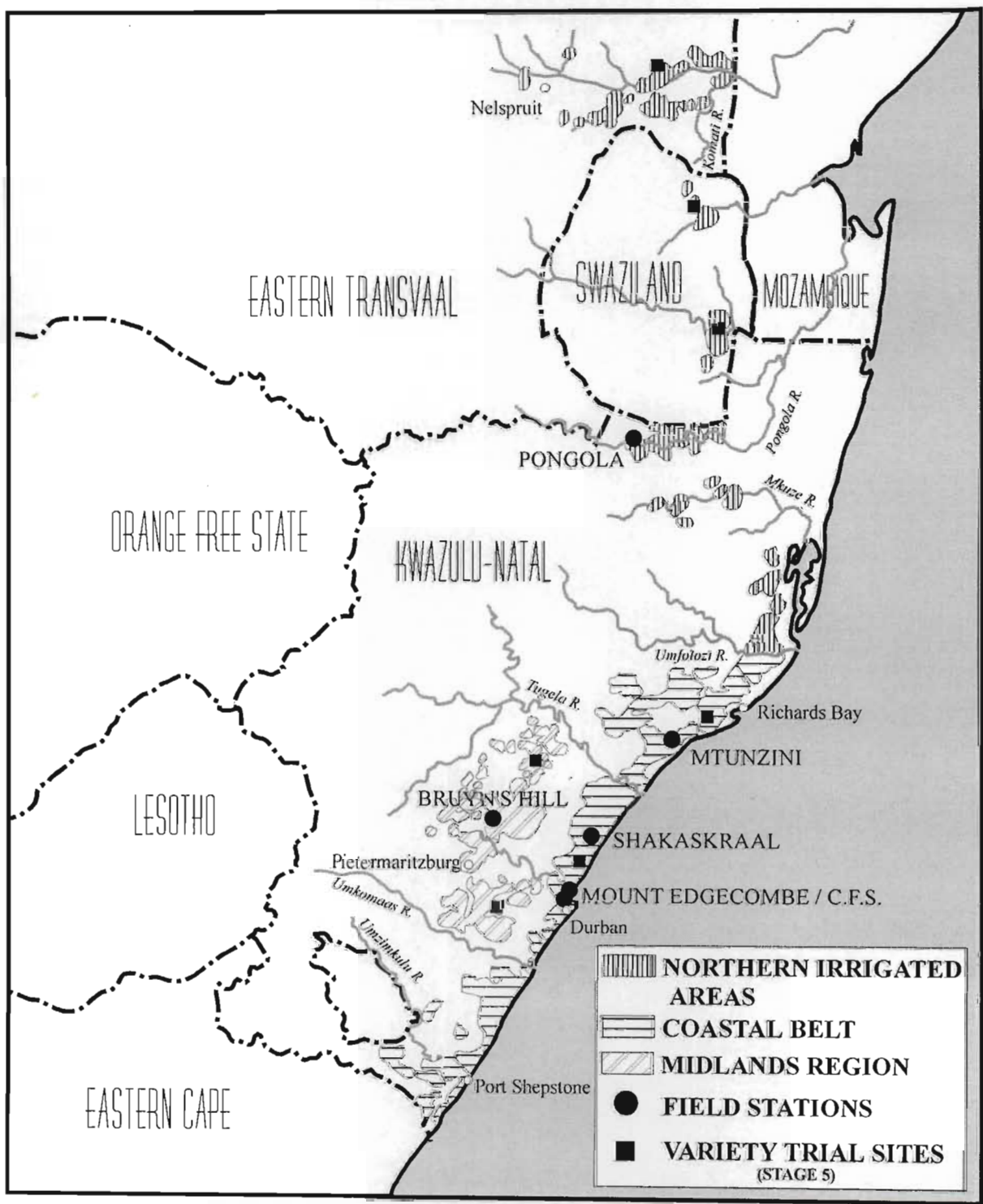


Figure A1.1 Sugarcane growing regions and selection sites of the South African Sugarcane Industry.

At each selection site there is a five stage selection programme. The first four stages are planted at the same location as that of Stage 1 and the Stage 5 trials are planted at all the other selection sites. Table A1.1 shows the different stages that a cultivar must go through prior to release. Many traits are selected for in this programme, the most important ones being sucrose, cane yield (t ha^{-1}), sucrose yield (t ha^{-1}), disease and pest resistance, ratooning ability, and erectness.

Table A1.1 The SASEX selection programme involving many selection stages, a number of different genotypes and years before a commercial cultivar is released to the Sugar Industry.

Selection stages	Number of offspring	Years	
		Raingrown sites	Irrigated sites
Terrace seedlings	180 000	0	0
Single stools (Stage 1)	135 000	1	1
Single lines (Stage 2)	12 000	3	2
Observation plots (Stage 3)	1 200	6	4
Primary cultivar trials (Stage 4)	180	8	6
Secondary cultivar trials (Stage 5)	60	10	8
Propagation plots	12	13	10
Bulking up stage	1	14	11
Release		15	12

When promising cultivars have been selected from Stage 5 they are planted in the propagation plots, which are much larger than the other selection stage plots. This is an evaluation stage where the cultivar is planted as a large stand of cane to test for diseases and pests and to increase the amount of seedcane. The bulking up stage further increases available seedcane and is planted on cooperative farms. This material is grown under permit. The following year, if a cultivar is suitable for release, the Department of Agriculture and Water Supply is requested to gazette its release and the cultivar then becomes available to the grower to plant commercially.

APPENDIX 2

SUGARCANE AGRONOMY

A2.1 Growing sugarcane

Sugarcane is vegetatively propagated by cutting the stalks into setts and planting them into a furrow. Cane setts must be covered with about 5 cm of soil. Soil samples are taken before planting and sent to the fertilizer advisory service (FAS) at the Experiment Station to be analysed. Fertilizer requirements are determined and the grower is advised. Fertilizer is applied at the time of planting. Weed control is essential for successful production of sugarcane. The average age of cane at harvest is between 12 and 24 months, depending on the location. Once harvested, cane is allowed to ratoon. Sugarcane can be ratooned for about nine crops before the quality of the juice begins to deteriorate. A top-dressing of fertilizer is necessary before the crop is ratooned. Leaf samples are collected from the ratoon crop and sent to FAS. Harvesting of cane takes place between April and December every year. Fields should remain fallow for at least six months before a new crop is planted. Cane setts must then be planted in the interrows. This promotes good soil conservation.

A2.2 Harvesting and milling sugarcane

Carbohydrates are produced by the sugarcane plant through photosynthesis. These carbohydrates are used by the plant for growth and are stored in the stalk to provide energy for the plant. Sugarcane grows vigorously through summer as the plant uses up the carbohydrates and then when growth slows down in winter the carbohydrates are stored in the stalks as sucrose.

After harvest, sugarcane is transported to the nearest sugar mill where the stalks are weighed and sent through the sugar milling process, producing raw sugar. An average commercial crop yields 65 t of cane of which about 9 t of cane produces 1 t of sugar. Sugarcane composition

is dependent on: cultivar; soil type; climatic conditions; pest and disease damage to the cane, time delays between harvest and cane crushing; and harvest method. Different components of sugarcane include:

Sucrose	:	12 - 14%
Fibre	:	12 - 16%
Water	:	70 - 75%

Raw sugar produced from the mills is then sent for refining or distribution. The remaining liquid is molasses which is used to make animal feeds or alcohol. Raw brown sugar can be refined into white sugar, exported, or packed raw for the domestic market. The many by-products from the milling process are used to make alcohol, bakers yeast, stock feed, fertilizer, chemicals (furfural) and pharmaceutical products. A by-product called bagasse is used to fuel the mill's boilers and can also be used to make paper.

APPENDIX 3

SITE DESCRIPTION

A3.1 Geographical location

Sugarcane is grown in South Africa along a belt that stretches from the Umtamvuna River on the KwaZulu-Natal / Eastern Cape border to Malelane in the Eastern Transvaal lowveld. In KwaZulu-Natal cane is grown along the coast and inland at Noodsberg, Mid-Illovo and Melmouth (Figure A3.1).

The trial was planted at the South African Sugar Association Experiment Station (29° 42' S, 31° 02' E) in Mount Edgecombe, Natal, Republic of South Africa. The trial site was 96 m above mean sea level. Climatological data was obtained from the Mount Edgecombe Meteorological site at the same location.

A3.2 Temperature

The long term mean (LTM) for the Mount Edgecombe Meteorological Site has been calculated over a 30 y period. The mean maximum temperatures are 27.3°C and 27.4°C for January and February, respectively. The mean minimum temperatures are 11.0°C and 11.2°C for July and June, respectively. The LTM annual variation in temperatures is presented in Figure A3.1.

Temperatures recorded at Mount Edgecombe varied during the investigation (Figure A3.2). The hottest months were January and February 1995 when the mean maximum temperatures were 32.6°C and 32.7°C respectively. The coldest months were June and July 1995 with mean minimum temperatures of 8.1°C and 9.6°C, respectively.

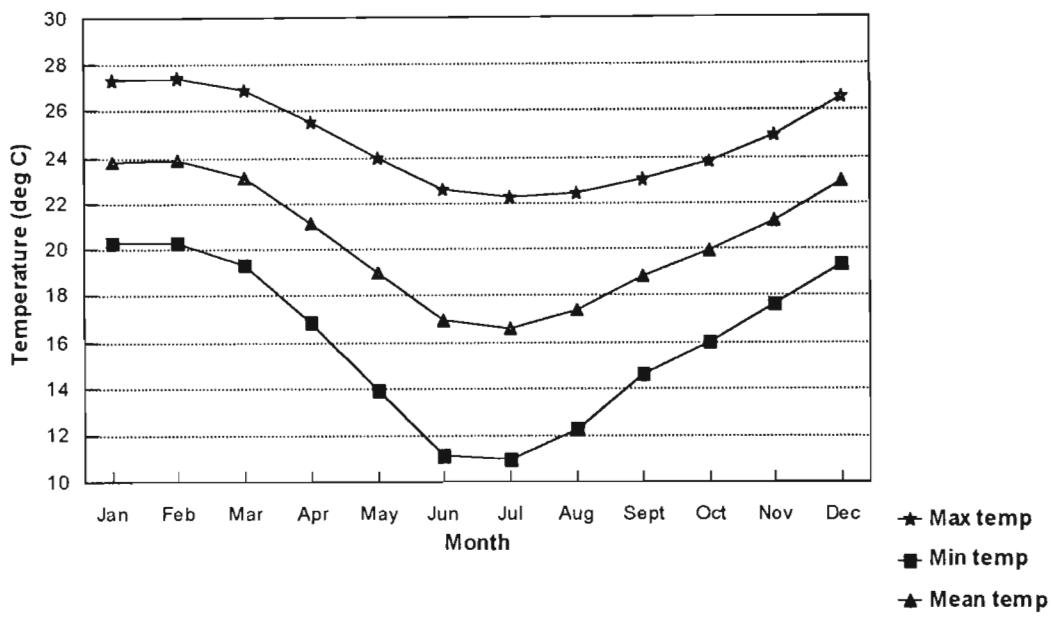


Figure A3.1 The long term (30 y) mean annual variation in temperatures obtained from the Mount Edgecombe Meteorological Site.

