

Evaluation and selection of different types of sugarcane varieties for
multi-purpose use from a population of inter-specific derived clones
in Mauritius

by

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Nomenclature

ACP	African-Caribbean-Pacific (sugar producing countries)
ANOVA	Analysis of Variance
ARIMA	autoregressive-integrated-moving average
Bagasse	Fibrous material left after cane juice extraction
BC1	First backcross between F1 and noble or commercial hybrids
BC2	Second backcross between BC1 and noble or commercial hybrids
BIOEN	Brazil Bioenergy Research Programme (Brazil)
BLUE	Best Linear Unbiased Estimation
BLUP	Best Linear Unbiased Prediction
Brix	Percentage of soluble solids in cane juice – an estimate of sucrose content
BSES	Bureau of Sugar Experiment Stations - Australia
CBS	Cane Breeding Station (West Indies)
CCS	Commercial cane sugar
CI	Coincidence Index
CRC SIIB	Cooperative Research Centre for Sugar Industry Innovation through Biotechnology
CSO	Central Statistical office
CTL	Cane tops and leaves
CV	Coefficient of variability
EU	European Union
F1	Crosses involving noble or commercial hybrids x wild relatives
H^2	Repeatability or broad-sense heritability
ICL	Independent Culling Level
IRSC	Industrial recoverable sucrose content
LMM	Linear Mixed Model
LSD	Least significant difference
Molasses	Viscous residue left after sugar crystals are centrifuged out
MREPU	Ministry of Renewable Energy & Public Utilities
MSIRI	Mauritius Sugar Industry Research Institute (Mauritius)
MVDA	Multivariate Data Analysis
Nobilization	Crossing and backcrossing of <i>Saccharum officinarum</i> clones or commercial hybrids with related wild species and genera
NSW	New South Wales - Australia
PC	Principal Component
PCA	Principal Component Analysis
pcscore n	Principal component score n
Pol	Net optical activity of different sugars measured with a polarimeter – a more precise way of measuring sucrose content than Brix
Ratoon	Regrowth of cane stubbles after harvest: A first ratoon crop thus is one obtained

	from new shoots springing from the cane stubbles after the first harvest.
R^2	Coefficient of determination
RCBD	Randomised Complete Block Design
SD	Standard Deviation
SRU	USDA-ARS Sugarcane Research Unit
SS	Sum of squares
tha ⁻¹	Tonnes per hectare
Trash	Dead dry fallen or clinging leaves
UPGMA	Unweighted Pair Group Method with Arithmetic mean
USDA -ARS	United States Department of Agriculture – Agricultural Research Service

Summary

Sugarcane is among the most efficient producers of biomass per unit area. Populations derived from crosses between sugarcane and related wild species provide a wide source of variation from which various types of canes with high biomass can be identified. To this end, the objective of this study was to characterise and identify high biomass genotypes for multiple uses from the local inter-specific derived germplasm collection. Sixty genotypes of different generations (wild, F1, BC1, BC2) were screened visually and on sucrose and fibre content from the population. They were evaluated in replicated trials with four commercial varieties used as controls. Traits of economic importance, particularly, sugar, fibre and different aboveground biomass yields were measured. Data on cane quality characters were taken at two sampling dates and characters were measured on both fresh and dry weights. The trials were followed up to the first ratoon crop.

The source data were validated and few genuine outliers observed were appropriately corrected. A total of 29 parametric traits were analysed individually in each crop cycle. Results showed good reliability of the trials with coefficient of variations within the acceptable limits and good repeatability (H^2) values for the majority of the traits. There was a good variation among genotypes allowing selection to operate effectively. Although precisions achievable were higher with dry weight measurements than their corresponding fresh weights, negligible differences were observed with selection simulations. It appeared that in the population of inter-specific derived clones, selection based on cane quality data collected at the pre-harvest season (April) was less efficient than those taken at early-harvest (July).

Multivariate data analyses efficiently summarised the data and identified groups of similar genotypes. Principal component analysis was very helpful in visualising the existing variations in the population. Six main clusters were obtained, of which three were of economic interest. Based on inherent variations in cane quality and biomass traits, four types of canes were defined for multiple uses. From Type 1 to Type 4 canes there was a continuous progress in fibre percent. The trait was negatively correlated to sucrose content and the high fibre canes were generally thinner and taller than the commercial controls. A selection algorithm was developed that identified 11 high potential genotypes simultaneously. Biomass yields of three genotypes exceeded those of the commercial controls by >40%. Fibre percent of one Type 4 cane reached 23% while that of the commercial varieties fluctuated at 13%. The results confirmed that high biomass varieties, with variable sucrose and fibre contents, could be obtained from the inter-specific populations. The different types of canes identified provided additional opportunities to exploit the total aboveground biomass of the crop for different end-uses, particularly for bioenergy production. The selection algorithm developed will be extended to the whole selection programme for classifying new sugarcane varieties.

CHAPTER 1

1 Introduction

1.1 *Sugarcane crop*

Sugarcane (*Saccharum* L. spp. hybrids) is an important tropical crop having C4 carbohydrate metabolism which, allied with its perennial nature, makes it one of the most productive cultivated plants. It is a large-stature grass that is cultivated primarily for its ability to partition carbon to sucrose in the stem in contrast with other cultivated grasses that usually accumulate their products in seeds. This unique feature was selected by man who first used its soft watery culm for chewing and subsequently, as the main plant source of sweetener for humans. Sugarcane is currently cultivated on more than 24 million hectares in tropical and subtropical regions of the world, producing up to 1.7 billion metric tonnes of crushable stems (see FAOSTAT: <http://faostat.fao.org/site/567/default.aspx>). It is mostly used to produce sugar, accounting for approximately 70% of the world's sugar supply.

Sugar production is not the only thing the plant does well. Together with certain of its tropical grass relatives, sugarcane is the finest living collector of sunlight known to man (Table 1.1). It is also considered as the most efficient species in the plant kingdom in terms of biomass production (Brumbley *et al.*, 2007). The crop is viewed as an ideal low-cost feedstock for renewable energy because it produces readily fermentable sugars and very high yields of green biomass. Industry and energy specialists now believe that in the very near future sugarcane fibre will attract high value due to the high degree of volatility in oil markets (driven by global supply-demand pressures) and potentially increased premiums given to renewable energy over fossil fuels. In addition, new technologies are emerging to convert cellulosic residues like sugarcane fibre and other agricultural by-products, such as sugarcane trash (dry and green leaves and plant tops left in the field during harvest), into valuable commodities that would be either degraded into small sugar molecules via enzymatic and/or physico-chemical processes to be fermented into ethanol, or used directly in power generation. These technologies are all in the scale-up phase and in the next few years will become commercial realities, changing the fate of cellulosic residues. If this occurs, then, some sugarcane varieties with high biomass yields and high fibre content, which in the past would have been discarded, could become very profitable.

Table 1.1: Examples of estimated solar energy capture efficiency (Klass, 2004)

Crop	Location	Conversion efficiency %
Switchgrass	Texas	0.22 – 0.56
Maize	Minnesota	0.79
Rice	New South Wales	1.04
Napier grass	Puerto Rico	2.78
Tropical forest	West Indies	1.55
Sugar cane	Hawaii; Java	2.24 and 2.59
Temperate grassland	New Zealand	1.02
Willow and Hybrid poplar	Minnesota	0.30 - 0.41

The sugar industry worldwide is thus at a crossroad as the traditional approach of sugar production is being reshaped by the biomass potential of the crop. Various sugarcane producing countries are showing interest in the creation of sugarcane varieties for multipurpose use. Sugarcane is furthermore being identified as a potential dedicated energy crop in regions where its cultivation is not a common practice. However, current varieties have not been optimised to achieve the required high biomass yield under a range of environments that will be necessary for an extensive production of biofuels. Therefore, the genetic improvement of the crop is essential to realize the national and international goals in the quest for environment friendly sources of energy.

1.2 Sugarcane in Mauritius

Mauritius is a tropical island about 890 km east of Madagascar in the Mascarene archipelago of the south-west Indian Ocean. Of volcanic origin, it has no mineral or oil reserves. It covers an area of 1840 km² and consists of a coastal plain rising gradually towards a central plateau bordered by mountain ranges. In summer (November to April) the climate is tropical whereas during the winter months it is sub-tropical. Temperatures range from 15^oC to 29^oC and rainfall is in the range of 900-5000 mm.

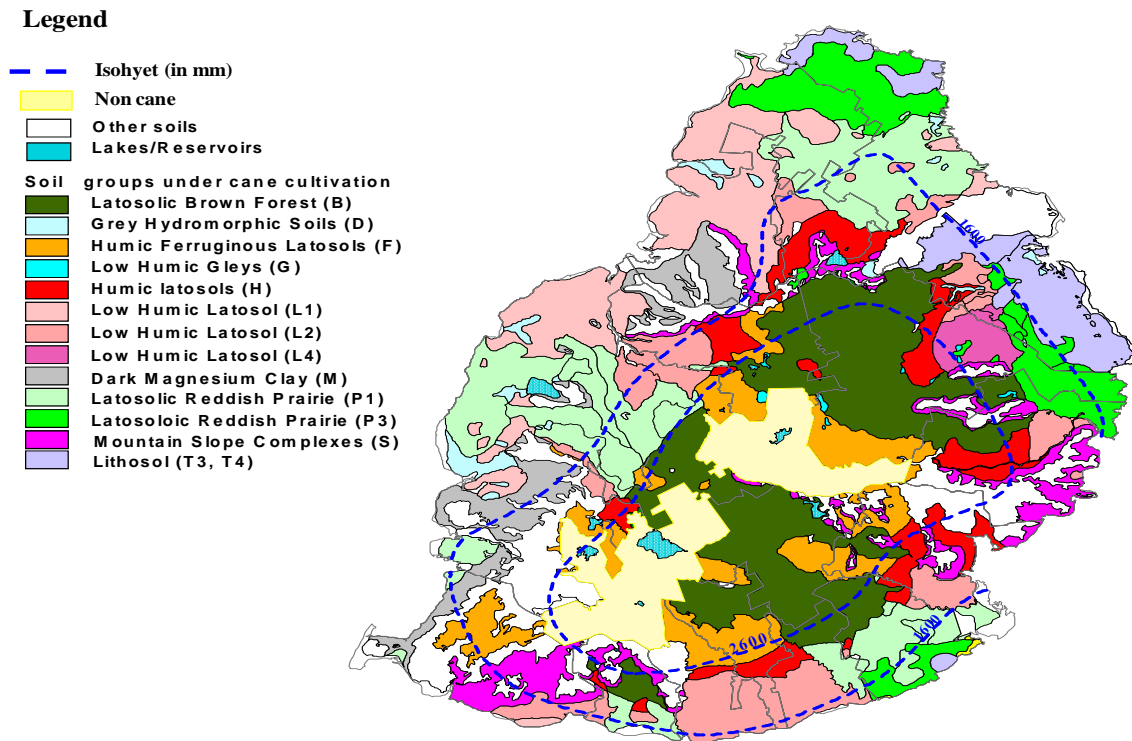


Figure 1.1: The soil map and agro-climatic zones of Mauritius (Parish and Feillafé – 1962)

There exists a mosaic of microclimates and soil types in Mauritius. There are three main agro-climatic zones: the super-humid central plateau (rainfall $>2500\text{mm}$), the humid or intermediate zone (rainfall $1500\text{-}2500\text{ mm}$) and the sub-humid regions (rainfall $< 1500\text{ mm}$) (Figure 1.1).

Sugarcane was introduced in the island in 1639 and was identified as the only major crop to resist the cyclonic conditions prevailing in the region. Currently, about 90% of the arable land and 35% of the total area of the island is devoted to growing sugarcane (CSO, 2008). Throughout its long life, the sugar industry has shaped the history and culture of the island. It has been the backbone of the Mauritian economy for decades. Today, although cane sugar accounts for merely 17% of the value of exports and 3% of the country's GDP (CSO, 2008), it remains a relatively sure agricultural investment due to long-established and consolidated vertical and horizontal integrations in the sector.

Mauritius forms part of the ACP (African-Caribbean-Pacific) developing countries that have benefited a preferential and guaranteed access to high prices in the European Union (EU) market under an agreed “Sugar Protocol”. The success of the sugar industry in these countries has contributed greatly to economic progress and the welfare of the nations in generating funds for investment in other economic activities. However, the Mauritius sugar industry and other ACP countries are now faced with new challenges which arise as a result of trade liberalisation world-wide, the EU sugar reform and the opening of the EU market to other non-ACP economies. The implementation of the new EU regime is having a deep impact on ACP suppliers due to the significant fall in revenue resulting from the drastic price cut, cumulating to 36% over a period of four years (2006-2009).

It is, therefore, imperative that the Mauritian sugar industry adapts to the new context and it is aiming to do so with the assistance of the government and through accompanying measures in the context of the EU support to the ACP sugar producing countries. The major concern is to urgently strengthen its competitive position at the international level by reducing the cost of production, increasing yield per unit area and maximising use of the crop biomass for the production of renewable bioenergy and other high value products.

The role of research, both strategic and applied, has been of paramount importance in the progress achieved so far in the sugar industry. Sugarcane improvement through breeding has been carried out for more than a century in Mauritius and is still the major thrust of research at the Mauritius Sugar Industry Research Institute (MSIRI). It is strongly believed that breeding and selection will be of continued fundamental importance in underpinning the future capacity of the sugar industry to meet the many challenges.

Well before the threat of the EU sugar reform, the island’s sugar industry had already intensified its effort in research and utilization of cane biomass for the generation of electricity and its export to the national grid (Baguant, 1984; Beeharry, 1996; Deepchand, 2000; Kong Win Chang *et al.*, 2001; Lau Ah Wing *et al.*, 2002). Efforts were also made towards the production of ethanol from cane sugar as a source of bio-fuel primarily for the export market. Since the early 1980s, the MSIRI had embarked on a genetic base-broadening programme that makes use of wild species to produce new parents and commercial varieties. In the mid to late 1980s, the programme also aimed at increasing the fibre content of varieties (MSIRI, 1985). In 2007, the MSIRI released a new variety, M1672/90, that can produce 15-25% more fibre than current ones without jeopardizing the sugar yield (MSIRI, 2008).

Progeny populations derived from crosses between sugarcane (*S. officinarum* or commercial cultivars) and diverse sources of related wild species provide a wide source of variation from which various

types of canes with high biomass can be identified. To this end, the MSIRI breeding programme is widening its scope to exploit sugarcane biomass through an expansion of the inter-specific crosses. The MSIRI has a germplasm collection of over 2000 clones that are used as parents in the sugarcane hybridisation programme. The collection includes locally bred and imported hybrids and wild relatives. Recently, 40 wild (*Saccharum spontaneum*) clones, 10 multipurpose hybrids with high fibre and eight high quality parent varieties with exceptionally high sucrose content were imported from West Indies Cane Breeding Station (CBS). This made up to a total of 106 wild clones in the local collection.

1.3 Objectives of study

The main objective of this study was to identify and characterise high biomass cultivars for multi-purpose use from the inter-specific derived parental germplasm collection. Sixty potentially high biomass genotypes were evaluated in replicated trials over two consecutive years (plant cane and first ratoon) and the variation in sugar and fibre contents, cane yield and other related traits were studied. The main objectives of the study were to:

- Investigate the variation in patterns of sucrose and fibre contents in sugarcane crop of different generations
- Study the correlations between various characters related to high fibre and biomass traits
- Devise a methodology for multivariate data analysis that can be relevant in selection for both high sugar and high fibre yielding varieties
- Devise selection methods and criteria, which could be reliably used in the sugarcane selection programme to screen different types of canes for multipurpose use, and
- Determine the most appropriate traits and time for data collection with respect to both sucrose and fibre accumulation.

CHAPTER 2

2 Literature review

2.1 *Sugarcane anatomy and composition*

2.1.1 Sugarcane anatomy

Figure 2.1 illustrates four distinct fractions of sugarcane biomass. The percentages in brackets represent the dry weight proportions reported by Van Dillewijn (1952) on a 12 month old crop in Hawaii.