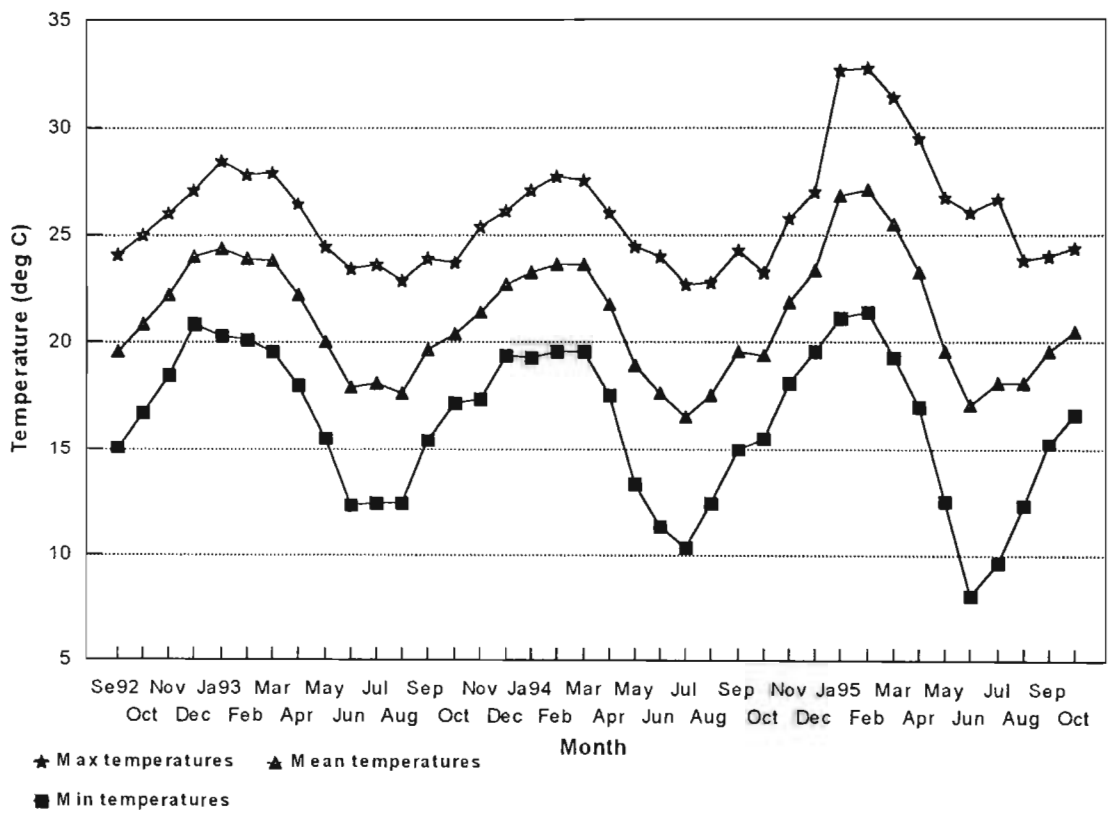


Figure A3.2 Minimum, maximum, and mean temperatures from September 1992 to October 1995 recorded at the Mount Edgecombe Meteorological Site.

A3.3 Rainfall

The mean annual rainfall for the period 1966 - 1995 on the Mount Edgecombe Experimental Farm was 994.6 mm. The years during which this trial was planted were extremely dry and variable, ranging from 487.6 mm (1992), to 920.8 mm (1993), to 762.9 mm (1994), to 1110.9 mm (1995). The wettest month recorded during this trial was December 1993 (197.7 mm) and the driest months were June and July 1995 (0 mm). Monthly rainfall recorded at this site, during the years of the trial, has been compared with the LTM (Figure A3.3).

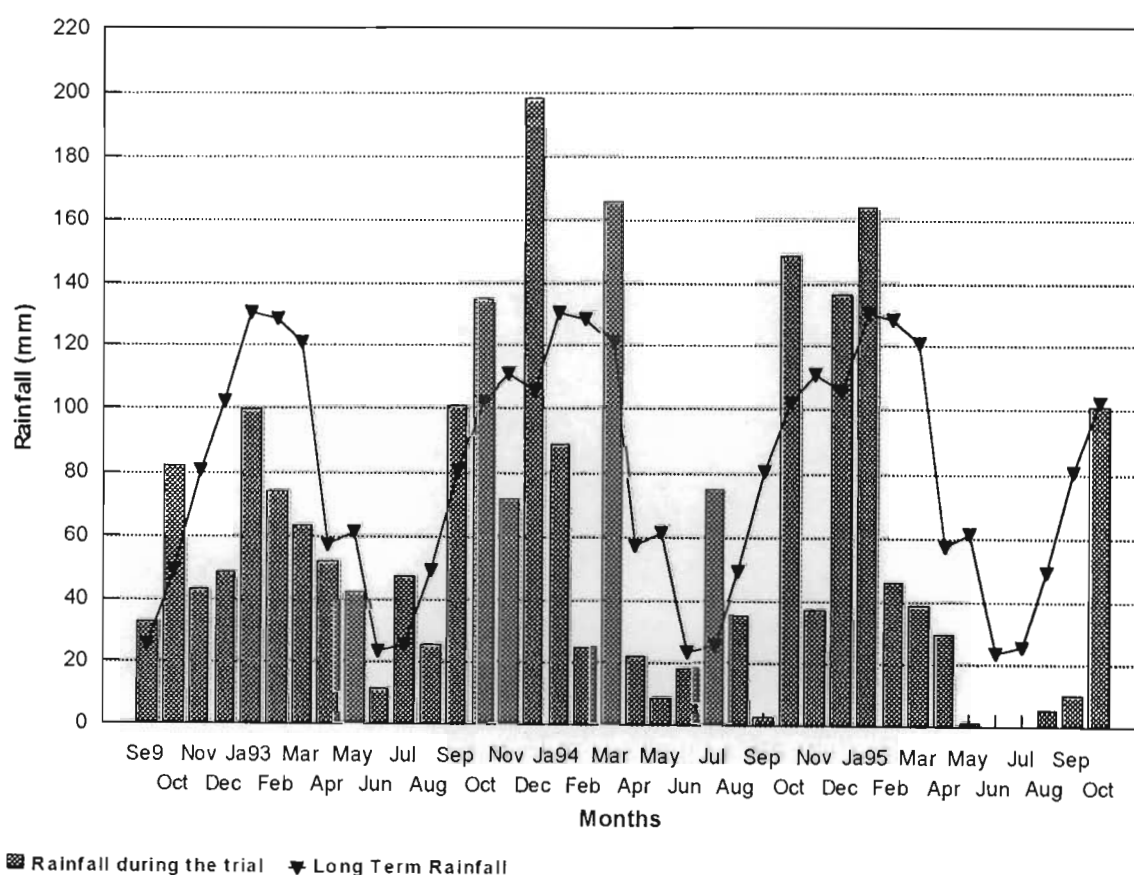


Figure A3.3 Long term rainfall variation, from September 1992 to October 1995 compared with that recorded during the trial at the Mount Edgecombe trial site.

A3.4 Relative humidity

The Relative Humidity (R.H.) recorded at Mount Edgecombe during this trial did not differ much from the LTMs (R.H. meaned for 30 y). The long term R.H. averaged 77% at 08h00 and 66% at 14h00. The only major difference between the trial period and the LTM was the R.H. recorded in June and July in 1995 where it dropped to 34% at 14h00, in comparison to the 53% and 54% of the LTMs for the same months. The highest monthly R.H. recorded during the trial period was 85% at 08h00 in February 1995 (Figure A3.4).

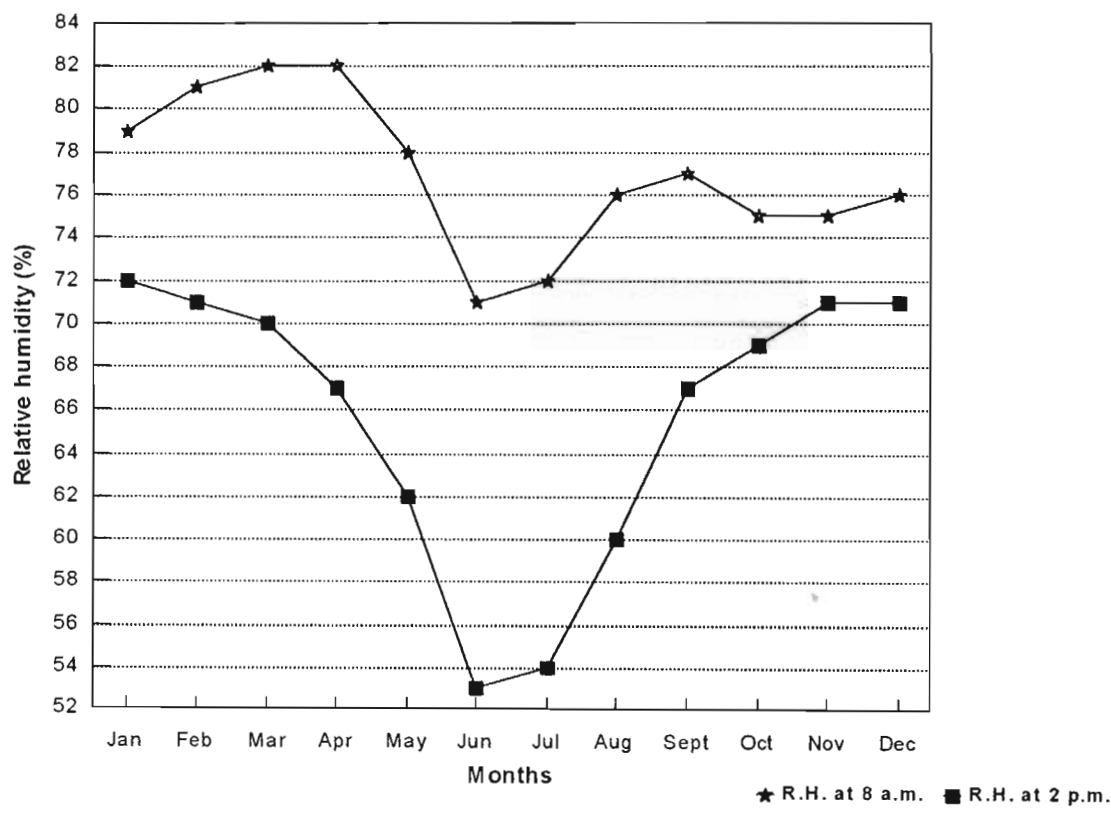


Figure A3.4 Long term relative humidities recorded at the Mount Edgecombe trial site.

A3.5 Soil conditions

Stage 1 and 2 trials were planted at SASSEX, Mount Edgecombe. These trials were planted in the Umzinto coastal low soil system. The soil series was Longlands - Kroonstad for both stages. Effective rooting depths were 600 mm for Stage 1 and 750 mm for Stage 2. This is a sandy clay loam soil type which is highly erodible and requires minimum tillage. It was advised that at harvest the trash and tops (i.e. all the remaining leaf material that is removed from the cane stalk before the cane is sent to the mill) must be left on the soil surface to protect against erosion. These soils compact very easily when wet and become capped when dry. Planting and harvesting took place in spring or early summer to ensure that the soil was well protected by the canopy in the rainy season. This soil type is inherently low in nitrogen, phosphorus and potassium. Soil samples were taken and sent to the FAS. Fertilizer was applied to the different fields at planting, according to rates recommended by FAS. Leaf samples were taken before the Stage 2 trial was ratooned to determine the topdressing rates.

APPENDIX 4

HERITABILITIES, CORRELATIONS, AND CLONAL REPEATABILITIES DETERMINED BY OTHER WORKERS

Many researchers of sugarcane have estimated broad (Table A4.1) and narrow (Table A4.2) sense heritabilities, genetic correlations between traits (Table A4.3) and clonal repeatabilities between plant and ratoon crops (Table A4.4).

Table A4.1 Broad sense heritabilities of sugarcane populations under raingrown and irrigated conditions.

Sugarcane Characteristic	Broad Sense Heritabilities (DGD)	Authors
Stalk population	0.58 [*]	George (1962) - Mauritius
	0.25 ± 0.14	Brown, Daniels and Latter (1968) - Fiji
	0.15	Cesnik and Vencovsky (1974) - Brazil
	0.74 ± 0.05	Hogarth, Wu and Heinz (1981) - Australia
	0.53 ± 0.09	Chavanne and Mariotti (1989) - Argentina
	0.67	Kang, Sosa, Jr and Miller (1990) - Florida, USA
	0.46 ± 0.06 (P)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
	0.50 ± 0.07 (R1)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
Stalk diameter	0.58 ± 0.06 (R2)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
	0.44 [*]	George (1962) - Mauritius
	0.57	Cesnik and Vencovsky (1974) - Brazil
	0.87 ± 0.02	Hogarth, Wu and Heinz (1981) - Australia
	0.64	Nageswara Rao, Rahman and Panduranga Rao (1983) - India
	0.51 ± 0.08	Chavanne and Mariotti (1989) - Argentina
	0.74 ± 0.05 (P)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
	0.78 ± 0.04 (R1)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
Stalk height	0.77 ± 0.04 (R2)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
	0.48 [*]	George (1962) - Mauritius
	0.56	Cesnik and Vencovsky (1974) - Brazil
	0.63 ± 0.07	Hogarth, Wu and Heinz (1981) - Australia
	0.57	Nageswara Rao, Rahman and Panduranga Rao (1983) - India
	0.41 ± 0.07	Chavanne and Mariotti (1989) - Argentina
	0.67 ± 0.05 (P)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
	0.58 ± 0.06 (R1)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
Cane mass (t/ha)	0.74 ± 0.05 (R2)	Milligan, Gravois, Bischoff and Martin (1990a)-Louisiana, U.S.A.
	0.59	Hogarth (1968a)
	0.81 ± 0.07	Hogarth (1971a)
	0.25 [†]	Bond (1975) - South Africa
	0.40 [†]	Bond (1975) - South Africa
	0.12 ± 0.05	Hogarth (1977) - Australia
	0.35 ± 0.13	Espinosa and Galvez (1980) - Cuba
	0.35 ± 0.06	Chavanne and Mariotti (1989) - Argentina
	0.49	Kang, Sosa, Jr and Miller (1990) - Florida, U.S.A.