- a) The *stubble* (4.5%) and underground *roots* (12.7%)
- b) The cane stalk free of tops and leaves is the *millable cane or stem* (49.2%) processed for sugar.
- c) The green immature *cane tops and leaves*(CTL for ease of reference) (9%) removed from the cane during harvest, and
- d) The dead and dry leaves known as *trash* (24.6%) or cane straw consisting of both attached and detached dry leaves

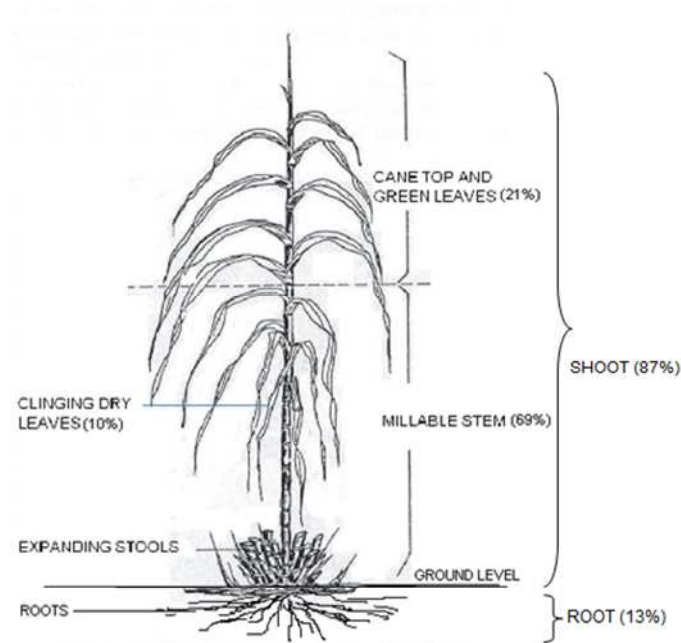


Figure 2.1: Schematic illustration of a sugarcane crop

More recently, under the local context, Beeharry *et al.* (1996) reported that the millable canes of commercial varieties accounted for around 69% on a fresh weight basis, the CTL accounted for another 21% and the trash accounted for around 10% of the total aboveground biomass. The

vegetative composition of cane plant is not uniform, but varies according to age, fertilisation, variety etc. The effect of age has been found dominant (Van Dillewijn, 1952).

Commercially, sugarcane is propagated vegetatively via stem cuttings. Germination of the lateral buds produces new plants that branch into stools consisting of a large number of tillers. Under good growth conditions, the plant will grow 4–5 meters in 12 months, with the extractable culms measuring 2–3 meters and containing 13–16% sucrose. Because it is a perennial crop, after harvest and under the right growing conditions, underground buds will sprout giving rise to a new crop. In most situations, four to eight crops are harvested before the yields become economically unsustainable and the field is renewed with the planting of a new crop.

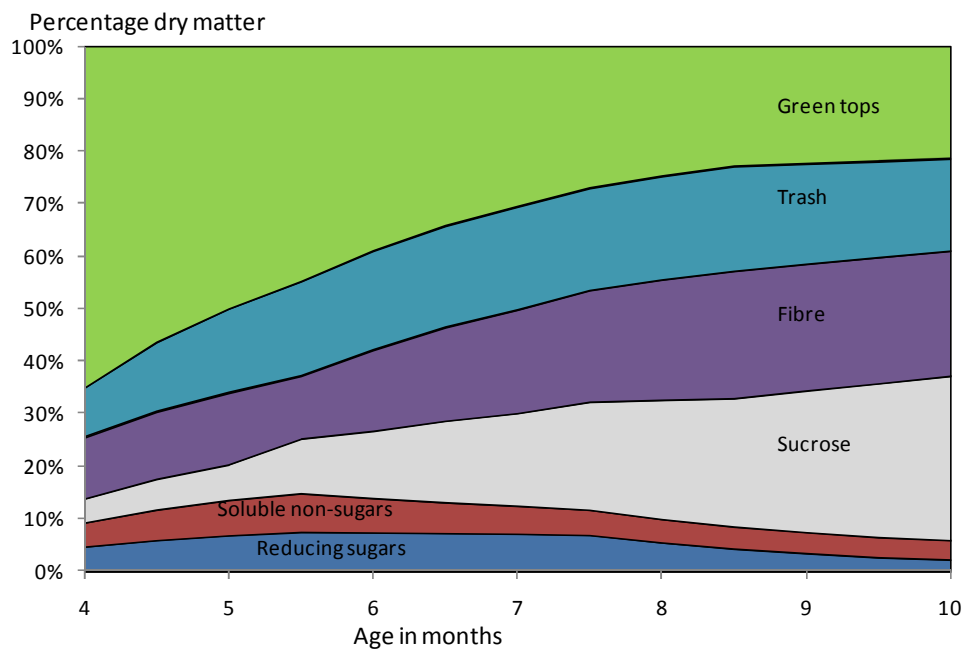


Figure 2.2: Evolution of percentage dry-matter composition of sugarcane across time (adapted from van Dillewijn, 1952)

Figure 2.2 broadly illustrates the evolution of dry matter proportion of the above ground biomass across the growth phase of the crop. The development of an adequate production apparatus in the form of leaves and roots is a necessary requisite for the formation of millable cane. This implies that during the early stages of its development, a cane plant consists largely of roots and leaves, the amount of millable cane being practically nil. According to van Dillewijn (1952), the dry weight of the green top remains more or less constant during the entire growing period of the plant, while the root system increases gradually but slightly. The growth of the latter as compared with that of the

whole plant is so small that in many cases it may be disregarded. Once the production apparatus has developed to a certain extent, the formation of millable stalks starts. It soon reaches a considerable rate which, with the exception of seasonal fluctuations, is maintained throughout a great part of the growing period. The formation of trash is closely related with cane formation, since the production of each node in the stem is associated with the formation of a leaf.

Generally, only the clean millable stem is cut and sent to the mill. The roots and the stubbles are left behind in the soil for regrowth. The CTL remain on the field or are used as livestock feed, either directly or in the form ensilage. In Mauritius, with mechanised harvest, they are also used as trash blanketing that controls weed growth and avoids evaporation of moisture content from the soil.

2.1.2 Cane stalk composition

A cross section of sugarcane stalk (Figure 2.3) shows two distinct fractions: the outer rind consisting of the epidermis and underlying tough, thick walled sclerenchyma cells, altogether termed as '**true fibre**' (Paturau, 1989), and the inner pith fraction largely consisting of thin-walled parenchyma cells (storage cells) and vascular bundles interspersed throughout the stalk. The vascular bundles are accompanied by adjacent sclerenchyma cells.

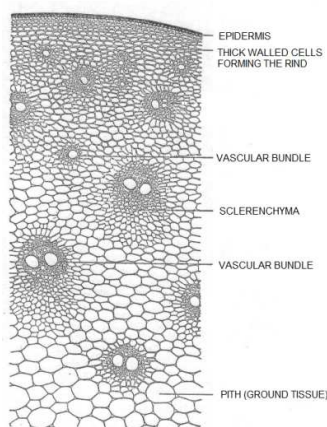


Figure 2.3: Cross section of a cane stem (van Dillewijn, 1952)

Mature trash-free cane stalks are generally composed of approximately 75% water (Table 2.1) and the remainder is divided between fibre and soluble solids. Commercial varieties in Mauritius have been found to be composed of about 13.0% sucrose and another 13.0% fibre in the cane stem (Paturau, 1989). The amount of each of these three components (water, fibre and soluble solids) is genetically determined and varietal differences are well known (Irvine, 1977).

The soluble solids comprise 75-92% sugars. *Sucrose* amounts to 70-88%, *glucose* (dextrose) 2-4% and *fructose* (laevulose) 2-4%. Other constituents of the juice, in order of abundance, are minerals, waxes, fats and phosphatides, and miscellaneous minor constituents.

Table 2.1: Composition of sugarcane and juice solids (Meade and Chen, 1977)

Millable cane	Cane (%)
Water	73-76
Solids	24-27
Soluble solids (Brix)	10-16
Fibre (dry)	11-16
Juice constituents	Soluble solids (%)
Sugars	75-92
Sucrose	70-88
Glucose	2-4
Fructose	2-4
Salts	3-4.5
Organic acids	1.5-5.5
Other organic non-sugars	
Protein	0.5-0.6
Starch	0.001-0.050
Gums	0.30-0.60
Waxes, fats, phosphatides	0.05-0.15
Other	3.0 – 5.0

2.1.3 Physiology of sucrose accumulation - a brief review

The sugarcane crop cycle has been reported to comprise distinct vegetative (tillering and elongation), ripening (sucrose accumulation) and senescence phases (Soopramanien, 1979). Figure 2.4 depicts the changes in sucrose concentration over the harvesting period in an early ripening commercial variety (S17). During the vegetative phase, dry matter is partitioned in favour of fibre and reducing sugars (glucose and fructose) as opposed to sucrose (Alexander, 1973). Under unfavourable conditions for growth, around 80% of the biomass fixed is deposited as sucrose in mature internodes (Glasziou and Bull, 1967; Soopramanien, 1979). A marked reduction in reducing sugars accompanies this ripening process (Julien and Delaveau, 1977; Mamet, 1992). After peak maturation, in general, very few new leaves are formed whilst older leaves senesce and sucrose storage slows down. The plant uses stored sucrose for maintenance and hence the sucrose concentration declines (Mamet, 1992). The effect is known to vary with normal non-flowering stalks, flowering stalks, flowering stalks that form side shoots and those that do not form side shoots (Van Dillewijn, 1952).

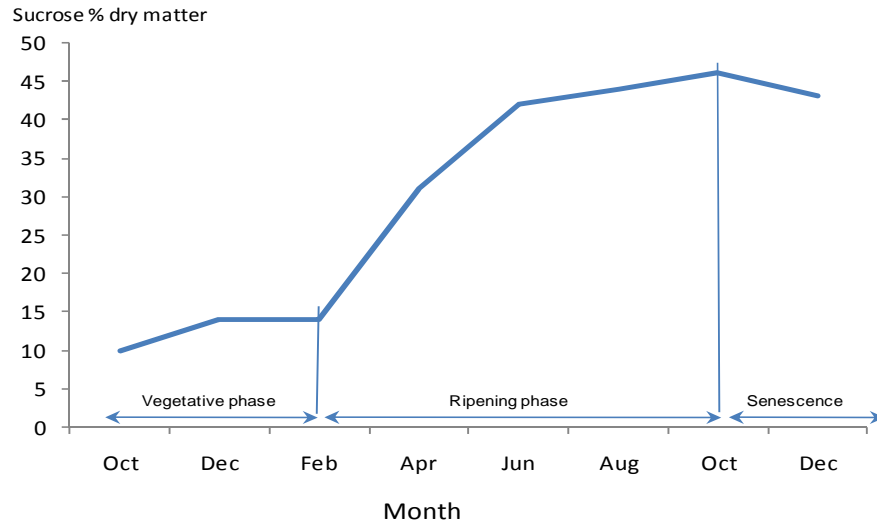


Figure 2.4: Changes in sucrose percent dry matter over the harvesting period in the early ripening commercial variety, S17 (Soopramanien, 1979)

Various studies done under the Mauritian context on sucrose accumulation pattern have shown that optimum ripening is influenced by climate, planting date, time of harvest and variety (Julien, 1974; Julien and Soopramanien, 1976; Julien and Delaveau, 1977; Mamet, 1992; Soopramanien and Julien, 1980). The ripening phase is considered to start with the onset of winter, about the month of May. The sugarcane harvest season extends from mid-June to November, with peak sucrose contents in most varieties being reached around the months of September and October. Different varieties mature at different periods within the harvesting season. Commercial varieties have thus been categorised in three major groups, the early-maturing, late-maturing and high-sucrose types. Recent studies have identified a fourth category, the very early type of varieties that start accumulating sucrose as from March (Nayamuth *et al.*, 2005).

2.2 *Sugarcane Taxonomy*

Sugarcane (*Saccharum* spp., $2n=100-130$) belongs to the *Andropogonae* tribe, which encompasses only polyploid species, and to the subtribe *Saccharinae* (Daniels and Roach, 1987). Current commercial cultivars are highly polyploid and aneuploid, with about 120 chromosomes. Sugarcane scientists have adopted the term ‘*Saccharum* complex’, originally coined by Mukherjee (1957), to describe a subset of genera within *Saccharinae* closely enough related to *Saccharum* to have contributed to its genetic background. Genera within the *Saccharum* complex include *Erianthus*, *Miscanthus*, *Narenga*, *Saccharum* and *Sclerostachya* (Amalraj and Balasundaram, 2005).

2.2.1 The *Saccharum* species

Six species have traditionally been included in the *Saccharum* genus by sugarcane geneticists:

- *S. officinarum* ($x = 10$, $2n = 80$; sweet chewing cane found in native gardens in New Guinea and other South Pacific islands)
- *S. spontaneum* ($x = 8$, $2n = 40-128$, wild cane found throughout Asia)
- *S. robustum* ($x = 10$, $2n = 60, 80$; putative ancestor of *S. officinarum* found most commonly on river banks in the same region)
- *S. edule* ($2n = 60-122$, produces aborted tassels, a delicacy in the same region)
- *S. barberi* ($2n = 116-120$, semi-sweet Indian cane)
- *S. sinense* ($2n = 81-124$, semi-sweet Chinese cane)

Of these, *S. edule*, *S. barberi*, and *S. sinense* are likely of natural inter-specific and/or inter-generic origin and should probably be relegated to horticultural group status (D'Hont *et al.*, 2002; Daniels and Roach, 1987). Irvine (1999) proposed further reducing the number of *Saccharum* species to two, namely *S. spontaneum* and *S. officinarum*, the latter encompassing all remaining species and inter-specific hybrids.

- *S. officinarum*

S. officinarum, probably originating from New Guinea, is also known as ‘noble’ cane. It is a group of thick, juicy canes that were initially cultivated in South East Asia and the Pacific islands before spreading over the inter-tropics between 1500 and 1000 BC (Plate 1.1). The clones accumulate very high levels of sugar in the stem but have poor vigour and disease resistance. A practical description of the species is that it possesses often colourful large-diameter stalks, broad leaves, short internodes, high sugar content, low fibre content, and is



Plate 1.1: Noble cane *S. officinarum*

relatively intolerant to the more sub-tropical environments where sugarcane is commercially grown, especially those where freezes can occur (Tew and Cobill, 2008).

– *S. spontaneum*

S. spontaneum is characterised by thin stalks with no or very little sugar and has a huge geographic distribution. It is far more genetically diverse than *S. officinarum*, and is highly polymorphic. Genotypes vary from short, grassy-appearing narrow-leafed types with no stalks, to large-stature types over 5 m in height and 3 cm in stalk diameter. *S. spontaneum* is highly adaptable and able to survive a wide range of abiotic stresses, including droughts, floods, saline conditions, and freezing temperatures (Mukherjee, 1950). *S. spontaneum* is regarded as wild cane with high fibre and low sugar levels. Because of its aggressive rhizomatous habit and its ability to propagate via seed dispersal, it is regarded as a noxious weed.



Plate 1.2: Wild cane *S. spontaneum*

– *S. robustum*

S. robustum is characterised by long, thick and woody stalks with little or no sugar and has been reported as occurring in natural populations in the Indonesian islands in New Guinea. It is probably the closest wild relative of *S. officinarum* in morphology and geographical distribution. The species is believed to have contributed towards the production of some Hawaiian and Canal Point varieties.



Plate 1.3: Wild cane *S. robustum*

– *Other Saccharum species*

S. edule is grown in subsistence gardens from New Guinea to Fiji for its edible, aborted inflorescence; its large, thick stalked canes contain no sugar. Some sparse molecular data support the hypothesis that *S. edule* corresponds to a series of mutant clones, which were identified in *S. robustum* populations and were preserved by humans (D'Hont *et al.*, 2008). The authors further confirm that *S. barberi* and *S. sinense* have hybrid origins and are the results of inter-specific hybridisations between representatives of two genetic groups of the *Saccharum* genus, *S. spontaneum* on the one side and *S. officinarum* or *S. robustum* on the other. Since the *S. barberi* and *S. sinense* have sweet stalks and the regions where they were formerly cultivated is outside the natural distribution range of *S. robustum*, the scenario of Brandes (1956) provides the simplest explanation for their origins: *S. officinarum*

cultivars were probably transported by humans to mainland Asia, where they naturally crossed with local *S. spontaneum* giving rise to *S. barberi* and *S. sinense* in India and China, respectively.