Sugarcane Characteristic	Broad Sense Heritabilities (DGD)	Authors
Cane mass (t/ha)	0.59 ± 0.06 (P)	Milligan, Gravois, Bischoff and Martin (1990a)
	0.71 ± 0.05 (R1)	Milligan, Gravois, Bischoff and Martin (1990a)
	0.64 ± 0.06 (R2)	Milligan, Gravois, Bischoff and Martin (1990a)
	0.10 ± 0.02 (1979)	Blose (1992) - South Africa
	0.26 ± 0.04 (1983)	Blose (1992)
	0.14 ± 0.03 (1985)	Blose (1992)
	0.27 ± 0.04 (1987)	Blose (1992)
	0.43	Bond and Van der Merwe (1992) - South Africa
Stool mass (kg)	0.52	Nageswara Rao, Rahman and Panduranga Rao (1983)
Fibre % cane	0.75 ^{**}	Brown (1965)
	0.57 ± 0.18	Hogarth and Cross (1987)
	0.86	Kang, Sosa, Jr and Miller (1990)
Brix value	0.32 [*]	George (1962)
	0.52 ^{**}	Brown (1965)
	0.88 ± 0.04	Hogarth (1971a)
	0.52	Cesnik and Vencovsky (1974)
	0.60 [†]	Bond (1975)
	0.17 [‡]	Bond (1975)
	0.53 ± 0.17	Hogarth (1977)
	0.75 ± 0.05	Hogarth, Wu and Heinz (1981)
	0.84 ± 0.17	Hogarth and Kingston (1983)
	0.75	Nageswara Rao, Rahman and Panduranga Rao (1983)
	0.63 ± 0.06 (P)	Milligan, Gravois, Bischoff and Martin (1990a)
	0.74 ± 0.05 (R1)	Milligan, Gravois, Bischoff and Martin (1990a)
	0.68 ± 0.05 (R2)	Milligan, Gravois, Bischoff and Martin (1990a)
	0.36 ± 0.05 (1979)	Blose (1992)
	0.75 ± 0.07 (1983)	Blose (1992)
	0.45 ± 0.06 (1985)	Blose (1992)
	0.52 ± 0.06 (1987)	Blose (1992)
	0.38	Bond and Van der Merwe (1992)
Sucrose % dry wt.	0.51 ± 0.16	Brown, Daniels and Latter (1968)
Ers % cane	0.91	Hogarth (1968a)
	0.51 ± 0.06 (1979)	Blose (1992)
	0.68 ± 0.07 (1983)	Blose (1992)
	0.59 ± 0.07 (1985)	Blose (1992)
	0.50 ± 0.06 (1987)	Blose (1992)
Pol % cane	0.54	Cesnik and Vencovsky (1974)
	0.57 ± 0.09	Chavanne and Mariotti (1989) - Argentina
	0.73 ± 0.12	Espinosa and Galvez (1980)

where:

^{*} = mean of four environments in Mauritius;

^{**} = mean of three trials in Fiji, wetland;

[†] = mean of two irrigated sites in South Africa;

[‡] = mean of two raingrown sites in South Africa;

P = plant crop result;

R1 = first ratoon result;

R2 = second ratoon result;

(1979)-(1987) = results from single line trials, under irrigated conditions, over four different years using intra-class correlations among half-sibs.

Table A4.2. Narrow sense heritability estimates of sugarcane populations under raingrown and irrigated conditions.

Characteristic	Narrow Sense Heritabilities	Authors
Stalk population	0.85 ± 0.04 (I)	Hogarth (1971a)
	0.82 ± 0.09 (II)	Hogarth (1971a)
	0.70 ± 0.10	Hogarth, Wu and Heinz (1981)
	0.32 ± 0.09	Gravois, Milligan and Martin (1991)-USA
	0.38 ± 0.08 (OHS)	Schnell and Nagai (1992) - Hawaii
	0.57 ± 0.08 (EHS)	Schnell and Nagai (1992)
	0.50 ± 0.12 (EHS)-op	Schnell and Nagai (1992)
Stalk diameter	0.66 ± 0.07	Hogarth, Wu and Heinz (1981)
	0.78 ± 0.27 ^s	Gravois and Milligan (1992)
	0.80 ± 0.05 (OHS)	Schnell and Nagai (1992)
	0.66 ± 0.08 (EHS)	Schnell and Nagai (1992)
	0.67 ± 0.17 (EHS)-op	Schnell and Nagai (1992)
Stalk height	0.79 ± 0.10 (II)	Hogarth (1971a)
	0.40 ± 0.13	Hogarth, Wu and Heinz (1981)
	0.09 ± 0.08	Gravois, Milligan and Martin (1991)
	0.68 ± 0.26 ^s	Gravois and Milligan (1992)
	0.56 ± 0.08 (OHS)	Schnell and Nagai (1992)
	0.34 ± 0.08 (EHS)	Schnell and Nagai (1992)
	0.25 ± 0.13 (EHS)-op	Schnell and Nagai (1992)
Stalk mass	0.89 ± 0.03 (I)	Hogarth (1971a)
	0.90 ± 0.05 (II)	Hogarth (1971a)
	0.29 ± 0.11	Hogarth (1977)-Australia
	0.22 ± 0.04 (1979)	Blose (1992)-South Africa
	0.41 ± 0.07 (1983)	Blose (1992)
	0.51 ± 0.08 (1985)	Blose (1992)
	0.19 ± 0.04 (1987)	Blose (1992)
	0.81 ± 0.27 ^s	Gravois and Milligan (1992)
Cane yield	0.77 ± 0.06 (I)	Hogarth (1971a)
	0.73 ± 0.13 (II)	Hogarth (1971a)
	0.06 ± 0.04	Hogarth (1977)
	0.18 ± 0.08	Gravois, Milligan and Martin (1991)
	0.06 ± 0.02 (1979)	Blose (1992)
	0.00 ± 0.00 (1983)	Blose (1992)
	0.10 ± 0.03 (1985)	Blose (1992)
	0.20 ± 0.04 (1987)	Blose (1992)
	0.17 ± 0.22	Schnell and Wu (1992)-Hawaii
Brix % cane	0.84 ± 0.04 (I)	Hogarth (1971a)
	0.95 ± 0.03 (II)	Hogarth (1971a)
	0.50 ± 0.17	Hogarth (1977)
	0.67 ± 0.08	Hogarth, Wu and Heinz (1981)
	0.61 ± 0.15	Hogarth and Kingston (1983)
	0.31 ± 0.08	Gravois, Milligan and Martin (1991)
	0.03 ± 0.02 (1979)	Blose (1992)
	0.57 ± 0.08 (1983)	Blose (1992)
	0.42 ± 0.07 (1985)	Blose (1992)
	0.14 ± 0.03 (1987)	Blose (1992)
	0.80 ± 0.27 ^s	Gravois and Milligan (1992)
	0.69	Milanés and Tejero (1992)
	0.57 ± 0.42 [†]	Tai, He, Gan and Miller (1992)
	0.37 ± 0.30 [†]	Tai, He, Gan and Miller (1992)

Characteristic	Narrow Sense Heritabilities	Authors
Ers % cane	0.23 ± 0.04 (1979) 0.85 ± 0.10 (1983) 0.50 ± 0.08 (1985) 0.31 ± 0.05 (1987)	Blose (1992) Blose (1992) Blose (1992) Blose (1992)
Purity	0.37 ± 0.08 0.57 ± 0.24 ^s 0.25 ± 0.27 0.37 ± 0.24 [†] 0.32 ± 0.29 [‡]	Gravois, Milligan and Martin (1991) Gravois and Milligan (1992) Schnell and Wu (1992) Tai, He, Gan and Miller (1992) Tai, He, Gan and Miller (1992)
Sucrose content	0.40 ± 0.08 0.61 ± 0.51 [†] 0.55 ± 0.38 [‡]	Gravois, Milligan and Martin (1991) Tai, He, Gan and Miller (1992) Tai, He, Gan and Miller (1992)
Sucrose yield	0.17 ± 0.07	Gravois, Milligan and Martin (1991)
Fibre % cane	0.45 ± 0.17 [†] 0.78 ² 0.91 ± 0.28 ^s 0.41 ± 0.30 [†] 0.62 ± 0.42 [‡]	Hogarth and Cross (1987) Hogarth and Cross (1987) Gravois and Milligan (1992) Tai, He, Gan and Miller (1992) Tai, He, Gan and Miller (1992)

where:

- ¹ = progeny analysis used to determine estimate
² = mid-parent-offspring regression analysis used to determine estimate
^s = heritabilities based on the data from one crop, three locations, and two replications
[†] = heritabilities based on a backcross of 3 F₁'s with 2 *S. spontaneum* males
[‡] = heritabilities based on a backcross of 2 F₁'s with 3 *S. spontaneum* males
(1979) = results from single line trials over four different years using intra-class correlations among
- (1987) half-sibs
(I) = heritabilities based on component of variance between full sibs, using Morley and Heinrichs (1960) modification of the nested design
(II) = heritabilities based on component of variance between full sibs, using a factorial design
(OHS) = randomly selected *S. officinarum* maternal half-sib families
(EHS) = elite Hawaiian commercial clone maternal half-sib families

Table A4.3 Genetic correlations among traits determined by sugarcane researchers around the world.

Trait combinations	r_g^{\dagger}	Authors
Stalk height vs stalk population	- 0.060	George (1962)
	0.406	Cesnik and Vencovsky (1974)
	0.377	Mariotti (1974)
	- 0.050	Kang, Miller and Tai (1983)
	0.447	Nageswara Rao, Rahman and Panduranga Rao (1983)
	0.243	Chavanne and Mariotti (1989)
	- 0.110	Milligan, Gravois, Bischoff and Martin (1990b)
	0.546	Ramesh and Varghese (1995)
	0.310	Singh and Khan (1995)
Stalk height vs stalk diameter	0.370	George (1962)
	0.097	Mariotti (1974)
	0.550	Cesnik and Vencovsky (1974)
	0.010	Kang, Miller and Tai (1983)
	- 0.233	Nageswara Rao, Rahman and Panduranga Rao (1983)
	0.008	Chavanne and Mariotti (1989)
	- 0.240	Milligan, Gravois, Bischoff and Martin (1990b)
	- 0.200	Gravois and Milligan (1992)
	0.550	Ramesh and Varghese (1995)
Stalk height vs stool mass	0.270	Singh and Khan (1995)
	0.620	George (1962)
	0.252	Mariotti (1974)
Stalk height vs stalk mass	0.393	Nageswara Rao, Rahman and Panduranga Rao (1983)
	0.734	Cesnik and Vencovsky (1974)
	0.252	Mariotti (1974)
	0.360	Kang, Miller and Tai (1983)
	0.549	Chavanne and Mariotti (1989)
	0.520	Milligan, Gravois, Bischoff and Martin (1990b)
	0.380	Gravois and Milligan (1992)
	0.812	Ramesh and Varghese (1995)
	0.510	Singh and Khan (1995)
Stalk height vs cane yield / plot	0.313	Mariotti (1974)
	0.572	Chavanne and Mariotti (1989)
	0.240	Milligan, Gravois, Bischoff and Martin (1990b)
	0.560	Singh and Khan (1995)
Stalk height vs brix	- 0.170	George (1962)
	- 0.262	Cesnik and Vencovsky (1974)
	0.318	Nageswara Rao, Rahman and Panduranga Rao (1983)
	- 0.020	Kang, Miller and Tai (1983)
	0.220	Milligan, Gravois, Bischoff and Martin (1990b)
	- 0.120	Gravois and Milligan (1992)
Stalk height vs pol in juice	0.025	Mariotti (1974)
	- 0.146	Chavanne and Mariotti (1989)
Stalk height vs fibre	0.590	Gravois and Milligan (1992)
Stalk population vs stalk diameter	- 0.120	George (1962)
	- 0.167	James and Falgout (1969)
	- 0.226 (PC)	Smith and James (1969)
	- 0.119 (FR)	Smith and James (1969)
	- 1.115	Cesnik and Vencovsky (1974)
	- 0.610	Kang, Miller and Tai (1983)
	- 0.408	Nageswara Rao, Rahman and Panduranga Rao (1983)
	- 0.444	Chavanne and Mariotti (1989)
	- 0.180	Milligan, Gravois, Bischoff and Martin (1990b)
	- 0.010	Singh and Khan (1995)
	0.169	James and Falgout (1969)
Stalk pop / stool vs stool mass	0.330	George (1962)
	0.596	Hogarth (1971b)
	0.711	Nageswara Rao, Rahman and Panduranga Rao (1983)

Trait combinations	r_k^+	Authors
Stalk pop / stool vs stalk mass	0.303 - 0.630 - 0.248 - 0.330 0.543 0.030	Mariotti (1974) Kang, Miller and Tai (1983) Chavanne and Mariotti (1989) Milligan, Gravois, Bischoff and Martin (1990b) Ramesh and Varghese (1995) Singh and Khan (1995)
Stalk pop / stool vs brix	- 0.220 0.086 0.159 (PC) 0.291 (FR) - 0.230 - 0.030 0.185 0.010	George (1962) James and Falgout (1969) Smith and James (1969) Smith and James (1969) Hogarth (1971b) Kang, Miller and Tai (1983) Nageswara Rao, Rahman and Panduranga Rao (1983) Milligan, Gravois, Bischoff and Martin (1990b)
Stalk number/plot vs stalk diameter	- 0.716 0.515	Mariotti (1974) Ramesh and Varghese (1995)
Stalk number/plot vs cane yield/plot	0.814 0.621	Mariotti (1974) Chavanne and Mariotti (1989)
Stalk number/plot vs pol in juice	- 0.031 - 0.269	Mariotti (1974) Chavanne and Mariotti (1989)
Stalk diameter vs stalk mass	0.898 0.915 0.900 0.823 0.700 0.840 0.864 0.600	Mariotti (1974) Cesnik and Vencovsky (1974) Kang, Miller and Tai (1983) Chavanne and Mariotti (1989) Milligan, Gravois, Bischoff and Martin (1990b) Gravois and Milligan (1992) Ramesh and Varghese (1995) Singh and Khan (1995)
Stalk diameter vs stool mass	0.440 0.150	George (1962) Nageswara Rao, Rahman and Panduranga Rao (1983)
Stalk diameter vs brix	0.070 - 0.135 - 0.139 (PC) - 0.050 (FR) 0.644 - 0.030 - 0.670 - 0.290	George (1962) James and Falgout (1969) Smith and James (1969) Smith and James (1969) Nageswara Rao, Rahman and Panduranga Rao (1983) Kang, Miller and Tai (1983) Milligan, Gravois, Bischoff and Martin (1990b) Gravois and Milligan (1992)
Stalk diameter vs pol in juice	0.220 0.088	Mariotti (1974) Chavanne and Mariotti (1989)
Stalk diameter vs fibre	- 0.233 - 0.590	James and Falgout (1969) Gravois and Milligan (1992)
Brix vs ash % juice	- 0.720	Hogarth and Kingston (1984)
Brix vs fibre % cane	0.080	Gravois and Milligan (1992)
Brix vs stool mass	- 0.060 0.241	George (1962) Nageswara Rao, Rahman and Panduranga Rao (1983)
Brix vs stalk mass	0.312 0.559 0.188 - 0.210 - 0.390 - 0.260	Hogarth (1971b) Hogarth (1971b) Cesnik and Vencovsky (1974) Kang, Miller and Tai (1983) Milligan, Gravois, Bischoff and Martin (1990b) Gravois and Milligan (1992)
Fibre % cane vs stalk mass	- 0.200	Gravois and Milligan (1992)

where:

(PC) = plant crop
(FR) = first ratoon crop

Table A4.4 Individual repeatability estimates of sugarcane populations between plant and ratoon crops.

Sugarcane trait	Repeatability estimate	Author
Stalk diameter	0.258	Loupe, Anzalone and Giamalva (1962)
	0.760	Roach (1968)
	0.551	Smith and James (1969)
	0.520	Miller and James (1975)
	0.597*	Vega, López and González (1983)
	0.750 ± 0.06	Schnell and Nagai (1992)
Stalk height	0.330*	Vega, López and González (1983)
	0.630 ± 0.07	Schnell and Nagai (1992)
Stalk population	0.504	Loupe, Anzalone and Giamalva (1962)
	0.611	Smith and James (1969)
	0.460	Miller and James (1975)
	0.530	Bond (1977)
	0.820	Tai, Miller, Gill and Chew (1980)
	0.436*	Vega, López and González (1983)
	0.720	Kang, Miller and Tai (1984)
	0.730 ± 0.06	Schnell and Nagai (1992)
Fibre % cane	0.260 [†]	Bond and Van der Merwe (1992)
Brix % cane	0.119	Loupe, Anzalone and Giamalva (1962)
	0.389	Smith and James (1969)
	0.620	Miller and James (1975)
	0.580	Bond (1977)
	0.840	Tai, Miller, Gill and Chew (1980)
	0.210	Kang, Miller and Tai (1984)
	0.860 [†]	Bond and Van der Merwe (1992)
	0.444**	Vega, López and González (1983)
	0.410	Milanés and Tejero (1992)
	0.359**	Vega, López and González (1983)
Pol % cane	0.920 [†]	Bond and Van der Merwe (1992)
	0.550	Milanés and Tejero (1992)
Purity	0.270	Bond (1977)
	0.171**	Vega, López and González (1983)
	0.800 [†]	Bond and Van der Merwe (1992)
Stalk mass	0.880	Tai, Miller, Gill and Chew (1980)
	0.050	Kang, Miller and Tai (1984)
Cane t ha ⁻²	0.700	Tai, Miller, Gill and Chew (1980)
	0.330	Kang, Miller and Tai (1984)
Cane mass (kg)	0.410	Bond (1977)
	0.400 [†]	Bond and Van der Merwe (1992)
Sugar t ha ⁻²	0.620	Tai, Miller, Gill and Chew (1980)
	0.330	Kang, Miller and Tai (1984)

where:

- [†] = measured on a family basis
 * = mean of 9 different clones
 ** = mean of 8 different clones

APPENDIX 5

DATA RECORDING METHODS

A5.1 Agronomic traits

Agronomic characters recorded included stalk diameter, population, height and mass:

i) stalk diameter was measured using a calibrator (a vernier caliper). The calibrator measured the diameter in cm^2 (Bond, 1979). One measurement was taken, half way up five different stalks of each stool at Stage 1. In Stage 2, one reading was taken, half way up four different stalks along the 5 m single line. This was done for both plant and ratoon crops. Stalk diameter was measured in the middle of an internode, assuming cylindrical stalks;

ii) stalk population was recorded by counting the number of stalks in each stool at Stage 1. At Stage 2 all stalks found in 4 m along the 5 m line were counted, the end stools were not included in the count because of end effects. Stools within the row tend to be smaller than the end stools because of greater competition between stools on either side. Stalk population was measured as stalks (stool)⁻¹ in Stage 1 and stalks m⁻¹ in Stage 2;

iii) stalk height was measured as the length (cm) of the stalk from ground level to the growing point. Stalk height at Stage 1 was determined from the mean of 10 stalks that were harvested at the end of the trial, for planting in Stage 2. In Stage 2 the stalk height was measured at the same time as the sucrose sampling. Stalk height was the mean of a 12 stalk sample; and

iv) stools were not weighed at Stage 1 but the mass was estimated using the following calculation designed at SASEX (Bond, 1979):

$$\text{Stool mass (kg)} = \frac{\text{Population (stalks per stool)}}{4} \times \frac{\text{Height (cm)}}{30} \times \frac{\text{Diameter (cm}^2\text{)}}{10}$$

Cane mass in Stage 2 was the field mass of each of the 5 m lines, added to the mass of the 12 stalk sample used to determine the sucrose (kg (5 m row)⁻¹).

A5.2 Millroom traits

At the time of sucrose sampling, a sample representing each of the single stools and each of the single lines was taken and sent to the SASEX millroom. In Stage 1 a two stalk sample was taken from all the stools to measure dry matter% cane, fibre% cane, brix% cane, brix% dry matter, pol% cane, purity, and ers% cane. A 12 stalk sample was taken from the middle of each of the lines in Stage 2 to measure the same traits.

The mass of the cane sample is first measured and then the sample is shredded. Juice is extracted from the shredded cane by the process of diffusion. The cane is repeatedly washed with hot water and thin juice to remove the sucrose. The spent fibre is then passed between rollers to squeeze out the remaining juice. Lime and flocculating agents are added to the heated juice and the impurities are allowed to settle. These impurities are removed from the bottom of the clarifier and filtered to recover any other clarified juice. A saccharimeter and refractometer reading are then taken from this clarified juice. The mass of the sample, and these two readings are then used to determine dry matter % cane (dm%), fibre % cane (fib%), brix % cane (brix%), brix % dry matter (brix%dm), purity (%), pol % cane (pol%) and ers % cane (ers%). These characters were determined as follows:

Sacch	=	saccharimeter reading
Refrac	=	refractometer reading
Cane mass	=	mass of cane sample sent to the millroom
Tray mass	=	mass of tray before the fresh mass of cane is added = M
Tray wet	=	mass of tray + 4 kg of fresh mass of cane sample = W
		(Water is removed from the 4 kg fresh mass sample)
Tray dry	=	mass of tray + dehydrated mass of sample = D

$$Pol\ ex = \frac{Sacch}{2(3.835 + (0.01477 \times Refrac) + (0.000055 \times Refrac))}$$

$$Dm\% = \frac{D - M}{W - M} \times 100$$

$$Fib\% = \frac{80(Dm\% - (3 \times Refrac))}{80 - Refrac}$$

$$Brix\% = Refrac(3 - (\frac{Fib\%}{80}))$$

$$Brix\%_{dm} = \frac{Brix\%}{Dm\%} \times 100$$

$$Pol\% = Pol\ ex(3 - (\frac{Fib\%}{80}))$$

$$Purity(\%) = \frac{Pol\ ex}{Refrac} \times 100$$

$$Ers\% = Pol\% - (0.485(Brix\% - Pol\% + (\frac{Fib\%}{8.5})))$$

These characteristics were recorded at specific times throughout the season for Stages 1 and 2, plant and ratoon crops (Table A5.1).

Table A5.1 Planting, ratooning, sampling and harvesting dates of the three trials at SASEX, Mount Edgecombe under raingrown conditions.

Selection stages	Planting / ratooning	Sampling	Harvesting
Stage 1 Single stools	Mid - September 1992	October 1993	October 1993
Stage 2 Single lines - plant	End - October 1993	November 1994	End - November 1994
Stage 2 Single lines - ratoon	End - November 1994	October 1995	October 1995

APPENDIX 6

FIELD TRIAL PLANS

A6.1 Introduction

This investigation was planted in two stages over three seasons at SASEX, Mount Edgecombe. The two stages were Stage 1 (Single Stool Stage) and Stage 2 (Single Line Stage). Stage 1 only had a plant crop, while Stage 2 included plant and first ratoon crops.

A6.2 Stage 1 - Single Stool Stage

The field plan of this trial was similar to that of the normal selection programme at SASEX. The main difference was that four stools of each of the parent cultivars of the selected crosses were planted. The parents were planted together in two replications in two different fields. The trial was planted in six different fields at SASEX, Mount Edgecombe. Setts (300 mm in length) were cut from the terrace seedlings. The stools were planted at 1 m spacing (1 m between the stools along the row). There was 1.2 m spacing between rows 1 and 2, then 2.4 m between rows 2 and 3, then 1.2 m again between rows 3 and 4, etc. The 2.4 m spacing between rows was to allow for easy access to the stools when recording traits. A control cultivar (NCo376) was planted every twenty first stool to allow for visual comparison when selecting cultivars to go onto the next stage (Figure A6.1).