2.2.2 The related genera

As indicated above, the *Saccharum* complex includes other genera that are expected to be sexually compatible at some levels (Daniels and Roach, 1987). The genera *Erianthus* and *Miscanthus* have attracted the attention of sugarcane breeders since the beginning of the 20th century because of desirable characteristics as described below.

The genus *Erianthus* ($2n = 20-60$) is distributed in India, South-East Asia to Japan, Indonesia and New Guinea (Daniels and Roach, 1987). Seven species are described. Clones of *Erianthus* are highly vigorous, tall with slender stalks of good diameter and display disease resistance, excellent ratooning ability and tolerance to both drought and water-logging (BSES, 1990; Cai *et al.*, 2005).



Plate 1.4: *Erianthus* sp.

The genus *Miscanthus* ($2n = 38-76$), is distributed from Tahiti through Eastern Indonesia, Indo-China to northern China, Siberia and Japan. The species vary from small wiry-leaved types to taller ones, occurring from sea levels in Indonesia to 3300 m in Taiwan (Berding and Koike, 1980; Lo *et al.*, 1978). Its main desirable feature is its superior overwintering ability in temperate climates along with its high biomass yield.



Plate 1.5: *Miscanthus* sp.

2.2.3 Contribution of molecular genetics to sugarcane evolution

The taxonomy of the sugarcane complex, based on morphology, chromosome numbers, and geographical distribution, has been controversial since the original classification of *S. officinarum* by Linnaeus in 1753 (Daniels and Roach, 1987; Irvine, 1999). Recent molecular data are beginning to help trace the domestication and early evolution of sugarcane. These data support the view that the genus *Saccharum* is a well-defined lineage that has diverged over a long period of evolution from the lineages to the *Erianthus* and *Miscanthus* genera (Grivet *et al.*, 2006) (Figure 2.5).

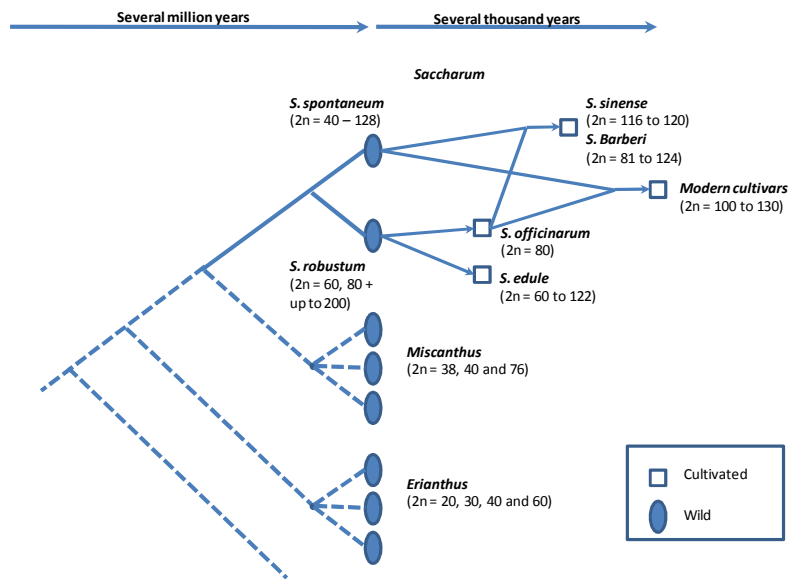


Figure 2.5: Scenario compatible with molecular data for sugarcane evolution and domestication (Adapted from Grivet *et al.* 2006)

According to Grivet *et al.* (2006), and supported by D'Hont *et al.* (2008), cultivated sugarcanes probably emerged from wild *Saccharum* species, and secondary introgressions with other genera were not likely pathways. The authors, however, believed that this did not mean that natural inter-generic hybridisations were impossible and might not account for some local peculiarities. Artificial inter-generic hybrids with these genera have been produced (D'Hont *et al.*, 1995; Piperidis *et al.*, 2000). With the advent of molecular genomics, the sugarcane genome has thus become less mysterious, although its complexity has been confirmed in many aspects. Shortcuts to genomic analyses have been identified thanks to synteny conservation with other grasses, in particular sorghum and rice. Over time, new tools have become available for understanding the molecular bases behind sugarcane productivity and a renewed interest has surfaced in its genetics and physiology (D'Hont *et al.*, 2008).

2.3 Sugarcane improvement through breeding

Improvement of sugarcane for increased sugar yield through classical hybridisation and selection has been a directed, ongoing process since 1888, following the observation in 1858 that sugarcane produced viable seed (Stevenson, 1965). Until the early 20th century, cultivated sugarcane varieties in most parts of the world consisted mainly of *S. officinarum* clones (plate 1.1), collected from Papua New Guinea and Indonesia.

2.3.1 Nobilization

In the early 20th century, breeders in India and Indonesia initiated programs that utilized inter-specific hybrids derived from crosses between *S. officinarum* and *S. spontaneum* (Daniels and Roach, 1987). The initial inter-specific hybrids were crossed back to *S. officinarum* clones or other hybrids to retain sufficiently high sugar content, in a process that was termed “**nobilization**” by sugarcane breeders (Bremer, 1961). The objective was mainly to dilute the side effects of the wild clones while trying to develop disease resistant varieties. These hybridizations not only solved many of the disease problems but they also provided spectacular increases in yield, improved ratooning ability, and adaptability for growth under various abiotic stresses (Roach, 1972).

Nobilization thus refers to the crossing of the wild canes to the noble cane *S. officinarum* (or commercial hybrids), and further backcrossing of progenies to the latter (Stevenson, 1965), and includes the planned introgression of the other *Saccharum* species and related genera into the noble cane (Figure 2.6).

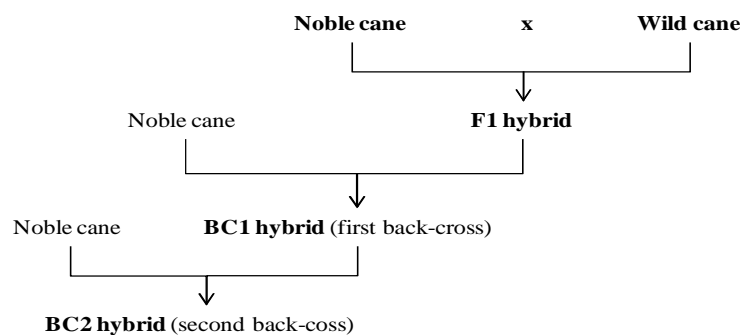


Figure 2.6: Genetic base-broadening through “nobilization”. The noble canes include the *S. officinarum* spp. or, commercial hybrids with high sucrose content.

2.3.2 Current sugarcane cultivars

Inter-specific hybrid varieties, termed as “wonder canes” (POJ 2364, POJ 2878, Co 206, Co 213), that resulted from early breeding activities, formed the genetic foundation of modern sugarcane varieties. All present-day cultivars (*Saccharum spp.*; $2n = 100-130$) are genetically complex and are derived from the interbreeding of these first inter-specific hybrids. Altogether, it is estimated that 19 *S. officinarum* clones (four with high frequency), a few *S. spontaneum* (two with high frequency) clones, and one *S. barberi* clone were involved in these inter-specific crosses (Arceneaux, 1967).

As stated by Tew and Cobill (2008), while most of the genomic composition of sugarcane is from *S. officinarum* (D'Hont *et al.*, 1996) most of the genetic diversity is thought to be contributed by *S. spontaneum*, since it is by far the more genetically diverse of the two species (Lima *et al.*, 2002). The use of wild species of sugarcane, *S. spontaneum*, can be cited as a classical example of the success in inter-specific breeding, having contributed to spectacular increases in cane and sugar productivity world-wide and in contributing genes for resistance to biotic and abiotic stresses (Ramdoyal and Badaloo, 2002; Roach and Daniels, 1987). Still, the narrow genetic base of modern sugarcane cultivars is recognised, and efforts to broaden it through continuous inter-specific crosses are considered vital in many sugarcane breeding programmes (Arceneaux, 1965; Ramdoyal and Badaloo, 2002; Roach, 1989).

Inter-generic hybridization has also been tried as a means to broaden the genetic base, to obtain commercially useful characteristics and to increase hybrid vigour. Although many attempts to cross between the inter-generic species may have been made in sugarcane research stations, limited publications are available. Two genera, namely *Erianthus* and *Miscanthus*, have received considerable attention of plant breeders. Among the *Erianthus* genus, *E. arundinaceus* has been of greatest interest because of its large stature, excellent ratoon yields, deep and extensive root system, tolerance to drought and floods, and resistance to diseases of importance in sugarcane. The genus *Miscanthus* has been attractive because of its superior overwintering ability in temperate climates and as an energy cane (Tew and Cobill, 2008). In addition, downy mildew (*Peronosclerospora sacchari*) resistance genes have been reported to be successfully transferred from *Miscanthus* to sugarcane (Chen and Lo, 1989).

2.3.3 Appraisal of the current introgression breeding programmes

Despite all the promises introgression breeding may hold, in general, it is difficult to estimate its impact or success in recent decades. It has also been noted that much effort has not led to commensurate commercial successes (Berding and Roach, 1987; Stalker, 1980). According to Wang

et al. (2008) the process of introgression in sugarcane breeding is therefore traditionally a long-term and risky investment. The time and risk factors have clearly acted to reduce the level of resources devoted in most sugarcane breeding programmes to introgression breeding despite general agreement among sugarcane breeders of its potential value. Much emphasis is laid on crosses that include *S. officinarum* hybrid parents with potentially high breeding values and appreciable agronomic characteristics.

2.4 An outline of sugarcane genetics

2.4.1 Sugarcane cytogenetics

As mentioned above, commercial sugarcane varieties are complex inter-specific hybrids originally bred by a process of nobilization. Generally, when *S. officinarum* (noble cane) is crossed with *S. spontaneum* (wild cane) the noble female parent contributes the somatic chromosome complement whilst the wild parent contributes the usual gametic complement, resulting in progeny with $2n + n$ chromosomes. The same modality of transmission occurs for the BC1 generation when the noble clone is used as the recurrent parent (Bremer, 1925; Price, 1957; Price, 1961). In later backcrosses and intercrosses, normal $n + n$ inheritance is restored and meiosis is essentially regular. However, *S. officinarum* transmits the gametic chromosome number when intercrossed with other noble varieties, when selfed or when crossed with *S. robustum* (Stevenson, 1965).

2.4.2 Quantitative genetics of sugarcane

In contrast to other crops, statistical techniques of biometrical genetic analysis have been applied to a limited extent in the study of variation of quantitative traits in sugarcane (Badaloo, 1997; Hogarth, 1987; Lawrence and Sunil, 1997; Lawrence *et al.*, 1997). This is partly explained by the complex inter-specific origin and the peculiar aspects of sugarcane from the cytological viewpoint. In addition, owing to other characteristics of sugarcane, such as cross fertilisation, heterozygosity, self sterility of some varieties, incompatibility of some varieties when crossed, male sterility and low pollen viability of many varieties, many biometrical designs of quantitative inheritance are not suitable for sugarcane (Badaloo, 1997). Hogarth (1968) reviewed the application of quantitative genetics theory to sugarcane breeding and concluded that diallel crosses were impractical because of incompatibility and male sterility. Instead, he found the factorial mating design (m males crossed with each of n females) and Burton and De Vane's (1953) method more applicable to sugarcane.

2.5 Sugarcane breeding and selection programme at the MSIRI

Sugarcane breeding programmes typically commence by the crossing of heterozygous parents to produce true seeds. Seedlings so derived are planted in nurseries and/or transplanted directly in the field for screening. Thereon the clones are propagated vegetatively through stem cuttings and evaluated over larger plots in successive selection stages, their numbers being reduced at each stage. At the early selection stages, genotypes are tested in unreplicated trials essentially due to the presence of a large number of clones in the population and a lack of planting materials to establish replicated trials. True multi-location and multi-year evaluations begin in variety trials after a few years of screening of the test genotypes and the reduction of the number of clones into a manageable population.

The production and testing of new sugarcane varieties range between 8 and 20 years (Skinner *et al.*, 1987). Numerous combinations of selection rates, criteria, plot sizes and trial designs exist. As sugarcane is a perennial crop, ratooning ability needs to be tested. Typically, four to eight ratoons are grown commercially but this varies in different countries. Usually, testing for ratooning ability is done over two to three ratoons only, and the effects of ratoons and years are generally completely confounded.

2.5.1 MSIRI hybridisation programme

At the MSIRI, about 2000 crosses representing 450 to 500 different genetic combinations are made each year with a view to produce sugarcane varieties that meet the requirements of growers and benefit the sugar industry at large. The choice of individuals to be used in crossing depends largely on two main criteria: (a) the agronomic characteristics and morphological traits of the parents, their reactions to five major sugarcane diseases prevailing in the island and their flowering behaviour, and (b) the breeding performance of the parents as revealed by progeny tests of previous crosses (Ramdoyal and Domaingue, 1994; Ramdoyal *et al.*, 1999).

Furthermore, in the MSIRI breeding programme, the proven variety mating system makes extensive use of elite varieties, which are the commercial varieties and promising clones in the final phase variety trials. In addition, as knowledge about the mode of inheritance of important traits becomes available, there is a parallel influence on parental selection in the local breeding programme (Aljanabi *et al.*, 2007; Badaloo, 1997; Bissessur, 1997; Domaingue *et al.*, 1988; Mamet *et al.*, 1996; Ramdoyal and Badaloo, 2002; Ramdoyal *et al.*, 2000). Since 1995, the programme has recourse to cross prediction methodologies for major agronomic characters through the systematic evaluation of sugarcane families in replicated trials at the seedlings stage.

For some specific characters like sucrose content, genotypes from crosses are recycled to the parental gene pool as early as possible (stage 3), thus reducing the generation interval. In addition, basic wild and noble germplasm are characterised for major traits to provide a sound basis for their utilisation in genetic base-broadening programme and the introgression of specific characters in commercial hybrids (Badaloo *et al.*, 1998). Introgression breeding generally constitutes 10% of the annual crossing programme.

2.5.2 MSIRI selection programme

Some 66 000 seedlings, produced annually, enter the selection programme that spans over 11-15 years. Genotypes are screened over six successive selection stages (Figure 2.7). The early stages comprise the seedling stage (stage 1), the first and the second clonal stages (stages 2 and 3 respectively) where genotypes are tested in unreplicated trials in the plant cane crop. Selected varieties from stage 3 are planted in three successive replicated selection trials (stage 4, T1 and T2 trials) where more precise evaluations are made in several environments. There is a progressive reduction in the number of genotypes assessed and a concomitant increase in plot size at each selection stage.