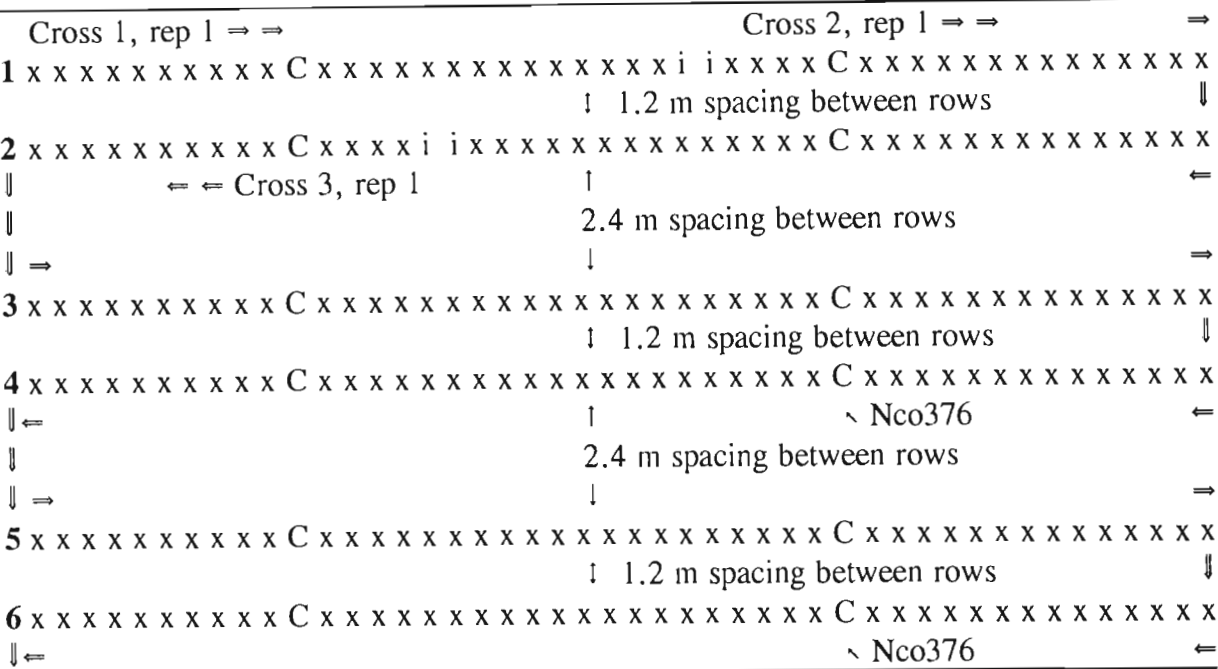


Figure A6.1 A detailed field plan of Stage 1, at SASEX, Mount Edgecombe required for accurate recording of field data.

- where:
- 1 = row number one
 - x = represents a single stool, an individual genotype
 - C = represents a control cultivar, Nco376, a single stool
 - i = red stalked cane cultivar, representing a change in cross

A6.3 Stage 2 - Single Line Stage

Setts were cut from the sugarcane stools in Stage 1 and planted in single 5 m lines in one field (field number 28) at the Mount Edgecombe Experiment Station (Figure A6.2). These lines were planted in banks across the field with each bank being separated from the next by a break of varying width. There were large (2 m wide) and small (1 m wide) breaks between banks. There was 1.2 m spacing between the lines. Guard rows were planted around the whole trial. Each of the parent cultivars was replicated six times and randomly planted within each of the replications. Each of the crosses was replicated four times, with eight offspring in each of the replications. The crosses were randomly planted within each of the four replications. A control cultivar, NCo376, was planted at regular intervals throughout the trial to help measure field variation.

C	* C	C	C	* C	C	C	* C	C	C	* C	C	C
11.01	P12	P20	7.10	7.11	P10	8.17	5.32	9.25	2.28	2.29	1.32	10.25
11.02	P7	P11	7.09	7.12	P16	8.18	5.31	9.26	2.27	2.30	1.31	10.26
11.03	P1	P6	C	7.13	P19	8.19	5.30	9.27	2.26	2.31	1.30	10.27
11.04	P14	P10	10.16	7.14	P20	8.20	5.29	9.28	2.25	2.32	1.29	10.28
11.05	P17	P5	10.15	7.15	P1	8.21	5.28	9.29	C	C	1.28	10.29
11.06	P2	P8	10.14	7.16	P18	8.22	5.27	9.30	13.32	6.25	1.27	10.30
11.07	C	P18	10.13	C	P5	8.23	5.26	9.31	13.31	6.26	1.26	10.31
11.08	3.80	P9	10.12	14.01	P13	8.24	5.25	9.32	13.30	6.27	1.25	10.32
C	3.70	P3	10.11	14.02	P12	C	C	C	13.29	6.28	C	C
6.01	3.60	P16	10.10	14.03	P6	7.17	8.32	11.25	13.28	6.29	12.32	\
6.02	3.50	P13	10.09	14.04	P2	7.18	8.31	11.26	13.27	6.30	12.31	5 m
6.03	3.40	P19	C	14.05	P15	7.19	8.30	11.27	13.26	6.31	12.30	
6.04	3.30	P15	5.16	14.06	P8	7.20	8.29	11.28	13.25	6.32	12.29	
6.05	3.20	P4	5.15	14.07	P14	7.21	8.28	11.29	C	C	12.28	
6.06	3.10	C	5.14	14.08	P3	7.22	8.27	11.30	3.32	14.25	12.27	
6.07	C	6.09	5.13	C	P11	7.23	8.26	11.31	3.31	14.26	12.26	
6.08	9.80	6.10	5.12	P10	P4	7.24	8.25	11.32	3.30	14.27	12.25	
C	9.70	6.11	5.11	P8	P17	C	C	C	3.29	14.28	C	
7.01	9.60	6.12	5.10	P6	P9	2.17	P13	7.25	3.28	14.29		
7.02	9.50	6.13	5.09	P5	P7	2.18	P20	7.26	3.27	14.30	5 m	
7.03	9.40	6.14	C	P1	C	2.19	P8	7.27	3.26	14.31		
7.04	9.30	6.15	9.16	P14	15.24	2.20	P17	7.28	3.25	14.32		
7.05	9.20	6.16	9.15	P17	15.23	2.21	P4	7.29	C	C		
7.06	9.10	C	9.14	P2	15.22	2.22	P16	7.30	15.32	\	5 m	
7.07	C	3.09	9.13	P7	15.21	2.23	P6	7.31	15.31	15.30		
7.08	14.80	3.10	9.12	P20	15.20	2.24	P14	7.32	15.29	15.28		
C	14.70	3.11	9.11	P12	15.19	C	P1	C	15.27	15.26		
1.01	14.60	3.12	9.10	P11	15.18	13.17	P3	4.25	15.25	C		
1.02	14.50	3.13	9.09	P16	15.17	13.18	P12	4.26				
1.03	14.40	3.14	C	P18	C	13.19	P19	4.27				
1.04	14.30	3.15	15.16	P9	12.24	13.20	P15	4.28				
1.05	14.20	3.16	15.15	P3	12.23	13.21	P9	4.29				
1.06	14.10	C	15.14	P13	12.22	13.22	P5	4.30				
1.07	C	8.09	15.13	P15	12.21	13.23	P11	4.31	5 m			
1.08	12.80	8.10	15.12	P4	12.20	13.24	P10	4.32				
C	12.70	8.11	15.11	P19	12.19	C	P2	C				
8.01	12.60	8.12	15.10	C	12.18	5.17	P18	\				
8.02	12.50	8.13	15.09	9.17	12.17	5.18	P7	5 m				
8.03	12.40	8.14	C	9.18	C	5.19	C					
8.04	12.30	8.15	1.16	9.19	11.24	5.20	1.24					
8.05	12.20	8.16	1.15	9.20	11.23	5.21	1.23					
8.06	12.10	C	1.14	9.21	11.22	5.22	1.22					
8.07	C	12.09	1.13	9.22	11.21	5.23	1.21					
8.08	2.80	12.10	1.12	9.23	11.20	5.24	1.20					
C	2.70	12.11	1.11	9.24	11.19	C	1.19					
15.01	2.60	12.12	1.10	C	11.18	3.17	1.18					
15.02	2.50	12.13	1.09	4.17	11.17	3.18	1.17					
15.03	2.40	12.14	C	4.18	C	3.19	C					
15.04	2.30	12.15	2.16	4.19	14.24	3.20						
15.05	2.20	12.16	2.15	4.20	14.23	3.21	5 m					
15.06	2.10	C	2.14	4.21	14.22	3.22						
15.07	C	11.09	2.13	4.22	14.21	3.23						
15.08	5.80	11.10	2.12	4.23	14.20	3.24						
C	5.70	11.11	2.11	4.24	14.19	C						
10.01	5.60	11.12	2.10	C	14.18	\						
10.02	5.50	11.13	2.09	10.17	14.17	5 m						
10.03	5.40	11.14	C	10.18	C							
10.04	5.30	11.15	P12	10.19	6.24							
10.05	5.20	11.16	P2	10.20	6.23							
10.06	5.10	C	P15	10.21	6.22							
10.07	C	13.09	P6	10.22	6.21							
10.08	4.80	13.10	P13	10.23	C							
C	4.70	13.11	P5	10.24								
13.01	4.60	13.12	P18	C	5 m							
13.02	4.50	13.13	P1	6.17								
13.03	4.40	13.14	P4	6.18								
13.04	4.30	13.15	P7	6.19								
13.05	4.20	13.16	P17	6.20								
13.06	4.10	C	P9	C								
13.07	C	4.09	P11	\								
13.08	P7	4.10	P3	5 m								
C	P2	4.11	P14									
P11	P18	4.12	P8	C								
P5	P10	4.13										
P9	P19	4.14										
P15	P12	4.15										
P6	P1	4.16										
P16	P3	C										
P14	P17	P20										
P4	P13	P16										
C	P20	P19										
	P8	P10										
5 m	C	C										

Figure A6.2 Cane setts were cut from Stage 1 and planted in Stage 2, as 5 m single lines, at Mount Edgecombe.

where: C = NCo376; * = 2 m breaks between banks; \ = 1 m break between banks; P1-P20 = parent varieties; 11.01 = cross number 11, offspring number 1; 11.02 = cross number 11, offspring number 2; etc.

APPENDIX 7

STATISTICAL ANALYSES OF TRIAL DATA

A7.1 Field adjustments

Two different methods were used for making field adjustments at the two different stages (Stages 1 and 2). Due to the layout of Stage 1 the only method of adjustment available was the use of the replications of the crosses themselves. At Stage 2 there were a number of possible ways to make field adjustments, one of which was chosen.

A7.1.1 Stage 1 - Single Stool Stage

The single stools were planted over six different fields at the Experiment Station. Each of the crosses was replicated twice. In each replication there were 16 offspring. The mean of the 16 offspring was used to get a mean for each of the replications. The mean of the cross was then calculated as the mean of the two replications (cross mean). Each of the 32 offspring in each cross was then multiplied by the cross mean divided by the replication mean.

A7.1.2 Stage 2 - Single Line Stage

The offspring and parents were planted in 5 m rows in banks across the field. There were 13 banks and the maximum number of rows planted to a bank was 84 rows. From the growth of the sugarcane it could be seen that there was field variation within banks and rows. The number of rows in each of the banks also varied. It was necessary to measure this field variation so that the offspring data could be adjusted and valuable comparisons could be made between crosses (Chapter 3). Figures A7.1 - A7.4 indicate how these adjustments were able to remove the field variation from the two traits, diameter and ers% cane. All the other traits gave similar plots before and after the adjustments.

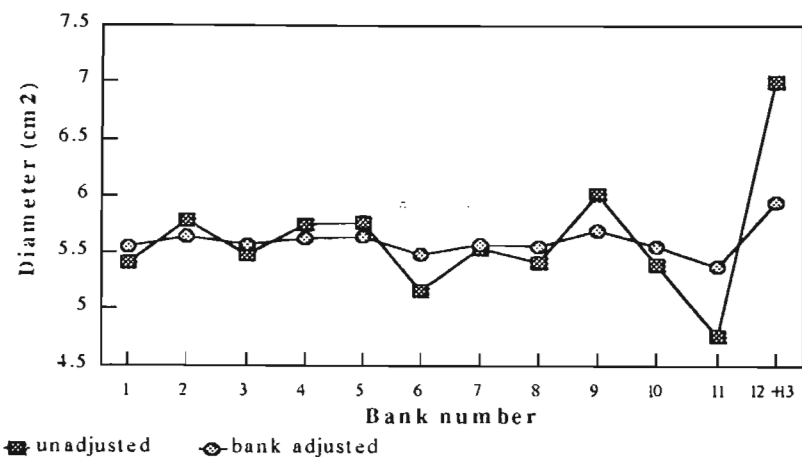


Figure A7.1 Field data of stalk diameter, in Stage 2, plant crop, adjusted for bank differences to remove field variation, compared with unadjusted field data.

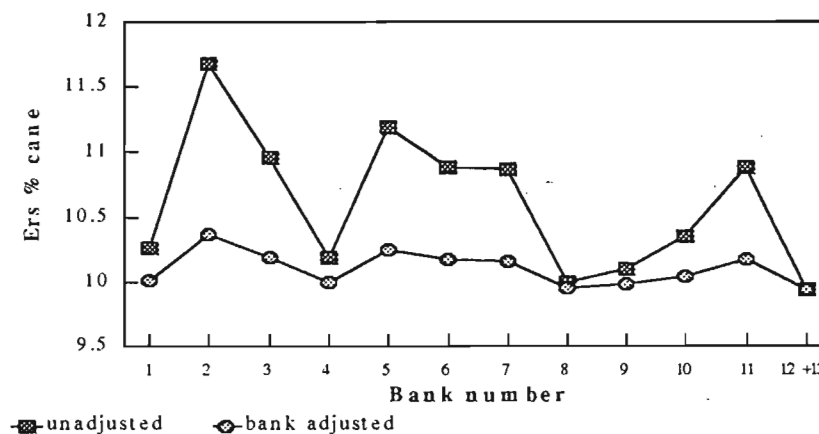


Figure A7.3 Field data of ers % cane, in Stage 2, plant crop, adjusted for bank differences to remove field variation, compared with unadjusted field data.

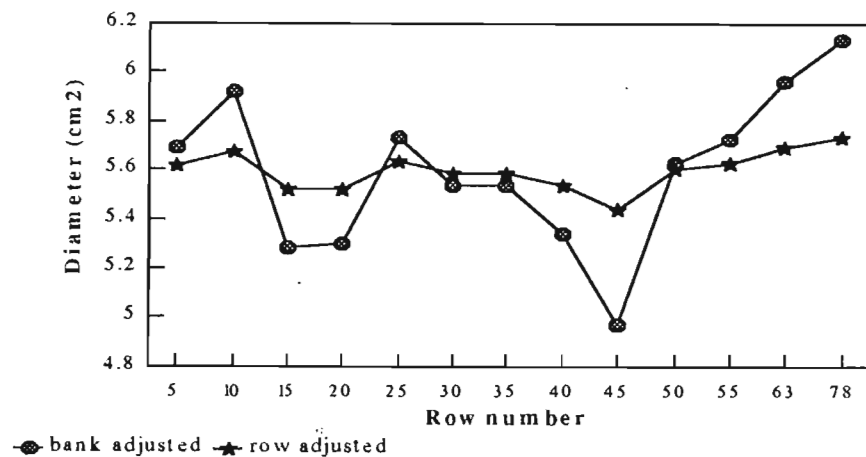


Figure A7.2 Bank adjusted data of stalk diameter, in Stage 2, plant crop, adjusted for row differences, compared with bank adjusted data.

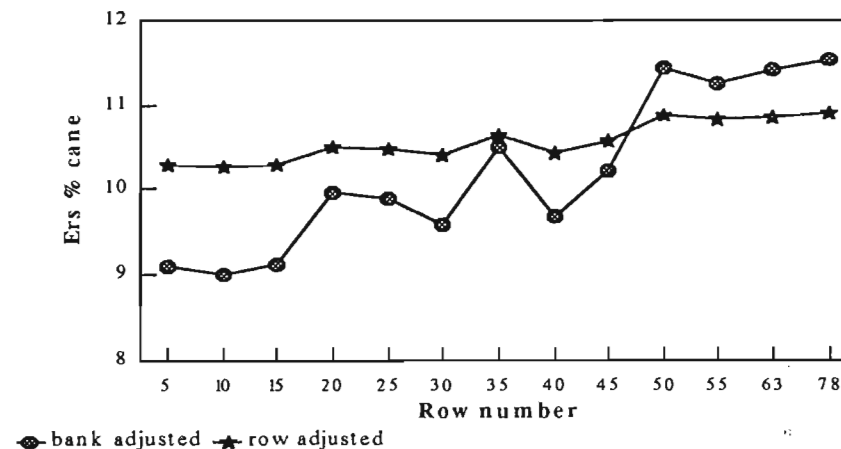


Figure A7.4 Bank adjusted field data of ers % cane, in Stage 2, plant crop, adjusted for row differences, compared with the bank adjusted data.

The effectiveness of these field adjustments was ascertained by calculating mean squares for all 15 crosses and the four replications from ANOVA tables, using the Genstat statistical package (Programme A7.1). F-values for crosses were determined from these mean squares and were compared before and after adjustments were made to ensure that adjustments removed the replication effect and field variation but not the cross variation (Table A7.1).

Programme A7.1 Genstat programme ascertaining the effectiveness of bank and row field adjustments for stalk diameter at Stage 2, plant crop.

Genstat 5 Release 3.1 (IBM-PC 80386/DOS) 15 November 1995
Copyright 1993, Lawes Agricultural Trust (Rothamsted Experimental Station)

```
1 JOB 'ANOVA TO TEST THE EFFECTIVENESS OF ADJUSTMENTS'
2 OPEN 'C: ADJUST.PRN'; CHANNEL=2; FILETYPE=INPUT; WIDTH=133
3 UNITS [60]
4 FACTOR [LEVELS=!(1...4)] REPS
5 FACTOR [LEVELS=!(1...15)] CROSS
6 READ [CHANNEL=2] CROSS,REPS,DIAM,BDIAM,RDIAM
```

WHERE:

```
CROSS = CROSS NUMBER;
DIAM = UNADJUSTED DATA FOR STALK DIAMETER;
BDIAM = BANK ADJUSTED DATA FOR STALK DIAMETER;
RDIAM = ROW ADJUSTED DATA FOR STALK DIAMETER.
```

```
8 BLOCKS REPS
9 TREATMENTSTRUCTURE CROSS
10 ANOVA [PRINT=A,M,%CV;FPROBABILITY=YES] DIAM,BDIAM,RDIAM,POP,BPOP,\
11 RPOP,HT,BHT,RHT,MASS,BMASS,RMASS
```

***** Analysis of variance *****

Variate: DIAM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPS stratum	3	0.3620	0.1207	0.69	
REPS.*Units* stratum					
CROSS	14	41.9053	2.9932	16.99	<.001
Residual	42	7.3975	0.1761		
Total	59	49.6648			

***** Tables of means *****

Variate: DIAM

Grand mean 5.596

CROSS	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00
	4.797	6.250	5.153	6.755	5.045	4.713	5.922	5.377	6.140	5.918
CROSS	11.00	12.00	13.00	14.00	15.00					
	7.427	4.302	4.548	5.693	5.907					

*** Standard errors of differences of means ***

Table CROSS

rep. 4

s.e.d. 0.2968

***** Stratum standard errors and coefficients of variation *****

Variate: DIAM

Stratum	d.f.	s.e.	cv%
REPS	3	0.0897	1.6
REPS.*Units*	42	0.4197	7.5

***** Analysis of variance *****

Variate: BDIAM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPS stratum	3	0.0838	0.0279	0.17	
REPS.*Units* stratum					
CROSS	14	34.7696	2.4835	14.84	<.001
Residual	42	7.0278	0.1673		
Total	59	41.8812			

***** Tables of means *****

Variate: BDIAM										
Grand mean 5.583										
CROSS	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00
	4.617	6.312	5.185	6.602	5.028	4.925	5.855	5.477	6.253	5.738
CROSS	11.00	12.00	13.00	14.00	15.00					
	7.085	4.460	4.590	5.555	6.060					

*** Standard errors of differences of means ***

Table	CROSS
rep.	4
s.e.d.	0.2892

***** Stratum standard errors and coefficients of variation *****

Variate: BDIAM			
Stratum	d.f.	s.e.	cv%
REPS	3	0.0432	0.8
REPS.*Units*	42	0.4091	7.3

***** Analysis of variance *****

Variate: RDIAM					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPS stratum	3	0.1921	0.0640	0.38	
REPS.*Units* stratum					
CROSS	14	30.2641	2.1617	12.99	<.001
Residual	42	6.9888	0.1664		
Total	59	37.4450			

***** Tables of means *****

Variate: RDIAM										
Grand mean 5.588										
CROSS	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00
	4.725	6.260	5.238	6.383	5.065	4.925	5.887	5.320	6.460	5.785
CROSS	11.00	12.00	13.00	14.00	15.00					
	6.835	4.485	4.635	5.738	6.078					

*** Standard errors of differences of means ***

Table	CROSS
rep.	4
s.e.d.	0.2884

***** Stratum standard errors and coefficients of variation *****

Variate: RDIAM			
Stratum	d.f.	s.e.	cv%
REPS	3	0.0653	1.2
REPS.*Units*	42	0.4079	7.3

The same determinations were carried out for the other 10 traits in Stage 2, plant crop (Table A7.1).

Table A7.1 Testing the effectiveness of the field adjustments of a sugarcane population at Stage 2, plant crop.

Sugarcane traits	Before field adjustments		After bank adjustments		After row adjustments	
	F-value	CV %	F-value	CV %	F-value	CV %
Diameter	16.99	7.5	14.84	7.3	12.99	7.3
Population	4.80	10.0	5.27	8.9	5.74	8.3
Height	2.37	12.3	2.05	11.9	2.07	9.3
Mass	3.75	17.1	3.38	16.6	3.46	14.9
DM % cane	2.80	4.6	2.26	4.6	3.23	3.2
Fibre % cane	6.41	6.1	5.03	6.2	5.79	5.6
Brix % cane	4.05	6.7	3.81	6.7	4.93	5.1
Brix % DM	7.86	4.5	6.74	4.6	6.84	4.3
Purity	5.08	2.8	4.87	2.7	5.92	2.1
Pol % cane	4.77	8.8	4.31	8.9	5.77	6.7
Ers % cane	5.22	11.0	4.67	11.1	6.18	8.5

where:

F-value = variance ratio

CV % = coefficient of variation

The CV % decreased slightly for all traits, while F-values either increased or decreased slightly depending on the trait. It could be deduced from these results that variation between crosses was not removed, and this indicates that the adjustments were successful. The adjusted data were then used to determine heritabilities, correlations and clonal repeatabilities.

A7.2 Regression analysis

Mid-parent-offspring regression was used to determine narrow sense heritabilities at Stage 1 and 2 (plant and ratoon crops). Measurements were first taken from all the progeny in the trial and their parents. The progeny measurements (y dependent variable) were then regressed on the mid-parent values (x independent variable) (Table A7.2 and A7.3).

Table A7.2 Mid-parent offspring regression of stalk diameter (cm²) for 12 sugarcane crosses at Stage 2, plant crop.

Cross	Mid-parent value	Offspring mean	Deviations from mean		Square		Product
	x	y	Mean - x	Mean - y	x ²	y ²	xy
1	5.31	4.73	-0.94	-0.94	0.88	0.87	0.88
2	7.02	6.26	0.77	0.60	0.59	0.36	0.46
3	5.70	5.24	-0.55	-0.42	0.30	0.18	0.23
4	7.93	6.39	1.68	0.72	2.83	0.52	1.22
5	6.14	5.07	-0.11	-0.60	0.01	0.35	0.07
6	5.49	4.93	-0.77	-0.74	0.58	0.54	0.56
7	6.01	5.89	-0.24	0.22	0.06	0.05	-0.05
8	6.90	6.46	0.65	0.80	0.42	0.63	0.52
9	6.95	5.78	0.70	0.12	0.49	0.02	0.09
10	5.91	6.84	-0.33	1.18	0.11	1.38	-0.39
11	5.00	4.64	-1.25	-1.03	1.55	1.06	1.28
12	6.62	5.74	0.37	0.08	0.14	0.01	0.03
Sum	74.94	67.94	0.00	0.00	7.97	5.97	4.88
Mean	6.25	5.66					

Regression coefficient (b) = $\frac{\sum xy}{\sum x^2} = \frac{4.88}{7.97} = 0.61$

y' is the estimated deviation of y corresponding to any x deviation, where x = mid-parent value,

y' - mean of y = b (x - mean of x)

y' = mean of y + b (x - mean of x)

= 5.66 + 0.61 (x - 6.25)

Therefore, to calculate y' values, values were substituted into the above equation so that when:

x = 4, y' = 4.29

x = 5, y' = 4.90

x = 6, y' = 5.51

x = 7, y' = 6.12

x = 8, y' = 6.74

The fitted values are then plotted on the same graph as the raw data and the goodness of fit determined (Table A7.3).

Table A7.3 Estimated offspring means (y') and their deviations from regression ($d(y.x)$) for stalk diameter calculated for 12 sugarcane crosses and their mid-parent values at Stage 2, plant crop.

Cross	Mid-parent value	Offspring mean	Estimated offspring mean	Deviation from regression	Square of deviation
	x	y	y'	$y - y' = d(y.x)$	$d(y.x)^2$
1	5.31	4.73	5.09	-0.36	0.13
2	7.02	6.26	6.13	0.13	0.02
3	5.69	5.24	5.33	-0.09	0.01
4	7.93	6.39	6.69	-0.31	0.09
5	6.14	5.07	5.60	-0.53	0.28
6	5.49	4.93	5.20	-0.27	0.07
7	6.01	5.89	5.52	0.37	0.14
8	6.90	6.46	60.60	0.40	0.16
9	6.95	5.78	6.09	-0.31	0.09
10	5.91	6.84	5.46	1.38	1.90
11	5.00	4.65	4.90	-0.27	0.07
12	6.62	5.74	5.89	-0.15	0.02
Sum				0.00	2.99

where:

Sum of squares of deviations (2.99) is the basis for an estimate of error in fitting the line.