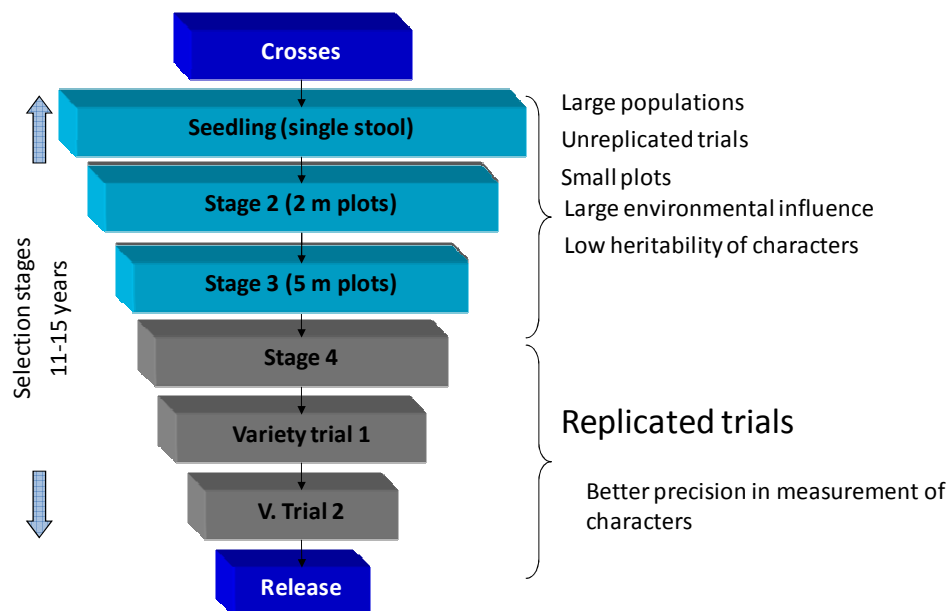


Figure 2.7: Sugarcane selection flowchart at the MSIRI

Genotypes planted in advanced selection trials are evaluated and characterised for 22 different traits. The genetic improvement of sugarcane is geared towards the development of new varieties with high cane yields, high sucrose content, resistance to the major diseases and pests, adapted to the various agro-climatic zones of the island, and suitability for harvesting at different periods of the milling season. Varieties should also demonstrate good ratooning capacity, are suitable for mechanized harvest, and also have suitable morphological attributes, high population density, good germination potential and efficient canopy cover. However, selection for a large number of characters is known to be inefficient and progress is often seriously limited. The gain from selection is often smaller than expected and frequently some characters included in the selection scheme show no measurable improvement (Skinner *et al.*, 1987). Moreover, with continuous improvement in sucrose content and sugar yield through breeding, the ceiling gets higher and more difficult to surpass with each new variety released to the planting community. In consequence, any new candidate with more or less equal performance to the existing commercial varieties and with an added value (e.g. self trashing, good ground cover, erect cane, specific adaptation) is of considerable commercial interest.

New genotypes are normally compared to commercial controls either in relative units of measurements or on a relative basis. In the MSIRI selection programme, control varieties are used at all clonal selection stages. The conventional design used in unreplicated sugarcane selection trials is based on a systematic arrangement of control varieties after every 5-6 rows of test genotypes. Recently, new Augmented Latin Square designs, as described by Lin and Poushinsky (1983), have been adopted to allow genotype yields to be adjusted for field variation in two dimensions while using much less proportion of the area as check plots (Ramdoyal and Santchurn, 2009). The new design also allows simultaneous comparison of test genotypes with more than one control variety.

Randomised block or lattice designs are used for the replicated selection trials (stage 4, variety trials 1 and 2). The mean of 4-6 commercial controls, with variable ripening behaviour, is used as the basis for comparison. This approach is in agreement with Simmonds (1979) who suggested that it was more efficient to use a range of commercial check varieties in replicated trials rather than depend on a single standard. This supported the conclusions of Pollock (1975), who found that standard varieties varied in stability, and that it was more efficient to select new varieties in comparison with the average of three standards. Similarly, Julien *et al.* (1983) stated that it was preferable to use a group of controls with different maturity characteristics rather than a single control variety. Use of a range of standards can be particularly important if a new disease adversely affect the performance of one standard (Skinner *et al.*, 1987).

2.5.3 Contribution of research in plant breeding to sugar production

In Mauritius the genetic improvement of sugarcane dates back to 1891, following the successful production of seedlings in Java 1888. With the establishment of the 'Station Agronomique' in 1893, a structured approach to breeding of new varieties was adopted leading to the selection of a series of varieties from an intra-noble crossing programme. The Department of Agriculture created in 1913 introduced a large number of sugarcane varieties from various countries including the 'wonder cane', POJ 2878, imported from Java, Indonesia, which became an important parent of many commercial varieties bred in Mauritius. An inter-specific programme involving the noble species, *S. officinarum*, and the wild *S. spontaneum* culminated in the development of the famous variety *M 134/32*, which occupied 92% of the area under cane in 1952 (Figure 2.8).

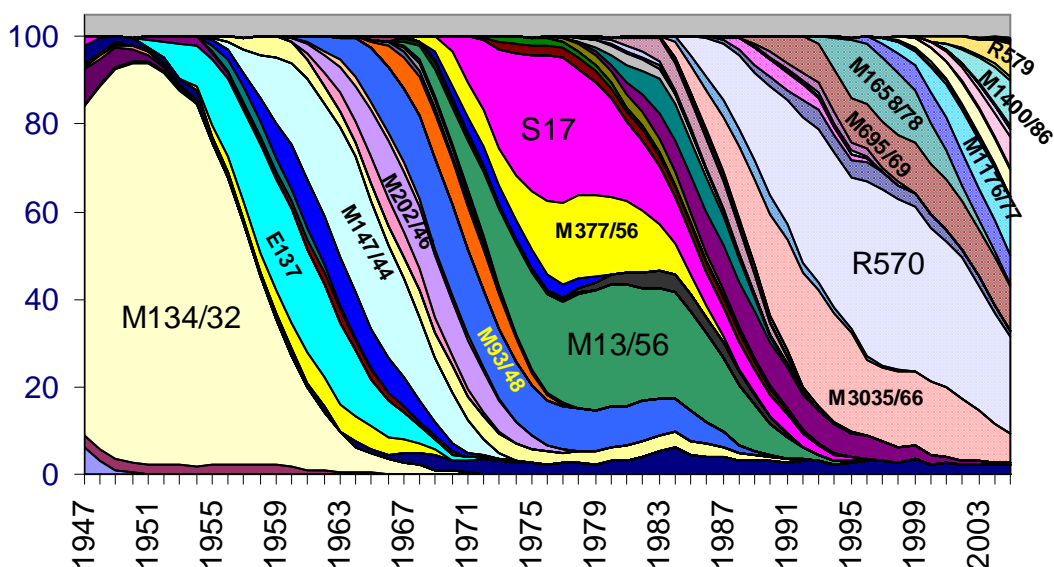


Figure 2.8: Evolution of varieties cultivated (%) in Mauritius: 1947 – 2005

Source: MSIRI annual reports 1947-2008.

Sugarcane breeding was further strengthened with the creation of the MSIRI in 1953, which became the sole organization entrusted with this activity. Since then, the MSIRI has released 66 varieties, equivalent to a rate of 1.2 varieties per year, of which 54 were developed from crosses made locally and 12 were introduced from other breeding stations and tested in MSIRI trials. The yields of cane and sugar have progressed with a rough increase of 200 kg cane and 44 kg sugar per hectare per year respectively over the last 60 years, excluding severe drought and cyclonic years (Figure 2.9). New varieties have certainly been instrumental in enhancing the sugar productivity per unit area.

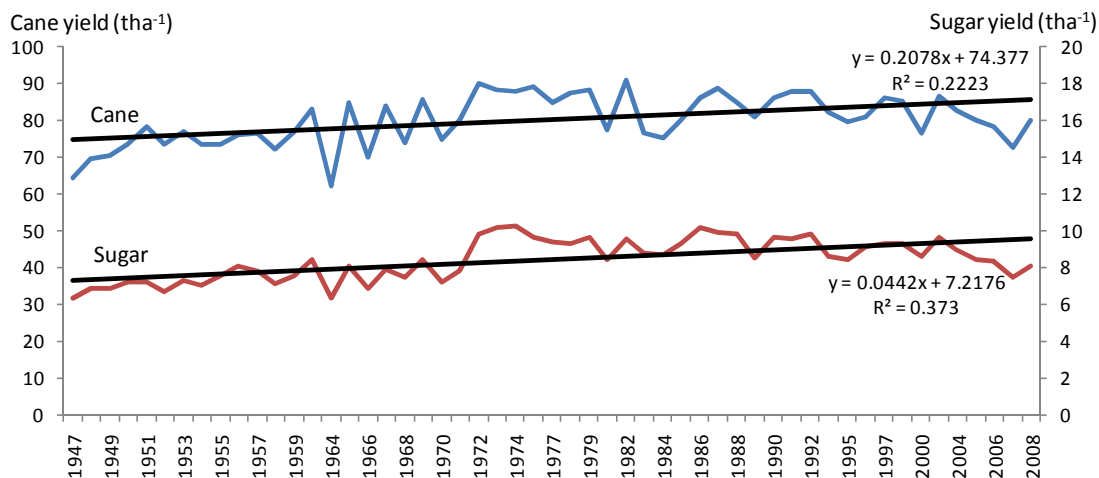


Figure 2.9: Cane and sugar yield (tha^{-1}) trends between 1947 and 2008 in miler planters land.

Source: MSIRI annual reports 1947-2008.

Most of the varieties released show specific adaptation to different soil types and the climatic conditions prevailing in Mauritius. They also show peak sucrose accumulation at specific period of the harvest season. The final decision for a large-scale cultivation of new varieties rests on farmers' own evaluation and appreciation. Assuming that a new cultivar exploited over more than 5% of the total area under sugarcane represents a successful adoption, then broadly one out of two varieties (27 in all) released by the MSIRI have largely contributed to the increased productivity. However, new varieties take at least 5-6 years to reach a considerable proportion. This is so because, in Mauritius, sugarcane is planted once and harvested over 8-9 consecutive years (plant cane crop and 7-8 ratoon crops). Hence, replanting is done in roughly 10% of the total sugarcane fields annually. As a consequence, the contribution of the recently released varieties is not accounted in the estimations.

Over the last two decades, two by-products have gained sizeable importance: *Bagasse* as a source of environment-friendly cane residue for the generation of electricity and *Molasses* for the production of ethanol as a gasoline mix in the transport sector.

Bagasse, also termed as ‘bagasse proper’, is the fibrous material left after juice extraction from milled cane stalks. It is composed of:

Moisture	: 46-52%	(av. 50.0%)
Fibre	: 43-52%	(av. 47.7%)
Soluble solids (mostly sugar)	: 2-6 %	(av. 2.3%)

Source: (Paturau, 1989)

The composition, however, varies according to the variety of cane, its maturity, the method of harvesting and finally the efficiency of the milling plants (Paturau, 1989). Bagasse represents about 21% of aboveground biomass. With 50% moisture, it is found to have a gross calorific value of 9.7-9.9 MJ/kg (Beeharry, 1996; Deepchand, 2000; Lau Ah Wing, 2008).

Molasses is the viscous residue (slurry) left after sugar crystals are centrifuged out. It represents around 2% of aboveground sugarcane biomass and can be relatively easily fermented into ethanol and other high-value products. It is also used for animal feed and the production of potable alcohol. Enzymatic hydrolysis of bagasse and trash followed by fermentation is another method to produce cellulosic ethanol, but appears to be a longer term solution under the local context.

2.6.2 Energy potential of sugarcane aboveground biomass

Assuming a ratio of 70:20:10 for cane stalk:CTL:trash (see section 2.1.1), then, for every 1000 kg of cane sent to the factory, the following products and calorific values can be obtained with existing commercial varieties:

Table 2.2: Estimated yields of biomass components and energy obtainable from 1000 kg of cane harvested

	Estimated % to total biomass	Yield (kg)	Bagasse equivalence correction factor*	Adjusted Yield (kg) at 50% moisture	Calorific value (GJ)
Millable cane	70	1000			
Recoverable sugar	8.2	117	-	-	
Bagasse	21.0	300	1.0	300	2.97
Molasses	2.0	29	-	-	
Water, scum and impurities	38.8	554	-	-	
Cane tops and leaves (CTL)	20	286	0.6	171	1.70
Trash	10	143	1.5	214	2.12
Total	100	1429		686	6.79

*: Correction factor for CTL and trash to bagasse equivalence with 50% moisture content (Beeharry, 1996).

The figures are only indicative and are based on some additional assumptions such as:

- The commercial varieties have 13% fibre content and 13% sucrose content measured on a fresh weight basis (see section 2.1.2).
- Bagasse, CTL and trash have the same gross calorific values on a dry weight basis
- The maximum industrially recoverable sugar is 90% of total sucrose in the cane stem. This factor is currently being used in the calculation of Industrial Recoverable Sucrose Content (IRSC) which is equivalent to Commercial Cane Sugar (CCS) used in several countries.

$$\text{IRSC} = (0.9 \times \text{Pol \% cane}) - 1.8$$

Where 0.9 is the extraction efficiency at the mill and 1.8 is the correction factor for the presence of extraneous matter (cane trash, soil, non-millable canes, etc.) sent to the mill. Pol % cane is a quick laboratory method of estimating sucrose content (see section 3.4) in the cane stem.

The energy potential derived from sugarcane aboveground biomass entails an integrated use of CTL, trash and bagasse. Apart from sugar and molasses, every tonne of cane sent to the mill, there is a potential of producing 686 kg of bagasse equivalent feedstock for the production of electricity; bagasse proper representing 44% (300 kg) and CTL and trash the remaining 64% (386 kg). While bagasse is readily available at the mill for immediate use, the latter two residues involve additional efforts of baling in the field, transport and shredding before exploitation. Studies are currently being carried out at the MSIRI on the energy output:input ratio and efficient use of CTL and trash as an alternative source of bioenergy.

In Mauritius, between four and five million tonnes of cane are sent to the factory annually. This implies 1.2-1.5 million tonnes of bagasse, 0.12-0.15 million tonnes of molasses and over 2 million tonnes of CTL and trash. Currently, with mechanised harvest, more trash and extraneous matter are

sent to the mill along with the cane stems. This has led to a lower sugar extraction rate and a higher proportion of bagasse.

Traditionally bagasse was burned in specially designed furnaces for raising process steam and for producing motive power for the manufacture of raw sugar. This activity was viewed as a way of disposing of the bagasse to avoid additional handling cost rather than as a fuel-saving alternative. One sugar factory, namely St. Antoine sugar estate, first exported electricity to the national grid in 1957 using surplus bagasse as fuel. It was a modest 280 MWh/year, believed to be the world's first commercial, electrical export to the grid from the sugarcane industry. In the 1980s, besides sugar production, energy generation from bagasse complemented by coal became a major activity of the sugar industry during the harvest season. Over the last two decades, the high degree of volatility of oil markets has increased the awareness amongst policy makers of the need to decrease dependence on fossil fuels by increasing use of sustainable energies.

2.6.3 Policy initiatives in the use of bagasse as a source of energy

Since mid 1980s, both government and the privately owned sugar industry agreed that to sustain the viability of the sugar industry, value added from within the sector had to be generated from enhanced use of sugar by-products. Various policy initiatives and fiscal measures have followed to this end (Table 2.3).

Table 2.3: Landmark on bagasse energy enhancement and other by-products

Year	Policy initiatives and fiscal measures	Emphasis
1985	Sugar Sector Action Plan	Bagasse energy policy evoked
1988	Sugar Industry Efficiency Act	Fiscal incentives
1991	Bagasse energy development Programme	Renewable energy policy
1997	Blue Print for Centralisation of milling activities	Investment in bagasse energy and ethanol production
2001	Sugar Sector Strategic Plan	Optimise use of sugarcane resources. Investments in co-generation units
2006	Multi-Annual Adaptation Strategy	Co-generation annexed to each plant (4 clusters)

Government support and involvement has been instrumental in the development of a cogeneration programme in Mauritius. First, in 1985, the Sugar Sector Action Plan Act was enacted to encourage the production of bagasse for the generation of electricity. The Sugar Industry Efficiency Act (1988)

provided tax incentives for investments in the generation of electricity. Three years later, the Bagasse Energy Development Programme (BEDP) for the sugar industry was initiated. In 1994, the Mauritian Government abolished the sugar export duty, an additional incentive to the industry. The centrepiece of the recent action plans was the establishment of four sugarcane clusters made up of sub-clusters which would be operational around four main sugar factories. The success of the four clusters was found to rest on a few critical factors, namely in descending order of importance:

- The operation of very efficient and sizeable sugar factories
- The adequate provision of energy in the form of steam and electricity,
- A reliable and sustainable supply of canes,
- The operation of efficient and flexible state-of-the-art installations to produce different types of sugars and to optimise the use of bagasse and molasses.

2.6.4 Achievements in the Mauritian sugar industry

In 2001 the Mauritian sugar industry started a very innovative restructuring exercise which called for factory centralization, rightsizing of labour force, increased year-round generation of electricity from bagasse cum coal, improvement of value-added through co-products development and the establishment of a comprehensive Research and Development programme to take full advantage of cane biomass utilization. It is expected that by year 2011, the whole production will be processed in four state-of-the-art sugarcane factories, annexed with bagasse cum coal high-pressure boiler power plants; two of them are already operational. Until late 1990's sugarcane was processed in 19 sugar factories. Currently, the number of factories operating has been reduced to six.

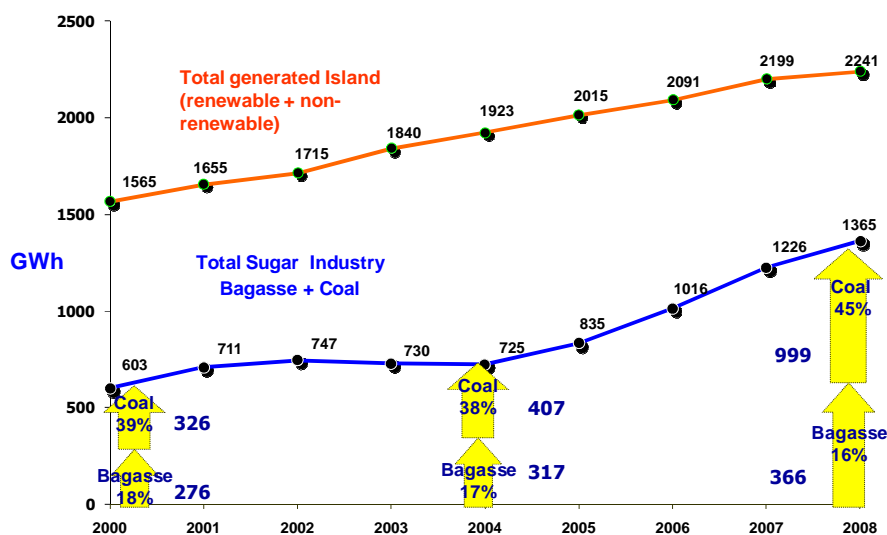


Figure 2.11: Electricity generated from sugar factory located power plants

Figure 2.11 illustrates electricity export from sugar factories since year 2000. By the year 2008, surplus electricity export to the grid, using bagasse as fuel, reached 366 GWh representing 16.4% of the total electricity produced in the island (MSIRI, 2008). With the export of around 300 GWh of cogenerated electricity to the grid, around 200,000 tonnes of coal are avoided, thus alleviating the burden on foreign exchange for such imports.

By year 2015, when the four state-of-the-art, bagasse cum coal power plants, annexed to energy efficient raw sugarcane factories will come online, the sugar industry is expected to double the amount of electricity export to 630 GWh. For every tonne of cane, surplus exportable electricity is expected to be 126 kWh/t, i.e. one of the highest in the world with the conventional steam system cycle (Lau Ah Wing, 2008).

The various policy initiatives and fiscal measures taken, especially in bagasse cogeneration, are considered a success story in Mauritius and in the African continent (Autrey, 2004; Deenapanray, 2009; Deepchand, 2005; Kong Win Chang *et al.*, 2001). Mauritius provides a model for emulation in ongoing and planned modern biomass energy projects in other African countries. Within the ACP group, the Mauritian sugar industry is considered to be extremely successful in the generation of electricity from sugarcane residues and is believed to be one of the most efficient at the world level (Wilson, 2006).

From an environmental life cycle perspective, sugarcane bagasse energy is associated with a net positive global benefit in that sugarcane is an annually renewable crop and contributes to a reduction in greenhouse gas emissions from energy which would have otherwise been generated from fossil fuels. The carbon dioxide released from the combustion of bagasse is re-absorbed in the ensuing crop and hence is carbon neutral. With the use of cane field residues for energy, more electricity can be generated, otherwise the residues decay and release methane, another greenhouse gas. In addition cogeneration also generates carbon emission credits that are potentially tradable under the Kyoto Protocol Clean Development Mechanism. The value of such credits could be as much as \$US 20 per tonne of carbon dioxide. The revenue derived therefore further enhances the financial viability of this renewable energy option.

2.6.5 New challenges of the sugar industry

A prerequisite to the sustained renewable long-term energy strategy is the generation of a critical mass of sugarcane biomass for cogeneration. Mauritius is a small island where prospects of increasing the

land area under sugarcane are non-existent. Figure 2.12 depicts the evolution of sugarcane crop harvested over the last 60 years. Following a sharp rise in the 1950s, at the expense of natural tropical forests, sugarcane cultivation reached its peak (around 82 000 ha harvested) in the 1960s. As from early 1980s, there has been a progressive reduction in the area devoted to the crop. This decline has been alarmingly sharp in the last decade. Some 11 258 ha of sugarcane lands, representing 15% of the area harvested in year 2000, have been either used for urbanisation, or to strengthen other sectors of the island's economy, or simply abandoned.

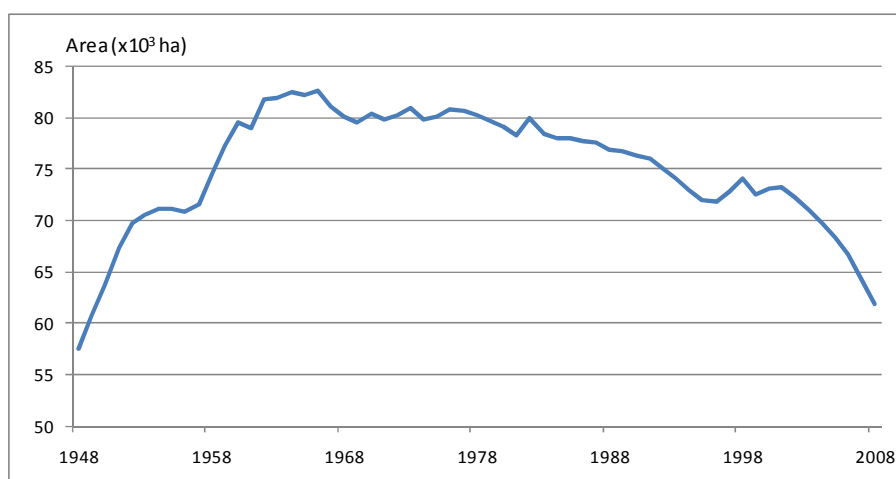


Figure 2.12: Evolution of sugarcane lands harvested in Mauritius

Source: MSIRI annual reports 1953-2008

In the coastal areas, mostly the marginal sugarcane lands have been converted into hotels and expensive residential under the “Integrated Resort Scheme” (IRS) and “Real Estate Scheme” (RES) projects. In the more central part of the island, much fertile sugarcane lands are being utilised in the construction of new cities and improvement of road infrastructure. In addition, in Mauritius, sugarcane is cultivated by three different categories of farmers (Table 2.4), based on the land area they occupy.

Table 2.4: Type of sugarcane farmers and percentage area cultivated by each category

Type of farmers	Acreage per owner	Percentage area under cane
Miller and Corporate-Planters	>100 ha	57
Large planters	10-100 ha	12
Small planters	<10 ha	31

Source: Sugar Industry Fund Board (SIFB) - 2007

The small farmers consists of some 28 000 individuals who have been identified as the most vulnerable group in sugarcane cultivation. Government's major concern is to effectively group them into clusters so that they benefit from economies of scale and remain in the sugar industry.

Still, given the trend, the Mauritian sugar industry is expected to operate with some 50 000 ha, or less, of sugarcane land by the year 2015. At such a level, it will be difficult to achieve the set national objectives. It is imperative then to have a concerted effort to enhance the long-term survivability of the local sugarcane industry. There is a need to investigate how to further increase energy export apart from the methods described above, while attempting to maintain a relatively high sugar yield.

2.6.6 High fibre varieties – a lifeline for Mauritius

In this respect, high fibre varieties with high biomass hold great promises and are being quoted as an important lifeline for the future (MREPU, 2009). A real 1% increase in fibre content, say from 13% to 14% fibre percent cane, would increase the bagasse yield from 300 kg to 323 kg for every tonne of clean cane sent to the mill (Table 2.5).

Table 2.5: Potential bagasse yield and energy conversion with every unit rise in fibre percent cane (assuming all other factors constant as at year 2008)

Fibre content	Bagasse (kg)	Electricity (GWh)	% to total electricity*
13	300	366	16%
14	323	394	18%
15	346	422	19%
16	369	450	20%
17	392	479	21%
18	415	507	23%
19	438	535	24%
20	462	563	25%
21	485	591	26%
22	508	619	28%
23	531	648	29%
24	554	676	30%
25	577	704	31%

*: Percentage calculated using year 2008 electricity production as the baseline

With all other factors kept constant as at year 2008, a 1% increase in fibre content island-wide would have produced 28 GWh of surplus electricity, without any substantial cost incurred and assuming that the extra bagasse so generated were all used for co-generation. This would have raised the percentage contribution of sugarcane biomass from 16% to 18% of total electricity generated in the year 2008. Similarly, a variety with 25% fibre and with same cane biomass as current commercial varieties

would theoretically produce some 700 GWh of electricity, which is equivalent to 30% of the 2008 island requirement. The CTL and trash would potentially contribute another 20%, exclusive of energy input for their collection, transport and processing and assuming the 70:30 ratio of cane to CTL and trash is maintained. This very simplistic linear extrapolation is, however, only indicative and does not account for various factors involved with handling and processing of higher fibre canes. Hence, one should be cautious in using the figures as such.

2.7 Studies on sugarcane varieties for high fibre and biomass

In the past, breeders consciously or unconsciously selected against fibre. Too high fibre content meant lowering the ratio of tonnes sugar made to tonnes fibre crushed. Bagasse and other extraneous matter, like trash, soil, leaves and cane tops are known to interfere with the milling efficiency and juice quality. Most of the efforts made in the past have been in increasing the level of sucrose concentration in the stem and cane yield.

Alexander (1985) was among the first to recognise the importance of growing high biomass varieties as an energy alternative. He estimated that large potential gains in both sugar and fibre production could be achieved from sugarcane if breeding programmes concentrated on total biomass production and not simply increasing the sugar concentration in the stem. Studies on high biomass canes with high fibre at the world level are relatively new. The biomass production of inter-specific and inter-generic hybrids is assumed to surpass that of existing cultivars.

The success of the Brazilian sugarcane industry in bioenergy production, particularly ethanol, and the high volatility of fossil fuel price have increased interest in producing sugarcane for ethanol throughout the world. The sustained capacity to improve and diversify its production by investing in research and development is one of the most important factors underlying the success and growth of Brazil's sugar/ethanol complex. The BIOEN programme in Brazil is aiming to integrate comprehensive research on sugarcane and other plant that can be used as biofuels sources, thus assuring Brazil's position among the leaders in the area of bioenergy.

The role sugarcane can play in helping the United States meet its need for renewable transportation fuel, electricity as well as food and feed is increasingly being recognised. Research being conducted at the USDA-ARS, Sugarcane Research Laboratory at Houma is geared to developing high biomass (sugar and cellulose) yielding varieties with levels of cold tolerance that will allow an expansion of the geographic range of adaptation to areas of the South beyond where sugarcane is traditionally grown. High fibre sugarcane genotypes were released for energy purposes in the U.S. in 2007. In Louisiana, three high-fibre sugarcane varieties, namely L 79-1002 (F1 hybrid from CP 52-68 × Tainan *S. spontaneum*), HoCP 91-552, and Ho 00-961 were released as high-fibre sugarcane cultivars. The latter two clones, developed at the USDA-ARS Sugarcane Research Unit, approach the sugar yields of current commercial standards (Table 2.6). Both have higher fibre content (16% vs. 13%) and biomass yields than conventional sugarcane varieties. The values were obtained from several years of field testing and machine-harvesting under Louisiana's temperate climate. In addition, the breeders have identified seven candidate energy cane varieties for further testing in different environments.

They also reported that the wild cane x sugarcane hybrids had significantly higher biomass yields and appeared to be more cold tolerant than commercial sugarcane varieties.

Tew and Cobill (2008), from SRU, further described three different types of canes that can be achieved through sugarcane breeding (Figure 2.13). Traditional sugarcane is grown primarily for the sugar. In the case of energy canes, the vegetative biomass is an important product, and this is either a by-product, in the case of the “Type I” energy canes, or the main product, in the case of the “Type II” energy canes. The differences among the cane types are in the relative composition of the cane stem, in terms of sugar, fibre and water content. High fibre canes have lower sugar and water content, and vice versa.

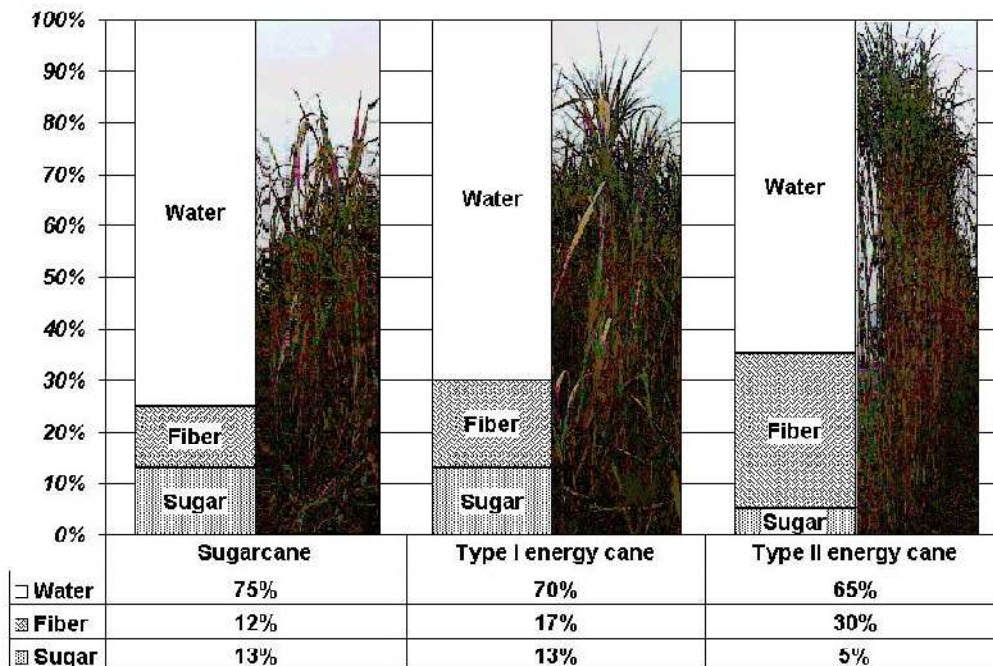


Figure 2.13: Variation in use and composition among sugarcane and Type I and Type II energy canes (Tew and Cobill, 2008)

The Cooperative Research Centre for Sugar Industry Innovation through Biotechnology (CRC SIIB), Australia, is working with the BSES Limited/CSIRO joint venture on a project to develop a special breed of 'Hi-Energy Canes' with the capacity to incorporate advances in biomass and bioenergy production. Some of the new varieties bred from the wild relatives have been crossed with current high-value, smut resistant, Australian varieties. It is hoped that offspring from these crosses could be commercially valuable for production of both sugar and energy.

In 2002, China, in collaboration with Australia, started a breeding and research programme aimed at utilizing *S. spontaneum* for biomass composition and yield components using DNA markers assisted methodology (Wang *et al.*, 2008). Parents and progeny from 43 biparental crosses between sugarcane and *S. spontaneum* clones were evaluated in field trials in China and Australia, along with several commercial cultivars. The collective results obtained in the study pointed to a series of broad strategies to exploit *S. spontaneum* clones for developing cultivars for future sugar or biomass production systems.