Corresponding degrees of freedom (d.f.) are $n-2 = 12 - 2 = 10$.

Mean square deviation from regression = sum of squares of deviations divided by d.f.
= $2.99 / 10 = 0.30$

Sample standard deviation from regression = square root of mean square deviation from regression
= 0.55

Sample standard deviation of b = sample standard deviation from regression divided by the square root of the sum of squares
= $0.55 / 2.82$
= 0.19

Therefore, h_a^2 for stalk diameter at Stage 2, plant crop = 0.61 ± 0.19 .

A7.3 Restricted maximum likelihood analysis

REML analysis of an unbalanced North Carolina design II was used to estimate h_a^2 and h_b^2 of

a sugarcane population (Programme A7.2). Positive constraints were set in the Genstat programme so that when the variance components were negative they were replaced with positive values. The estimated variance components of the interaction terms: reps×females; reps×males; and reps×females×males, were pooled with the residual variance, when heritabilities were determined. The following pages provide the output of the GENSTAT programme used to estimate variance components and heritabilities of stalk diameter in the plant crop of Stage 2.

Programme A7.2 Mean squares, genetic effects, variance components, and heritabilities of stalk diameter determined by REML analysis of an unbalanced North Carolina design II (Reps*Females*Males) at Stage 2, plant crop.

```

1 JOB 'REML ANALYSES OF SL TRAITS - PLANT AND RATOON ADJUSTED DATA'
2 UNITS [384]
3 FACTOR [LEV=4] REPS
4 FACTOR [LEV=4] BLOCKS
5 FACTOR [LEV=3] PLOTS
6 FACTOR [LEV=8] LINES
7 FACTOR [LEV=12] CROSSES
8 FACTOR [LEV=8] FEMALES
9 FACTOR [LEV=9] MALES
10 OPEN 'C:FACTOR.PRN'; CHANNEL=2; FILETYPE=INPUT; WIDTH=133
11 OPEN 'C:FACTOR1.PRN'; CHANNEL=3; FILETYPE=INPUT; WIDTH=133
12 READ [CHANNEL=2] REPS, BLOCKS, PLOTS, LINES, CROSSES, FEMALES, MALES, DIAM, RDIAM, POP, RPOP, HT, RHT,
    MASS, RMASS, DM%, RDM%, FIB%, RFIB%, BRIX%, RBRIX%
14 READ [CHANNEL=3] REPS, BLOCKS, PLOTS, LINES, CROSSES, FEMALES, MALES, BRIX%DM, RBRIX%DM, PURITY,
    RPURITY, POL%, RPOL%, UERS%, ERS%, RUERS%, RERS%
17 VCOMP RANDOM=REPS*FEMALES*MALES;CONSTRAINTS=POSITIVE
18 FOR X = DIAM, RDIAM, POP, RPOP, HT, RHT, MASS, RMASS, DM%, RDM%, FIB%, RFIB%, BRIX%, RBRIX%, BRIX%DM,
19 RBRIX%DM, PURITY, RPURITY, POL%, RPOL%, UERS%, ERS%, RUERS%, RERS%
21 REML [PRINT=MEANS,COMP,EFF,STR,DEV] X
22 VARIATE [VALUES=0,2,2,0,0,0,0,0] N1
23 VARIATE [VALUES=0,1,1,0,0,1,0,1] D
24 VFUNCTION [RANDOM=REPS*FEMALES*MALES] NUMERATOR=N1;DENOMINATOR=D
where: N1 = additive genetic variance component; and D = phenotypic variance component
26 VARIATE [VALUES=0,2,2,0,0,4,0,0] N1
27 VARIATE [VALUES=0,1,1,0,0,1,0,1] D
28 VFUNCTION [RANDOM=REPS*FEMALES*MALES] NUMERATOR=N1;DENOMINATOR=D
where: N1 = genotypic variance component; and D = phenotypic variance component'
*** Estimated Variance Components *** (for stalk diameter, at Stage 2, plant crop)
Random term           Component           S.e.
REPS                   0.0001            0.0176
FEMALES                0.3672            0.2892
MALES                  0.1403            0.2181
REPS.FEMALES           0.0001            0.0522
REPS.MALES             0.0001            0.0673
FEMALES.MALES          0.0001            0.2048
REPS.FEMALES.MALES     0.1063            0.0991
*units*                0.9517            0.0734

*** Approximate stratum variances ***
Mean Squares      Effective d.f.
REPS               1.8097         3.00
FEMALES            16.3496         6.87
MALES              6.9833         3.98
REPS.FEMALES       1.7999        13.64
REPS.MALES         1.7993         9.00
FEMALES.MALES      1.8007         0.15
REPS.FEMALES.MALES 1.7976        10.36
*units*            0.9517        336.00

```

* Matrix of coefficients of components for each stratum *

REPS	96.00	0.00	0.00	17.32	13.33	0.00	8.00	1.00
FEMALES	0.00	34.20	14.32	8.55	3.58	32.00	8.00	1.00
MALES	0.00	0.00	37.60	0.00	9.40	32.00	8.00	1.00
REPS.FEMALES	0.00	0.00	0.00	17.31	6.82	0.00	8.00	1.00
REPS.MALES	0.00	0.00	0.00	0.00	17.22	0.00	8.00	1.00
FEMALES.MALES	0.00	0.00	0.00	0.00	0.00	32.00	8.00	1.00
REPS.FEMALES.MALES	0.00	0.00	0.00	0.00	0.00	0.00	8.00	1.00
units	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

*** Deviance: -2*Log-Likelihood ***
Deviance d.f.
1107. 376

*** Table of effects for Constant ***
1
5.850

Table has only one entry: standard error 0.2632

*** Table of predicted means for Constant ***
1
5.850

Table has only one entry: standard error 0.2632

Procedure VFUNTION:

Term	Component	Coefficients	
		Numerator	Denominator
Constant		0	0
REPS	0.00009517	0	0
FEMALES	0.3672	2.000	1.000
MALES	0.1403	2.000	1.000
REPS . FEMALES	0.00009517	0	0
REPS . MALES	0.00009517	0	0
FEMALES . MALES	0.00009517	0	1.000
(REPS . FEMALES) . MALES	0.1063	0	0
Units	0.9517	0	1.000

Function value = 0.6955 (narrow sense heritability estimate)
Standard error = 0.4329

Procedure VFUNTION:

Term	Component	Coefficients	
		Numerator	Denominator
Constant		0	0
REPS	0.00009517	0	0
FEMALES	0.3672	2.000	1.000
MALES	0.1403	2.000	1.000
REPS . FEMALES	0.00009517	0	0
REPS . MALES	0.00009517	0	0
FEMALES . MALES	0.00009517	4.000	1.000
(REPS . FEMALES) . MALES	0.1063	0	0
Units	0.9517	0	1.000

Function value = 0.6958 (broad sense heritability estimate)
Standard error = 0.3177

From similar outputs to that presented above, heritabilities of the other traits, for plant and ratoon crops of Stage 2, were determined. The genetic effects, variance components, heritabilities, and genetic coefficients of variation were presented in Chapter 3.

Table A7.4 Mean squares of a sugarcane population under raingrown conditions for 11 traits at Stage 2, plant crop, using REML analysis of an unbalanced North Carolina design II.

	Stalk diameter (cm ²)	Stalk population (stalks per 5 m line)	Stalk height (cm)	Cane mass (kg per 5 m line)	Dry matter % cane	Fibre % cane	Brix % cane	Brix % dry matter	Purity (%)	Pol % cane	Ers % cane
MS _r d.f.	1.81 3.00	24.44 3.00	4403.00 3.00	510.20 3.00	23.85 3.00	7.69 3.00	5.64 3.00	48.80 3.00	66.34 3.00	9.37 3.00	11.11 3.00
MS _f d.f.	16.35 6.87	38.17 5.44	4753.10 6.86	2117.90 6.87	40.84 6.96	24.14 6.27	5.99 4.49	216.74 6.83	68.34 4.95	10.67 4.63	14.24 4.73
MS _m d.f.	6.98 3.98	104.06 4.95	1804.60 2.77	761.90 2.82	9.99 3.68	33.80 4.51	51.05 6.23	101.90 4.12	402.81 5.88	77.76 6.08	95.92 6.01
MS _{rf} d.f.	1.80 13.64	20.34 18.72	1802.70 15.13	381.00 14.23	6.08 16.12	5.32 15.97	3.56 18.78	33.69 17.61	21.27 14.25	4.19 16.81	5.02 16.21
MS _{rm} d.f.	1.80 9.00	12.72 7.43	1802.60 8.56	380.90 8.84	5.78 8.21	8.08 12.80	2.48 8.48	46.26 12.65	36.45 12.71	3.56 7.97	4.51 8.15
MS _{fm} d.f.	1.80 0.15	15.19 0.61	1803.00 1.37	760.90 1.31	5.78 0.37	4.50 0.21	2.05 0.28	13.42 0.05	16.33 0.17	3.54 0.28	4.51 0.27
MS _{rfm} d.f.	1.80 10.36	12.70 6.85	1802.10 9.30	380.60 9.93	5.77 8.67	2.87 4.24	2.05 5.74	13.38 2.74	16.30 6.05	3.53 8.22	4.51 8.65
MS _e d.f.	0.95 336.00	12.69 336.00	262.60 336.00	171.60 336.00	2.78 336.00	2.87 336.00	0.94 336.00	13.37 336.00	7.85 336.00	1.24 336.00	1.54 336.00
MS _c d.f.	6.35 369.00	58.45 369.00	5670.00 369.00	1314.10 369.00	20.41 369.00	19.14 369.00	9.03 369.00	106.70 369.00	81.87 369.00	12.52 369.00	15.57 369.00

where:

MS_r = mean square of replications, MS_f = mean square of female parents, MS_m = mean square of male parents

MS_{rf} = mean square of interaction between replications and female parents, MS_{rm} = mean square of interaction between replications and male parents

MS_{fm} = mean square of interaction between female and male parents, MS_{rfm} = mean square of interaction between replications, female and male parents

MS_e = mean square of residual error, MS_c = mean square of combined residual error (replication interaction terms combined with residual error)

d.f. = degrees of freedom

Table A7.5 Mean squares of a sugarcane population under raingrown conditions for 11 traits at Stage 2, ratoon crop, using REML analysis of an unbalanced North Carolina design II.

	Stalk diameter (cm ²)	Stalk population (stalks per 5 m row)	Stalk height (cm)	Cane mass (kg per 5 m line)	Dry matter % cane	Fibre % cane	Brix % cane	Brix % dry matter	Purity (%)	Pol % cane	Ers % cane
MS _r d.f.	1.87 3.00	25.11 3.00	916.00 3.00	345.20 3.00	5.06 3.00	7.53 3.00	8.17 3.00	76.28 3.00	27.46 3.00	6.92 3.00	7.33 3.00
MS _f d.f.	10.50 6.52	129.54 6.39	5205.80 6.85	1202.20 6.12	28.08 6.63	27.90 6.46	11.45 4.88	210.48 6.11	79.98 4.97	15.67 4.68	20.64 4.69
MS _m d.f.	10.41 4.40	89.64 2.62	2445.00 3.99	938.70 2.52	16.85 2.89	29.17 4.18	55.82 5.64	345.23 4.57	516.97 5.98	100.12 5.94	135.20 5.97
MS _{rf} d.f.	1.37 13.28	22.31 16.30	783.50 14.45	221.10 14.51	3.28 13.01	6.91 13.60	4.64 14.70	50.53 14.45	18.88 15.44	5.89 13.97	7.31 13.62
MS _{rm} d.f.	2.19 13.00	22.03 10.09	936.60 11.14	222.30 8.83	6.35 13.98	7.88 10.43	4.64 8.70	50.53 8.78	29.80 12.95	5.89 8.91	7.31 9.00
MS _{fm} d.f.	1.07 0.08	83.05 1.99	618.30 0.17	934.80 2.36	13.54 1.48	6.35 0.36	4.64 0.48	50.55 0.32	10.93 0.06	5.89 0.38	7.32 0.33
MS _{rfm} d.f.	1.07 6.72	15.24 6.61	617.10 7.40	219.70 9.66	2.44 6.02	6.34 8.97	4.64 9.60	50.50 9.78	10.91 4.60	5.89 10.12	7.31 10.38
MS _e d.f.	1.07 336.00	15.23 336.00	376.60 336.00	219.50 336.00	2.44 336.00	2.52 336.00	1.00 336.00	12.99 336.00	6.01 336.00	1.28 336.00	1.56 336.00
MS _c d.f.	5.70 369.00	74.81 369.00	2337.20 369.00	882.60 369.00	14.51 369.00	23.65 369.00	14.92 369.00	164.55 369.00	65.60 369.00	18.94 369.00	23.50 369.00

where:

MS_r = mean square of replications, MS_f = mean square of female parents, MS_m = mean square of male parents
MS_{rf} = mean square of interaction between replications and female parents, MS_{rm} = mean square of interaction between replications and male parents
MS_{fm} = mean square of interaction between female and male parents, MS_{rfm} = mean square of interaction between replications, female and male parents
MS_e = mean square of residual error, MS_c = mean square of combined residual error (replication interaction terms combined with residual error)
d.f. = degrees of freedom

A7.4 Correlations among traits

Genetic, phenotypic, and environmental correlations were determined among traits by REML. The REML output for stalk diameter (x_1) correlated to stalk population (x_2) in Stage 2, plant crop is presented in Programme A7.3:

Programme A7.3 Genetic, phenotypic, and environmental correlations between stalk diameter and stalk population (Stage 2, plant crop) using REML analysis of an unbalanced North Carolina design II (Reps*Cross factorial mating design).