Table 2.6: High biomass clones identified in different countries

Country	Genotypes	Cane yield (tha ⁻¹)	Brix % cane	Fibre % cane
USA	LCP 85-384	70.6	18.0	13.0
	L 79-1002	82.2	13.0	26.0
	HoCP 91-552	87.1	17.0	16.0
	Ho 00-961	77.5	17.0	16.0
Japan	NCo310	635	18.7	13.7
	NiTn18	836	18.7	14.5
	97S109	<i>1597</i>	16.9	14.9
	99GA112	<i>1310</i>	14.4	24.8
	00GS260	<i>1218</i>	17.4	18.9
Thailand	K 84-200	91.4	11.96	13.12
	MPT 99-582	102.4	13.16	15.92
	MPT 99-648	78.6	8.99	15.17
	MPT 00-478	137.4	4.84	21.89
West Indies	Commercial varieties	52	18.0	16.0
	WI79460	113	13.1	26.1
	WI79461	127	15.4	24.3
	WI80534	105	12.2	24.3
	B69689	89	13.9	26.3
	IS76163	89	9.8	33.5

In bold: commercial varieties; italics: Cane yield estimated in kg/acre from small plots

(Tew and Cobill, 2008; Terajima, 2007; Rao, 2009; Rao, 2007)

Terajima *et al.* (2005; 2007) recently carried out inter-specific and inter-generic crosses with *S. spontaneum*, *Sorghum bicolor*, *Miscanthus spp.* and *Erianthus spp.* to develop high-biomass sugarcane clones for multipurpose use in Japan. Their findings (Table 2.6) confirmed that sugar production could be maintained at present levels and three-times more biomass ethanol could be generated compared with that in the conventional process. In addition, they demonstrated that a carbon-neutral process could be achieved using the new process.

Rao and Weerathaworn (2009), from Mittr Phol Sugarcane Research Institute, Thailand, selected 21 test genotypes, from their ongoing breeding programme, based on their fibre content, sucrose content and other agronomic traits. Eleven cultivars were subjected to further analyses in different environments. Significant differences were observed for sucrose and fibre yield as compared with a commercial control variety, K 84-200 (part of results presented in Table 2.6). The studies indicated that selection for multipurpose cultivars with improved fibre and sugar yield was achievable within their current breeding programme.

In India, the Sugarcane Breeding Institute, Coimbatore, is also exploring the utilization of wild relatives of sugarcane in energy generation and as an alternate source of raw material for the paper industry (Amalraj *et al.*, 2008). The germplasm collection of the wild species, *Erianthus arundinaceus*, was evaluated for its performance under cultivation, biomass production, stalk yield, fibre content and juice quality. Out of 88 clones evaluated, 23 with high fibre–pith ratio were selected. Based on proximate analysis, six clones were selected for further tests and trials. Studies on fibre content, bagasse yield, biomass yield and pulping showed that the *Erianthus* species was superior to sugarcane as a source of energy and fibre. So far no systematic evaluation of this naturally growing species has been done for its biomass production, energy content, fibre yield and juice quality, and no commercial cultivation for its co-products has been attempted.

Confronted with the EU sugar reforms, ACP sugar industries started restructuring in an attempt to survive and prevent closure. The common theme among all the restructuring plans had been energy production. In the Caribbean regions, Jamaica announced plans to start production of ethanol for the transportation sector. Barbados indicated that it would increase the acreage under sugar production and plant more of the fuel cane varieties to utilise the bagasse in electricity generation and also manufacture 24 million litres of fuel ethanol. Belize and Guyana also planned to introduce cogeneration.

In 2002, the West Indies CBS identified several potential high biomass varieties in their large inter-specific derived germplasm collection (Table 2.6). They were considered to be suitable for the production of bagasse to be used as a source of fuel in the generation of electricity. From the first trials and additional material subsequently identified, three varieties (fibre range: 23.6-25.6% cane) were selected and planted on a larger scale.

The success of the Mauritius sugar industry in the efficient use of sugarcane by-products, mainly bagasse, has been highlighted in the previous sections. In summary, investments on modern sugar factories and power plants that can process more efficiently higher fibre varieties are already

operational in contrast to other ACP countries where new state-of-the-art cogeneration plants are not yet built (Lau Ah Wing, 2008). Moreover, policy measures are well advanced in the successful exportation of electricity generated from bagasse to the grid. From the sugarcane breeding point of view, one high fibre variety, M1672/90, was identified from the conventional breeding programme and released to the planting community in 2007. The genetic base-broadening programme has recently been strengthened with the introduction of new *S. spontaneum* and high fibre clones in the gene pool. This study broadly aims to identify potential high biomass varieties from the existing inter-specific derived germplasm collection. A methodology for screening different types of varieties for multi-purpose use that can be used in the routine selection programme is also envisaged. Full descriptions of the various objectives of this study have been given in section 1.3.

CHAPTER 3

3 Materials and methods

3.1 *The planting materials*

The MSIRI has maintained, since the 1980s till date, about 450 parents that were selected from the inter-specific programme that made use of wild *Saccharum sp.* and other related genera. This collection of parents included those of the first generation of inter-specific crosses, *S. officinarum* or Commercial x *S. Spontaneum* or other wild species (F1), the first generation backcrosses, F1 x Commercial (BC1) and second generation backcrosses, BC1 x Commercial (BC2). They are routinely used in the hybridisation programme for broadening the genetic base of varieties. These genotypes vary in their fibre content; most of them have fibre content above that of current commercial varieties. This collection was graded visually for high yield and farmers acceptability on a scale of 1 to 5, where 1 = very poor and 5 = best in performance.

A total of 57 genotypes were screened for their high biomass potential and included 22 F1, 29 BC1 and 6 BC2 clones (Table 3.1). Forty nine of them involved inter-specific crosses between *S. officinarum* (or commercial hybrids) with *S. spontaneum*, four with *S. robustum* and one with *S. sinense* clones. Three genotypes were obtained from inter-generic crosses involving the *Erianthus* genera. Two of them, however, proved to be selfing of *Erianthus sp.*, after investigation using microsatellite markers (Joomun *et al.*, 2005). For convenience, since the two progenies were derived from crosses, they were maintained in the F1 category. Furthermore, two clones of the genera *Erianthus arundinaceous* and one inter-generic *Miscanthus* clone, SM81022, imported from Taiwan, were considered for the trial. Four commercial varieties, R570, M695/69, M1176/77, M1400/86, widely cultivated across the island, were included as controls.

3.2 *The experimental site*

Trials were implemented in April 2005 in the humid zone, at Pamplémousses Experiment Station in the north of the island. The site is situated at an altitude of 79 metres, has low humic latosol soil (L soil) and receives a mean rainfall of 1352 millimetres (based on the last 30 year's data).

Table 3.1: Entries, generations (wild, F1, BC1, BC2 and commercial) and their parentage

Genotypes	Parents		Cross codes	Crosses*
	Female	Male		
F1 crosses (<i>S. officinarum</i> or Commercial x related wild clones)				
M1256/87	M555/60	IS76178	CEr0	Commercial/ <i>Erianthus</i> sp. (Inter-generic)
M1156/00	IK7647	IK7647	Er0	<i>Erianthus</i> sp./ <i>Erianthus</i> sp. (selfed)
M1162/00	IK7647	IK7647	Er0	
SM81022	-	-	CM11	Commercial/ <i>Miscanthus</i> sp. (Inter-generic)
M1005/86	CHNS	SENN	NSn0	<i>S. officinarum</i> / <i>S. sinense</i> (Inter-specific)
M1008/86	NG57233	IJ76424	NR0	
M1010/86	NG57233	IJ76424	NR0	<i>S. officinarum</i> / <i>S. robustum</i> (Inter-specific)
M3254/87	E69991	IK76	CS0	
M1227/87	N14	IK7610	CS0	
M2230/86	S17	IK7610	CS0	
M1235/87	SP716113	IK7610	CS0	
M1240/87	SP716113	IK7610	CS0	
M1241/87	SP716113	IK7610	CS0	
M1695/88	F146	IK76100	CS0	Commercial hybrid/ <i>S. Spontaneum</i> (Inter-specific)
M1230/87	N14	IS76216	CS0	
M1231/87	N14	IS76216	CS0	
M1242/87	NCO376	IS76216	CS0	
M1249/87	SP716113	IS76216	CS0	
M1011/86	NCO376	SSS	CS0	
M36/85	IS76147	IK7686	NS0	
M37/85	IS76147	IK7686	NS0	
M982/86	NG5777	IS76216	NS0	<i>S. officinarum</i> / <i>S. spontaneum</i> (Inter-specific)
M2118/78	M26/20	UBA	NS0	
BC1 (1st generation backcrosses, F1 x Commercial)				
M897/89	IJ76432	M25/85	NNS1	<i>S. officinarum</i> // <i>S. officinarum</i> / <i>S. spontaneum</i>
M816/86	M2124/78	F149	CNR1	Commercial// <i>S. officinarum</i> / <i>S. robustum</i>
M3309/87	CP67412	POLS334/87	CNR1	
M377/91	M1000/86	CP722086	CNS1	
M385/91	M1000/86	CP722086	CNS1	
M905/89	N55805	M2121/78	CNS1	
M933/89	N55805	M2121/78	CNS1	
M718/89	CL41142	M24/85	CNS1	
M1303/87	E69991	M376/84	CNS1	
M1372/87	N16	M376/84	CNS1	
M1748/88	N55805	M376/84	CNS1	
M1750/88	N55805	M376/84	CNS1	
M1384/87	E69991	M386/84	CNS1	
M1395/87	E69991	M386/84	CNS1	
M1396/87	E69991	M386/84	CNS1	
M3305/87	E69991	M386/84	CNS1	
M872/89	E69991	M39/85	CNS1	Commercial// <i>S. officinarum</i> / <i>S. spontaneum</i>
M1529/88	N55805	M39/85	CNS1	
M993/89	N55805	M39/85	CNS1	
M3279/87	N14	M398/84	CNS1	
M1424/87	RB70141	M398/84	CNS1	
M1005/89	N55805	M41/85	CNS1	
M1017/89	N55805	M41/85	CNS1	
M3266/87	D684	M428/84	CNS1	
M3271/87	D684	M428/84	CNS1	
M3273/87	D684	M428/84	CNS1	
M1472/87	SP716113	M428/84	CNS1	
M1281/87	CP48103	POLS334/87	CNS1	
M1459/87	CP48103	POLS334/87	CNS1	
BC2 (2nd generation backcrosses, BC1 x Commercial)				
M733/90	M587/70	M1335/87	CNS2	
M768/90	M587/70	M1335/87	CNS2	
M799/90	R575	POLS389/90	CNS2	Commercial/2/ <i>S. officinarum</i> / <i>S. spontaneum</i>
M812/90	R575	POLS389/90	CNS2	
M816/90	R575	POLS389/90	CNS2	
M819/90	R575	POLS389/90	CNS2	
Wild clones (<i>Erianthus arundinaceus</i>)				
IJ76403	UNKNOWN	UNKNOWN	Er	Genera related to sugarcane
IK7648	UNKNOWN	UNKNOWN	Er	
Commercial controls				
M1176/77	N55805	CP5530	C	
M695/69	NCO376	M907/61	C	
R570	H328560	R445	C	Commercial clone
M1400/86	M744/70	R570	C	

*: Crosses: / = 1st cross; // = 2nd cross with progenies of 1st cross; /n/ = n successive crosses with progenies of 1st cross

3.3 The experimental design and layout

The 60 test genotypes were evaluated in five sub-trials with homogeneous plot size of two adjacent rows of 5 m length. The intra-row spacing was at 1.5 m and the alley between two plots in the same row was 1 m wide. The resolvable lattice design (Yates, 1936) was used with four intra-blocks and three replicates (Figure 3.1). The design is commonly used at the first replicated trial stage (the 3rd clonal stage) of the sugarcane selection programme in Mauritius. The four commercial controls (numbered 1 to 4 in Figure 3.1) were included in each trial for comparison purposes. The trials were implemented contiguously in a relatively flat and uniform field.

		Rep 1				Rep 2				Rep 3			
		Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10	Col 11	Col 12
Sub-trial 1	Row 1	58	24	16	9	47	9	40	20	2	17	24	1
	Row 2	31	59	17	20	3	16	4	24	58	59	40	3
	Row 3	40	37	3	4	31	59	37	17	20	4	47	9
	Row 4	1	2	63	47	2	58	63	1	63	31	16	37
Sub-trial 2	Row 5	11	3	1	49	3	4	2	54	54	33	4	1
	Row 6	53	33	29	54	49	52	48	25	3	18	2	64
	Row 7	27	25	4	48	11	27	29	53	29	48	25	52
	Row 8	2	64	18	52	1	33	64	18	27	11	49	53
Sub-trial 3	Row 9	60	10	2	4	46	10	15	21	15	3	1	60
	Row 10	15	36	21	46	36	62	2	13	21	41	56	13
	Row 11	1	13	3	56	60	56	4	1	10	30	36	62
	Row 12	30	14	62	41	3	30	14	41	46	14	2	4
Sub-trial 4	Row 13	3	23	42	55	51	26	45	22	38	23	2	45
	Row 14	1	26	45	51	1	50	3	43	22	55	28	1
	Row 15	22	38	34	43	34	4	38	42	4	34	50	43
	Row 16	50	28	4	2	28	55	2	23	51	3	42	26
Sub-trial 5	Row 17	19	5	4	61	19	2	4	3	35	44	61	39
	Row 18	3	6	2	57	8	32	57	44	5	57	8	12
	Row 19	12	32	8	39	39	35	12	6	1	32	3	6
	Row 20	35	7	44	1	5	61	7	1	4	19	7	2

Figure 3.1: Layout of five sub-trials planted contiguously in the field.

Number of sub-trials = 5

Number of replicates = 3

Commercial control varieties (standards) = 4, numbered 1 – 4 in bold

Test genotypes = 60; numbered 5-64

Preliminary analyses with the different measured traits showed that the precision gained using the lattice design was minimal. Generally, the gain was at 0%-4% as compared to a randomised complete block design (RCBD). In consequence, the simpler RCBD model was adopted for the various analyses.

3.4 Data collected

The trials were well watered and the data were collected from the plant cane in 2006 and first ratoon crops in 2007. They have been summarised in Table 3.2 and further described below.

Table 3.2: A summary of traits measured at plant cane and first ratoon

Traits	Remarks
Cane quality characters	
Brix % (FW)*	<ul style="list-style-type: none"> • Cane quality characters obtained from 2 sampling dates <ul style="list-style-type: none"> – pre-harvest (April) and – early-harvest season (July)
Pol. % (FW)	
Fibre % (FW)	
Dry matter % cane	<ul style="list-style-type: none"> • Six canes sampled at random per plot • Samples milled: juice extract and fibre content analysed
Brix % (DW)*	
Pol. % (DW)	
Fibre % (DW)	
Cane morphology	
	<i>Measured at first sampling date</i>
Cane diameter (mm)	Five millable stalks measured at random per plot
Cane Height (cm)	Five millable stalks measured at random per plot
Stalk number	Total number of mature stalks per plot
Ground cover	Rated 1 to 5, five months after planting and first harvest
Lodging	Rated 1 to 5
Trashing ease	Rated 1 to 5
Visual grade	Rated 1 to 5
Yield parameters (tha⁻¹)	
Cane yield (FW)	End of July at plant cane and 1 st ratoon
Cane yield (DW)	Cane yield x dry matter % cane
CTL** yield (FW)	CTL fresh and dry weights measured in two replicates from 3 canes per plot taken at random at first sampling date
CTL yield (DW)	
Sugar yield	Cane yield x pol % cane
Cane fibre yield	Cane yield x fibre % cane
Total aboveground biomass (FW)	Cane yield + CTL yield fresh weight
Total aboveground biomass (DW)	Cane yield + CTL yield dry weight

*: In brackets: - FW = measurements in fresh weights; DW = measurements in dry weights

** : CTL was composed of non-millable cane tops, green and clinging dry leaves

Rated non-parametric characters: 1 = very poor; 5 = best performance

For convenience, the source data were classified into three major groups:

1. Cane quality characters analysed from milling of clean cane stalks at the laboratory
2. Cane morphology characters comprising cane diameter, height and cane number, and other non-parametric characters rated on a 5-point scale
3. Biomass characters essentially obtained from weighing the aboveground parts, namely cane stems, cane tops and leaves and derived characters like sugar and fibre yields.

The whole data set could further be categorised as *primary characters*, represented by variates that were effectively measured in the field and laboratory, and *derived characters*, as those that were estimated from the former. Cane quality and biomass characters could also be grouped in terms of *fresh* and *dry weights*.

3.4.1 Cane quality characters

At each crop cycle, two samples of millable cane stems were taken. A pre-harvest sampling was done in April, two months before the harvest season. The second sample was taken in July that coincides with the early-harvest season of sugarcane in Mauritius. Six clean canes were taken at random from each plot, weighed and sent to the mill for the analysis of juice and fibre content. The canes were cut at ground level and at the apical meristem (as only the millable portion of the cane is used for yield estimates). The whole sampling operation at each sampling date was completed in one day. Variables derived from the laboratory analyses were classified as cane quality characters.

The following analyses were performed in the laboratory on a standard weight (329g) of disintegrated cane. This standard weight has been calculated so that the polarimeter reading is numerically equivalent to the pol % cane (MSIRI, 1968). The canes were disintegrated in a cane chipper and 329g of chipped cane were then crushed further in a high speed wet disintegrator with 990ml of water and 10ml of 5% sodium carbonate solution (to prevent inversion of sucrose). The extracted juice was used to determine Brix % cane and pol % cane, whereas the cane particles were then washed and dried to constant weight for the determination of fibre content as described below.

- a) Brix % cane, which is equivalent to the proportion of total soluble solids, was derived from the diluted Brix measured in the laboratory according to the following formula:

$$\text{Brix \% cane} = \text{diluted Brix} \times \left(4.04 - \frac{\text{fibre \% cane}}{100} \right)$$

- b) Pol % cane is the apparent sucrose content of the juice as determined by polarisation using the method of de Saint Antoine (MSIRI, 1968). This estimate is determined with a polarimeter that measures the net optical activity of different sugars and is very reliable in ripe cane but may be unreliable in unripe cane (Mamet, 1992).
- c) Fibre % cane was obtained by direct determination of the fibre content of the cane according to the method of de Saint Antoine and Froberville (MSIRI, 1964):

$$\text{Fibre \% cane} = \frac{\text{fibre net weight}}{329} \times 100$$

d) Dry matter % cane was the sum total of soluble and non-soluble solids:

$$\text{Dry matter \% cane} = \text{Brix \% cane} + \text{fibre \% cane}$$

e) Derived laboratory characters

Table 3.3 gives the formulae used to calculate the dry weight estimates of the cane quality traits.

Table 3.3: Calculation of cane quality traits on a dry weight basis

Derived variate	Formulae
Brix % dry matter	$= \frac{\text{Brix \% cane}}{\text{Dry matter \% cane}} \times 100$
Pol % dry matter	$= \frac{\text{Pol \% cane}}{\text{Dry matter \% cane}} \times 100$
Fibre % dry matter	$= \frac{\text{Fibre \% cane}}{\text{Dry matter \% cane}} \times 100$

3.4.2 Morphological and non-parametric characters

After the first sampling in April, the canes were trashed and the morphological and non-parametric characters were recorded. Morphological characters included number of millable stalks per plot, cane diameter and cane height. Non-parametric characters involved ground cover, growth habit, ease of trashing and breeders' preference. More details on the measurements are given below:

- Number of stalks per plot was counted and extrapolated to stalk number per hectare.
- Cane height and cane diameter were measured on five canes taken randomly from the middle part of the plots. The average values were used for statistical analyses.
- Observations were made on non-parametric characters using a 5-point-scale index, where 1 = very poor, 2 = poor, 3 = average, 4 = good and 5 = best in performance.

Ground cover was rated five months after planting and after first harvest. Since the plots constituted of two rows of 5 m with an inter-row spacing of 1.5 m, genotypes with crop canopy fully covering the within plot inter-rows were rated as 5 (best performance). The ratings decreased progressively with decrease in canopy cover. Lodging was rated visually based on the extent of inclination of the cane stems towards the ground. A totally lodged genotype was rated 1 and one with fully erect canes as 5. Clinging dry leaves of individual clones were manually stripped to rate the ease of trashing. Breeder's preference, also termed as visual grade (VG), was a value given by the breeder based on the overall appearance of the clone and integrated the crop vigour, stalk diameter, stalk number, stalk

height and other visible parameters broadly acceptable to farmers. Least desirable genotypes were rated as 1 and the most desirable ones as 5.

3.4.3 Biomass characters

Biomass characters involved all aboveground traits that were weighed and extrapolated to tonnes per hectare (tha^{-1}). These involved mature trash free cane stems devoid of non-millable parts, namely immature cane tops, green and clinging dry leaves, commonly termed as CTL in this study. The latter were measured separately during sampling. Fallen dry leaves during growth of the crop were not considered as there was no assurance that the trash observed in a plot (particularly in the inter-plots) was from the genotypes planted therein.

a) Cane yield

Plot weight was taken by end July for each crop cycle and cane yield in tonnes per hectare was estimated.

$$\text{Cane yield fresh weight } (\text{tha}^{-1}) = \frac{\text{plot weight (kg)}}{15 \text{ m}^2 \times 1000 \text{ kg}} \times 10\,000 \text{ m}^2$$

Cane yield dry weight was estimated by the product of cane yield fresh weight and dry matter % cane obtained from the laboratory (see Table 3.3). Hence,

$$\text{Cane yield dry weight } (\text{tha}^{-1}) = \frac{\text{cane yield fresh weight} \times \text{Dry matter \% cane}}{100}$$

b) CTL yield

At the first sampling date, the weight of CTL was taken randomly from three canes of each plot. CTL constituted all the cane parts above the cane meristem and clinging dry leaves. Due to shortage of resources, measurements were made over two replicates only. The CTL samples were oven dried at 105 °C for 24 h and the dry weights were recorded. Fresh weights were taken in kilogram to the nearest 100g and dry weights were measured in gram. The values were standardised to tonnes per hectare as per the following formula:

$$\text{CTL fresh weight } (\text{tha}^{-1}) = \frac{\text{CTL fresh weight (kg)}}{3 \text{ canes} \times 1000 \text{ kg}} \times \frac{\text{stalk number per plot} \times 10000 \text{ m}^2}{15 \text{ m}^2}$$

CTL dry weight standardisation included a division of 1000 in the above formula to correct for the measurement in gram.

c) Derived biomass characters

Four biomass variables were derived as described in Table 3.4.

Table 3.4: Biomass characters derived from cane quality and cane yield traits

Derived variate	Formula
Sugar yield*	Pol % cane x cane yield (tha^{-1})
Fibre yield*	Fibre % cane x cane yield (tha^{-1})
Total biomass yield (fresh weight)**	Cane yield fresh weight + CTL yield fresh weight (tha^{-1})
Total biomass yield (dry weight)**	Cane yield dry weight + CTL yield dry weight (tha^{-1})

*: For each crop cycle, two yield data were derived from two sampling dates

** : Total biomass was calculated from two replicates, as CTL biomass was measured in two replicates only

3.5 The statistical analyses

Overall, at each crop cycle, 29 parametric traits were obtained that were used for statistical analyses. These included traits measured at two sampling dates and on fresh and dry weights. The non-parametric traits, particularly the morphological characters rated on 5-point scales, were important during final selection of the test genotypes.

Basically, the statistical analyses involved stepwise procedure in the determination and selection of the best genotypes for different end-uses. This involved:

- Data validation, analysis of variances and calculation of means,
- Multivariate data analysis involving several traits,
- Identification of different types of canes from the population,
- Simultaneous selection of clones for multiple uses, and
- Determination of the traits and time of data collection for most effective selection of the different types of canes.

Details of the statistical methods related to each of the above steps have been given in the relevant results chapters for ease of reference.

CHAPTER 4

4 Data validation, analysis of variances and calculation of means

The methodology of the analyses, which will be developed in the following sections, involves the stepwise procedure of:

- 4.1 Validation of trial data and identification of outliers
- 4.2 Analysis of variances and data quality assessment
- 4.3 Calculation of means and adjustment for sub-trial effects

4.1 Validation of trial data and identification of outliers

4.1.1 Introduction

An outlier is an observation that is numerically distant from the rest of the data. They can occur by chance in any distribution, but they are often indicative of either measurement error or that the population has a heavy tailed distribution. Outliers cannot be ignored; in fact, they can indicate special cases that open new areas of research. But they can have a disproportionate effect on the results of a regression analysis or ANOVAs.

Current sophisticated statistical packages like ASReml (Gilmour *et al.*, 2008) have graphical diagnostic tools to aid in outlier identification. The AGROBASE Generation IITM software (Agronomix Software Inc., 2005), used in this study, currently lacks a standard methodology for the identification of outliers (personal communication – Dr. Mulitze, 2009). It was thus felt necessary to devise a methodology to check for the validity of the data based on a statistically sound method.

4.1.2 Methodology

4.1.2.1 Data verification at data entry level

During measurement of traits and data entry, care was taken to minimise errors. The records were entered twice by two different individuals and subsequently validated. Furthermore, minimum and maximum values of each trait were checked and all inconsistencies were confirmed from the source document and corrected.

4.1.2.2 Use of residuals

Before starting in-depth analyses, there was a last opportunity to check the validity of the data. The methodology was similar to the approach described by Fox *et al.* (1997b). This involved the residuals, which were the discrepancies between the observed data and those values that could be expected based on genotype means. Formally, the residual for the i -th genotype in j -th block is

$$\varepsilon_{ij} = y_{ij} - \hat{y}_{ij}$$

where \hat{y}_{ij} is the predicted value from the model for the data values y_{ij} .

For the RCB design adopted in this study, the appropriate model used was

$$\varepsilon_{ij} = Y_{ij} - T_i - B_j + \mu$$

Where ε_{ij} = residual values

Y_{ij} = i th genotype value in j th block

T_i = i th treatment mean

B_j = jth block mean

The residuals were calculated starting from the block x genotype array as follows:

1. The mean for each block was calculated
2. The block mean was subtracted from each value in that block, repeating the procedure for each block.
3. The mean of the values resulting from (2) was calculated for each genotype
4. The genotype mean was subtracted from all values for each genotype

The remaining values, termed as residuals, from each trial were standardised using the formula:

$$Z_i = \frac{X_i - \mu}{\sigma}$$

Where Z_i = standardised value
 X_i = individual residual values
 μ = Mean of residuals, which was equal to 0 in this case
 σ = Standard deviation of residuals

The residuals from each trial were assumed to be normally distributed with mean 0 and standard deviation of 1; $\varepsilon_{ij} \sim N(0,1)$. Under such a distribution, nearly 100% of the variation ranges between -3.3 and 3.3. The standardised residuals also summed to zero within rows and within columns. Hence if a particular block or genotype included a large positive residual, it was also likely to include largish negative residuals. The overall consequence was that the effects were often masked in the presence of more than one outlier. It was only when the first outlier was corrected that the second value became apparent. For this reason, although values greater than 3.3 were good candidates as outliers, all standardised residuals with magnitude greater than 2.5 were visited in the quest for genuine outliers.

Individuals were also checked based on previous experience of the performance of known genotypes. As an example, commercial varieties used as controls were relatively rich in sucrose concentration and low in fibre content as compared with their related wild clones. Similarly, *Erianthus sp.*, are known to have very high fibre content and very low sucrose concentration.

4.1.2.3 Data correction methodology

The candidate outliers' values were re-verified from the source file. Those considered as genuine were deleted and an estimate worked out using the method of Yates (Sokal and Rohlf, 2000). The formula for missing values is:

$$X_{ij} = \frac{rB'_i + tT'_j - G'}{(r-1)(t-1)}$$

Where r = number of blocks

t = number of treatments

B'_i = the observed block total after deletion of outlier

T'_j = the observed treatment total in which the missing value occurred

G' = The observed grand mean without the outlier

The approach for more than one outlier per trial was to use dummy values, such as the general average, as substitute for all the missing values. The latter would then be computed with successive iterations until the estimates were constant.

4.1.2.4 Traits verified

The five sub-trials were individually scrutinised at each sampling date and crop cycle. Traits that were subject to verification were essentially the continuous primary characters that constituted the source document. Measures were taken to automatically update the derived characters following alterations made in the primary traits.

4.1.3 Results

4.1.3.1 Simulation studies

Simulation studies were made in two sub-trials where three genuine outliers were observed from the source during data handling (Table 4.1). In sub-trial 4, the control variety, R570, had an abnormally high fibre percent cane of 18.4 in replicate 1 while in the other two replicates, it was around 11. Under normal conditions, fibre content of this commercial variety should be around 12%. The standardised residual of the outlier was at 4.04.

Table 4.1: Identification of outliers using residuals – trait: fibre % cane

Sub-trials	variety	Replicates			Residuals			Standardised residuals		
		I	II	III	I	II	III	I	II	III
Sub-trial 4	M1176/77	8.1	9.1	9.5	-0.53	0.17	0.36	-0.44	0.14	0.30
	M1249/87	14.5	17.5	17.6	-1.78	0.93	0.85	-1.46	0.76	0.70
	M1256/87	13.8	16.6	17.9	-2.09	0.46	1.63	-1.72	0.38	1.34
	M1372/87	14.9	16.5	18.9	-1.63	-0.29	1.91	-1.34	-0.24	1.57
	M1395/87	15.0	16.0	15.4	-0.19	0.45	-0.26	-0.15	0.37	-0.22
	M1400/86	9.0	9.4	10.5	-0.41	-0.26	0.67	-0.34	-0.21	0.55
	M1695/88	14.6	13.4	14.7	0.61	-0.84	0.23	0.50	-0.69	0.19
	M2230/86	16.7	18.0	15.0	0.40	1.40	-1.80	0.33	1.15	-1.48
	M3273/87	14.6	14.7	15.8	-0.18	-0.36	0.54	-0.15	-0.29	0.44
	M3279/87	14.5	14.7	14.2	0.27	0.21	-0.48	0.22	0.17	-0.39
	M3309/87	15.7	15.7	15.0	0.43	0.19	-0.62	0.35	0.16	-0.51
	M695/69	10.3	11.7	11.1	-0.52	0.66	-0.14	-0.43	0.55	-0.12
	M718/89	13.2	12.4	12.2	0.85	-0.28	-0.57	0.69	-0.23	-0.47
	M733/90	11.3	12.9	13.8	-1.13	0.24	0.89	-0.93	0.20	0.73
	M816/86	15.2	14.1	14.0	0.99	-0.38	-0.61	0.81	-0.31	-0.50
	R570	18.4	11.4	11.3	4.92	-2.31	-2.60	4.04	-1.90	-2.14
Sub-trial 5	IJ76403	22.4	21.8	15.9	2.47	0.94	-3.42	1.16	0.44	-1.61
	IK7648	21.8	23.1	9.9	3.66	4.01	-7.67	1.72	1.89	-3.61
	M1005/86	19.9	17.4	19.4	1.13	-2.29	1.16	0.53	-1.08	0.55
	M1005/89	13.3	11.6	13.4	0.67	-1.99	1.32	0.32	-0.93	0.62
	M1017/89	11.6	13.2	14.3	-1.32	-0.66	1.97	-0.62	-0.31	0.93
	M1176/77	9.5	19.1	8.4	-2.72	5.99	-3.26	-1.28	2.82	-1.54
	M1240/87	15.3	16.4	17.6	-1.03	-0.86	1.89	-0.49	-0.41	0.89
	M1400/86	9.5	11.6	9.8	-0.66	0.49	0.17	-0.31	0.23	0.08
	M1472/87	15.7	15.3	18.5	-0.67	-2.05	2.71	-0.31	-0.96	1.28
	M1748/88	18.4	18.1	16.7	0.82	-0.44	-0.39	0.39	-0.21	-0.18
	M3254/87	12.2	13.1	13.6	-0.63	-0.64	1.27	-0.30	-0.30	0.60
	M3305/87	15.9	15.8	16.8	-0.13	-1.14	1.28	-0.06	-0.54	0.60
	M695/69	9.8	10.4	10.4	-0.30	-0.59	0.89	-0.14	-0.28	0.42
	M819/90	13.7	12.7	13.2	0.61	-1.29	0.68	0.29	-0.61	0.32
	M933/89	9.4	11.5	10.8	-1.03	0.14	0.89	-0.49	0.07	0.42
	R570	10.8	13.0	11.6	-0.87	0.36	0.51	-0.41	0.17	0.24

Bold: suspected outliers; shaded: Absolute SD of residuals >2.5

In sub-trial 5, two outliers were visually identified (Table 4.1 trial 5, in bold). The first one was observed in genotype IK7648, an *Erianthus sp.* known for its high fibre content (above 20% fresh weight). The value of 9.9 in replicate 3 was particularly low. The second outlier in the same sub-trial was observed in another commercial variety, M1176/77. Like R570, this variety is known for its relatively high sucrose content and low fibre concentration. However, the fibre percent of 19.9 in replicate 3 was abnormally high. Presence of more than one outlier mitigated the impact on standardised residuals. Although the aberrant values in sub-trial 5 were larger than that in sub-trial 4, the standardised residuals were smaller (-3.61 and 2.82 versus 4.04). Hence, the verification of data

for possible outliers as from standard residual of 2.5 could rightly be justified, particularly for more than one outlier per trial.