Genstat 5 Release 3.1 (IBM-PC 80386/DOS) 11 December 1995
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```

1 JOB 'CORRELATION ANALYSIS'
2 UNITS [384]
3 FACTOR [LEV=4] REPS
4 FACTOR [LEV=4] BLOCKS
5 FACTOR [LEV=3] PLOTS
6 FACTOR [LEV=8] LINES
7 FACTOR [LEV=12] CROSSES
8 FACTOR [LEV=8] FEMALES
9 FACTOR [LEV=9] MALES
10 OPEN 'C:FACTOR.PRN'; CHANNEL=2; FILETYPE=INPUT; WIDTH=133
11 OPEN 'C:FACTOR1.PRN'; CHANNEL=3; FILETYPE=INPUT; WIDTH=133
12 READ [CHANNEL=2] REPS, BLOCKS, PLOTS, LINES, CROSSES, FEMALES, MALES, DIAM, RDIA, POP, RPOP, HT,
    RHT, MASS, RMASS, DM%, RDM%, FIB%, RFIB%, BRIX%, RBRIX%
15 READ [CHANNEL=3] REPS, BLOCKS, PLOTS, LINES, CROSSES, FEMALES, MALES, BRIX%DM, RBRIX%DM, PURITY,
    RPURITY, POL%, RPOL%, ERS%, RERS%
19 pointer [values=DIAM,POP,HT,MASS,DM%,FIB%,BRIX%,BRIX%DM,PURITY,POL%,ERS%] x
21 for i=1...11
22   for j=2...11
23     if j>i
24       calc x1 = x[i]
25       calc x2 = x[j]
26       calc x12 = x[i]+x[j]
27       vcomp rand=REPS*CROSSES
28       reml [pr=c,m,s,d] x1
29       vkeep [sigma2=e1] CROSSES;comp=c1
30       reml [pr=c,m,s,d] x2
31       vkeep [sigma2=e2] CROSSES;comp=c2
32       reml [pr=c,m,s,d] x12
33       vkeep [sigma2=e3] CROSSES;comp=c3
34       calc rg=((c3-c2-c1)/2)/sqrt(c1*c2)
35       calc rp=((c3-c2-c1)/2)+((e3-e2-e1)/2))/sqrt((c1+e1)*(c2+e2))
36       calc re=((e3-e2-e1)/2)/sqrt(e1*e2)
37       print rg,rp,re
38     endif
39   calc j=j+1
40   endfor
41   calc i=i+1
42   endfor

```

Stage 2 - plant crop: stalk diameter vs stalk population

where: x_1 = stalk diameter, x_2 = stalk population, $x_{12} = x_1 + x_2$,
 c_1 = cross variance for stalk diameter, c_2 = cross variance for stalk population,
 c_3 = cross variance (stalk diameter + stalk population), e_1 = error variance for stalk diameter,
 e_2 = error variance for stalk population, e_3 = error variance (stalk diameter + stalk population),
 rg = genotypic correlation, rp = phenotypic correlation, re = environmental correlation.

***** REML Variance Components Analysis *****

Response Variate : x_1
 Random model : REPS+CROSSES+REPS.CROSSES
 Fixed model : Constant
 Number of units : 384
 No absorbing factor

*** Estimated Variance Components ***

Random term	Component	S.e.
REPS	-0.0131	0.0037
CROSSES	c1 = 0.4992	0.2317
REPS.CROSSES	0.0549	0.0438
units	e1 = 0.9517	0.0734

*** Approximate stratum variances ***

		Effective d.f.
REPS	0.1317	3.00
CROSSES	17.3662	11.00
REPS.CROSSES	1.3911	33.00
units	0.9517	336.00

* Matrix of coefficients of components for each stratum *

	REPS	CROSSES	REPS.CROSSES	*units*
REPS	96.00	0.00	8.00	1.00
CROSSES	0.00	32.00	8.00	1.00
REPS.CROSSES	0.00	0.00	8.00	1.00
units	0.00	0.00	0.00	1.00

*** Deviance: -2*Log-Likelihood ***

Deviance	d.f.
1107.	380

***** REML Variance Components Analysis *****

Response Variate : x_2 = stalk population
 Random model : REPS+CROSSES+REPS.CROSSES
 Fixed model : Constant
 Number of units : 384
 No absorbing factor

*** Estimated Variance Components ***

Random term	Component	S.e.
REPS	0.10	0.20
CROSSES	c2 = 1.51	0.84
REPS.CROSSES	0.15	0.45
units	e2 = 12.69	0.98

*** Approximate stratum variances ***

		Effective d.f.
REPS	23.53	3.00
CROSSES	62.23	11.00
REPS.CROSSES	13.92	33.00
units	12.69	336.00

* Matrix of coefficients of components for each stratum *

	REPS	CROSSES	REPS.CROSSES	*units*
REPS	96.00	0.00	8.00	1.00
CROSSES	0.00	32.00	8.00	1.00
REPS.CROSSES	0.00	0.00	8.00	1.00
units	0.00	0.00	0.00	1.00

*** Deviance: -2*Log-Likelihood ***

Deviance	d.f.
2082.	380

***** REML Variance Components Analysis *****

Response Variate : x_{12} = stalk diameter + stalk population
 Random model : REPS+CROSSES+REPS.CROSSES
 Fixed model : Constant
 Number of units : 384
 No absorbing factor

```

*** Estimated Variance Components ***
Random term      Component      S.e.
REPS              0.05          0.18
CROSSES          c3 =      0.64          0.50
REPS.CROSSES      0.54          0.50
*units*          e3 =     11.39          0.88

*** Approximate stratum variances ***
                                     Effective d.f.
REPS                                20.28          3.00
CROSSES                             36.12         11.00
REPS.CROSSES                         15.73         33.00
*units*                             11.39        336.00

* Matrix of coefficients of components for each stratum *
      REPS      96.00      0.00      8.00      1.00
      CROSSES    0.00     32.00      8.00      1.00
      REPS.CROSSES 0.00      0.00      8.00      1.00
      *units*    0.00      0.00      0.00      1.00

*** Deviance: -2*Log-Likelihood ***
      Deviance  d.f.
      2044.    380

Correlations:
      rg      rp      re
      -0.7901  -0.3996  -0.3246

```

Correlations among other traits for plant and ratoon crops were calculated using the same equations and were presented in Chapter 3 (Table 3.4 and 3.5, respectively).

A7.5 Clonal repeatabilities between seasons

Clonal repeatabilities were determined for the different seasons. Mean squares for the population planted over two different seasons were determined from ANOVA tables. F-values were calculated by dividing the mean squares of the individuals by the error mean squares of the different traits. The F-values were then substituted into the following equation to determine clonal repeatabilities:

$$\text{Clonal repeatability} = \frac{F - 1}{F}$$

where: F = F-value = variance ratio

The GENSTAT statistical output for calculating the F-values and clonal repeatabilities for stalk diameter and stalk population compared over two different seasons is presented on the following page (Programme A7.4).

Programme A7.4 F-values and clonal repeatabilities for all individuals in 15 crosses under raingrown conditions for stalk diameter and stalk population, between Stages 1 and 2, plant crop, and Stage 2, plant and ratoon crops.

```

1 JOB 'CLONAL REPEATABILITY OF TRAITS OVER SEASONS:  - STAGE 1 VS STAGE 2 (PLANT)
               - STAGE 1 VS STAGE 2 (RATOON)
               - STAGE 2 (PLANT) VS STAGE 2 (RATOON)'
5 OPEN 'C:REPEAT.PRN';CHANNEL=2;FILETYPE=INPUT;WIDTH=133
6 OPEN 'C:REPEAT1.PRN';CHANNEL=3;FILETYPE=INPUT;WIDTH=133
7 UNITS [898]
8 FACTOR [LEVELS=!(1...2)] YEARS
9 FACTOR [LEVELS=!(1...449)] INDIVS
10 FACTOR [LEVELS=!(1...2)] YEARS1
11 FACTOR [LEVELS=!(1...449)] INDIVS1
12 READ [CHANNEL=2] INDIVS,YEARS,DIAMETER,POPULATION,HEIGHTT,MASS,DM%,FIB%,BRIX%,BRX%DM,PURITY,POL%,ERS%
14 READ [CHANNEL=3] INDIVS1,YEARS1,DIAM1,POP1,HT1,MASS1,DM%1,FIB%1,BRIX%1,BRX%DM1,PURITY1,POL%1,ERS%1

```

STAGE 1 VS STAGE 2 (PLANT):

```

16 BLOCKS YEARS
17 TREATMENTSTRUCTURE INDIVS
18 ANOVA [PRINT=A,%CV] DIAMETER,POPULATION,HEIGHT,MASS,DM%,FIB%,BRIX%,BRX%DM,PURITY,POL%,ERS%

```

***** Analysis of variance *****

```

Variate: DIAMETER
Source of variation    d.f.      s.s.      m.s.      v.r.
YEARS stratum          1    107.6794  107.6794  183.25
YEARS.*Units* stratum
INDIVS                 448   1343.8839    2.9997    5.11 = F-value
Residual               448    263.2447    0.5876
Total                  897   1714.8081

```

Clonal repeatability of stalk diameter = $(5.11 - 1) / 5.11 = 0.804$

***** Stratum standard errors and coefficients of variation *****

```

Variate: DIAMETER
Stratum              d.f.      s.e.      cv%
YEARS                 1        0.4897    8.3
YEARS.*Units*        448        0.7666   13.0

```

***** Analysis of variance *****

```

Variate: POPULATION
Source of variation    d.f.      s.s.      m.s.      v.r.
YEARS stratum          1    2764.84   2764.84   154.11
YEARS.*Units* stratum
INDIVS                 448   19145.96    42.74    2.38 = F-value
Residual               448    8037.21    17.94
Total                  897   29948.02

```

***** Stratum standard errors and coefficients of variation *****

```

Variate: POPULATION
Stratum              d.f.      s.e.      cv%
YEARS                 1        2.481    13.7
YEARS.*Units*        448        4.236   23.5

```

STAGE 2 (PLANT) VS STAGE 2 (RATOON):

```

31 BLOCKS YEARS2
32 TREATMENTSTRUCTURE INDIVS2
33 ANOVA [PRINT=A,%CV] DIAM2,POP2,HT2,MASS2,DM%2,FIB%2,\
34 BRIX%2,BRX%DM2,PURITY2,POL%2,ERS%2

```

***** Analysis of variance *****

Variate: DIAMETER2

Source of variation	d.f.	s.s.	m.s.	v.r.
YEARS2 stratum	1	13.9274	13.9274	35.60
YEARS2.*Units* stratum				
INDIVS2	479	1195.3157	2.4954	6.38
Residual	479	187.3953	0.3912	
Total	959	1396.6383		

Clonal repeatability of stalk diameter = $(6.38 - 1) / 6.38 = 0.843$

***** Stratum standard errors and coefficients of variation *****

Variate: DIAMETER2

Stratum	d.f.	s.e.	cv%
YEARS2	1	0.1703	3.0
YEARS2.*Units*	479	0.6255	11.0

***** Analysis of variance *****

Variate: POPULATION2

Source of variation	d.f.	s.s.	m.s.	v.r.
YEARS2 stratum	1	400.727	400.727	63.27
YEARS2.*Units* stratum				
INDIVS2	479	12794.840	26.712	4.22
Residual	479	3033.912	6.334	
Total	959	16229.479		

***** Stratum standard errors and coefficients of variation *****

Variate: POPULATION2

Stratum	d.f.	s.e.	cv%
YEARS2	1	0.9137	5.9
YEARS2.*Units*	479	2.5167	16.2

Clonal repeatabilities for all the traits are presented in Table 3.8 in Chapter 3.