4.1.3.2 Application of algorithm to the data

Each trait per trial was subjected to the data validation procedure. Figure 4.1 shows a sample of such graphs as obtained for the plant cane crop stage in one sub-trial. Candidate outliers could easily be spotted as dots outside the range of +2.5 and -2.5 in the scatter plots.

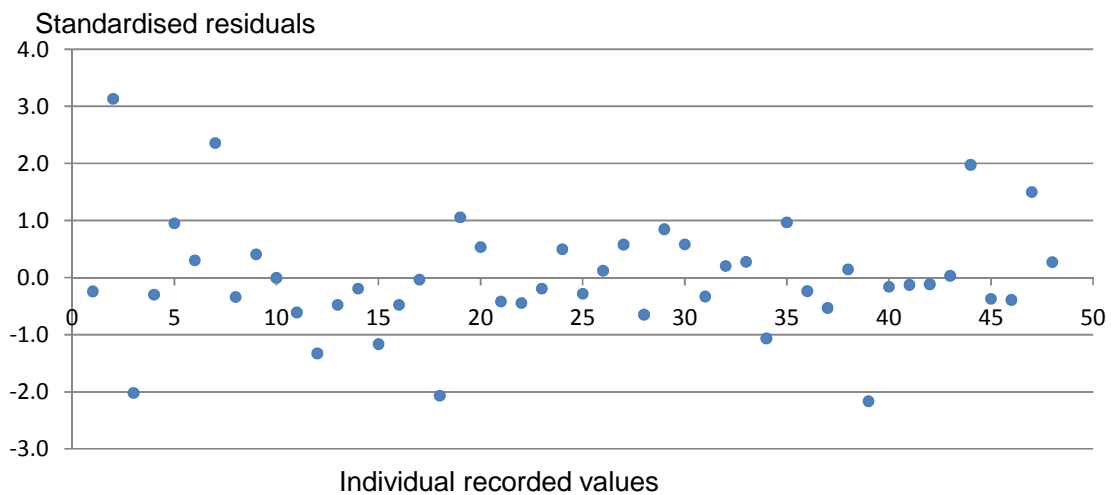


Figure 4.1: Scatter diagram of standardised residuals of cane yield fresh weight at plant cane in one sub-trial

The majority of the characters had standard deviations between -3 to 3, with mean values at zero. The probable outliers are listed in Table 4.2. Out of a total of 13600 parametric data analysed, 156 standardised residual values had magnitudes greater than 2.5.

Table 4.2: Range of standard deviations of standardised residuals (minimum and maximum) and number of observations with absolute values >2.5 in the five sub-trials treated individually

Variables*	Plant cane				First ratoon			
	Abs. Res.** > 2.5	% to total	Min.	Max.	Abs. Res. > 2.5	% to total	Min.	Max.
First sampling date								
Brix % (FW)	3	1.3%	-2.94	2.93	0	0.0%	-2.53	3.21
Pol % (FW)	1	0.4%	-2.43	2.83	5	2.1%	-2.69	2.99
Fibre % (FW)	3	1.3%	-3.26	4.04	8	3.3%	-3.61	2.70
Dry matter %	2	0.8%	-3.83	3.92	4	1.7%	-3.14	2.70
Brix % (DW)	5	2.1%	-3.91	3.10	4	1.7%	-2.88	4.55
Pol % (DW)	3	1.3%	-2.94	2.43	7	2.9%	-2.84	3.42
Fibre % (DW)	5	2.1%	-3.10	3.91	4	1.7%	-4.55	2.88
Second sampling date								
Brix % (FW)	3	1.3%	-3.25	2.62	4	1.7%	-3.50	3.40
Pol % (FW)	3	1.3%	-3.31	2.84	4	1.7%	-3.02	3.70
Fibre % (FW)	0	0.0%	-2.37	4.06	2	0.8%	-3.95	2.93
Dry matter %	3	1.3%	-2.74	3.52	3	1.3%	-3.47	3.47
Brix % (DW)	2	0.8%	-4.01	2.10	1	0.4%	-3.22	3.40
Pol % (DW)	1	0.4%	-3.75	2.28	1	0.4%	-3.01	2.83
Fibre % (DW)	2	0.8%	-2.10	4.01	1	0.4%	-3.87	3.22
Cane morphology								
Cane diameter (mm)	3	1.3%	-2.40	2.68	0	0.0%	-2.39	2.36
Cane height (cm)	4	1.7%	-2.61	2.52	2	0.8%	-3.23	3.60
Cane number/ha	5	2.1%	-3.69	2.63	3	1.3%	-3.73	3.75
Cane yield (tha⁻¹) (FW)	4	1.7%	-2.55	3.13	5	2.1%	-2.49	2.79
Derived biomass parameters (tha⁻¹) from first sampling date								
Cane yield (DW)	4	1.7%	-2.47	3.15	3	1.3%	-2.52	3.78
Fibre yield	2	0.8%	-2.33	3.67	4	1.7%	-2.48	3.83
Sugar yield	5	2.1%	-2.56	3.35	6	2.5%	-2.62	3.29
Derived biomass parameters (tha⁻¹) from second sampling date								
Cane yield (DW)	1	0.4%	-2.20	3.19	3	1.3%	-2.35	2.96
Fibre yield	1	0.4%	-2.42	3.44	4	1.7%	-2.66	2.77
Sugar yield	6	2.5%	-2.52	3.65	5	2.1%	-2.81	2.99

*: FW = fresh weight; DW = Dry weight

** : Absolute residuals

The 156 values were re-verified from source and 17 primary measured characters were considered as genuine outliers (Table 4.3). They were mostly observed in cane quality characters, believed to have occurred during manual recording in the laboratory. Most of them had extremely high (>3.3) or extremely low (<-3.3) standard deviations. The 17 abnormal values also influenced the derived

variables, thus raising the total number of outliers identified to 56. The latter represented 0.4% of the total data entries. The outliers were also randomly obtained in different traits, sub-trials, sampling dates and crop cycles. In most cases, none or only one outlier was observed per sub-trial. On two occasions, two outliers were observed in the same year, sub-trial and trait. In those two situations, as was expected, the residuals of the aberrant values were lesser than 3.3.

Table 4.3: List of genuine outliers with standardised residuals shaded

Trait	sub-trial	Sample	genotype	Replicates			Standardised residuals		
				1	2	3	rep1	rep2	rep3
Plant cane crop									
Fibre % cane	4	1	R570	18.36	11.40	11.28	4.04	-1.90	-2.14
Fibre % cane	5	1	IK7648	21.79	23.07	9.91	1.72	1.89	-3.61
BRIX % cane	3	2	M1400/86	13.89	9.02	15.49	0.78	-3.25	2.47
BRIX % cane	3	2	M1424/87	14.20	8.77	10.73	2.55	-2.01	-0.54
Fibre % cane	2	2	M1176/77	19.12	10.52	11.40	4.06	-2.18	-1.88
Fibre % cane	3	2	M1424/87	11.73	20.91	9.70	-0.64	2.78	-2.13
Fibre % cane	3	2	M36/85	17.42	14.86	24.56	-0.24	-2.35	2.59
Fibre % cane	5	2	M1400/86	11.12	19.57	10.94	-1.97	3.52	-1.55
CTL (FW)	2	-	M1011/86	75.70	17.36		3.68	-3.68	
First ratoon crop									
Fibre % cane	1	1	M993/89	11.98	5.26	12.77	1.72	-3.61	1.89
Fibre % cane	3	1	M1162/00	20.24	13.07	20.33	1.73	-3.48	1.75
BRIX % cane	3	2	M905/89	11.83	16.42	11.99	-1.64	3.40	-1.77
BRIX % cane	4	2	M1372/87	9.85	5.15	10.75	1.00	-3.50	2.50
Fibre % cane	1	2	M1008/86	10.67	17.02	17.42	-3.95	1.80	2.15
Pol % cane	3	2	M905/89	9.80	15.45	10.20	-1.91	3.70	-1.79
Cane height	2	-	SM81022	194.60	281.20	173.40	-1.93	3.60	-1.67
Cane number (/ha)	1	-	M1230/87	84000	120000	50000	-0.63	3.75	-3.12

The 17 outliers were deleted and the estimates of the resulting missing values were worked out as per the methodology described in section 4.1.2.3. The derived characters were automatically updated.

4.1.4 Discussion

According to Fox *et al.* (1997b) errors in manual recording are estimated to occur with a frequency of one per 100 numbers. In this study, the frequency of outliers observed was as low as 0.4%, i.e. one in 250 numbers. This low frequency was attributable to the dual data entry by two independent individuals and validation operations prior to the analysis of the data.

Still, errors are often unpredictable and arise from multiple sources. The approach using the residual method before running various statistical analyses was found very effective. It should be noted, however, that the methodology described for data validation apply to the correction of only a few outliers per trial and only to allow the analysis of variance to be completed.

From the statistical point of view, finding estimates of missing values imply an adjustment in the analysis of variances for the correction of bias on contrasts. The degrees of freedom for the total and the error terms should both be reduced by one for each calculated missing observation, while the sum of squares of treatments should be accordingly adjusted. As mentioned by Sokal and Rohlf (2000), the correction for sum of squares of treatments would only be necessary for border-line cases of significance. From the breeding point of view, adjustments for the correction for bias can become highly burdensome with large data sets and if done routinely in a plant breeding programme. For this reason, such adjustments in the above data were avoided, but could ultimately be integrated in the algorithm, should precision in significance testing become very crucial.

4.2 *Analysis of variances and data quality assessment*

4.2.1 Introduction

The primary purpose of a plant breeding trial at an advanced stage of selection is to assess differences among genotypes for particular traits. The initial technique in the analysis of most experimental data is the analysis of variance (ANOVA). This has two purposes. First, it provides a subdivision of the total variation between the experimental units into separate components, each component representing a different source of variation, so that the relative importance of the different sources can be assessed. Second, and more important, it gives an estimate of the underlying variation between units which provides a basis for inferences about the effects of the applied treatment (Mead, 1988).

The prediction of genotype yield from trial data requires an underlying statistical model which provides an estimate of the mean yield and the distribution of possible yields (Kempton and Fox, 1997). For a single variety trial, where genotypes are arranged in blocks to control plot heterogeneity, prediction of the genotype yield is usually based on the additive model:

$$\text{Plot yield} = \text{Trial mean} + \text{Block effect} + \text{Genotype effect} + \text{plot error}$$

More formally, the model can be written as

$$y_{ij} = \mu + b_i + g_j + \varepsilon_{ij}$$

Where y_{ij} is the observed yield of the i -th genotype in the j -th block, μ is the average yield for the whole set of experimental units, b_i is the effect of the i -th block, g_j is the effect of the j -th genotype, and ε_{ij} is the residual effect corresponding to y_{ij} .

Two basic assumptions imply by the use of the model. The assumption of additivity implies that both the differences between blocks and the differences between genotypes remain consistent over a trial. The second assumption which simplifies the principles and practice of the analysis of blocked experiments is that the error terms, which represent the random variation between plots, are homogeneous in size and unaffected by the particular genotype. In other words, although the genotypes may differ in average yields, the yield variation between plots should be similar for all of the genotypes in the trial (Mead, 1997).

Apart from significance tests, ANOVA provides several possibilities of assessing the quality of the data based on the relative proportion of the different components of variation. The objectives of this study were to analyse the variations in the individual parametric traits at two crop cycles, test for

significance of genotype component of variance and assess the quality of the data by evaluating each component of variation using standard statistical parameters.

4.2.2 Materials and methods

4.2.2.1 The data and software used

The materials used for the analyses were those described in chapter three and subsequently corrected for outliers in chapter four. The resulting 29 parametric traits were individually processed in each of the five sub-trials at plant cane and first ratoon crops.

Overall, 290 different ANOVA runs were made using AGROBASE Generation IITM (Agronomix Software Inc., 2005) as statistical software. AGROBASE Generation IITM is a comprehensive, fully relational, 32-bit database software system for agronomy research and plant breeding. The software is licensed as a basic system, with additional modules that clients may license according to their needs. The Agronomic System is the foundation of AGROBASE Generation IITM, and provides all the basic functions and features to conduct agronomy and plant breeding trials. An example of output generated by the AGROBASE software is given on next page.

ANALYSIS OF VARIANCE (an illustration of output generated by AGROBASE software)
2006PSES1, Sub-trial 1

Variable: CYFW (Cane yield fresh weight): Tuesday, July 7, 2009, 15:00:10

Source	df	SS	MS	F-value	Pr>F
Total	47	29320.19			
BLOC	2	1168.936	584.468	2.91	0.0700
ENTRY	15	22123.33	1474.889	7.34	0.0000
Residual	30	6027.924	200.931		

Grand mean = 70.841 R-squared = 0.7944 C.V. = 20.01%

LSD for ENTRY = 23.6369, S.E.D. = 11.5738, r= 3.0, Herit. = 0.679

t (2-sided a=0.050, 30 df) = 2.0423 MSE = 200.93080

Genetic variance = 424.6526, Phenotypic variance = 625.5834

Standard error of heritability = 0.1129, Bias = 0.0416

ENTRY Level	Averages	Cv	Rank	
16	112.18	8.7	1	M993/89
4	100.65	2.5	2	R570
3	94.04	33.7	3	M1400/86
14	89.53	10.8	4	M872/89
8	81.4	18.2	5	M1230/87
5	81.16	11.8	6	M2118/78
1	80.69	17.7	7	M695/69
2	80.44	45	8	M1176/77
9	68.42	11	9	M1231/87
12	57.76	11.8	10	M1459/87
15	54.89	16.4	11	M897/89
11	52.58	15.9	12	M1281/87
10	48.09	31.4	13	M1241/87
6	47.18	12.5	14	M37/85
7	43.2	7.3	15	M1008/86
13	41.25	16.3	16	M3266/87

Table of original data for CYFW across complete blocks (reps)

Entry	Rep1	Rep2	Rep3	Mean	Variety/entry name
1	71.87	73	97.2	80.69	M695/69
2	122.13	56.67	62.53	80.44	M1176/77
3	126.93	91.53	63.67	94.04	M1400/86
4	101.47	102.6	97.87	100.65	R570
5	92	77.6	73.87	81.16	M2118/78
6	46.47	53.4	41.67	47.18	M37/85
7	46.67	42.47	40.47	43.2	M1008/86
8	64.73	93	86.47	81.4	M1230/87
9	71.27	74.13	59.87	68.42	M1231/87
10	65.07	43	36.2	48.09	M1241/87
11	62.2	47.2	48.33	52.58	M1281/87
12	60.13	63.07	50.07	57.76	M1459/87
13	47.4	42.27	34.07	41.25	M3266/87
14	90.33	98.8	79.47	89.53	M872/89
15	58.93	61.13	44.6	54.89	M897/89
16	105.2	108.07	123.27	112.18	M993/89
	77.05	70.5	64.98	70.84	Trial & rep averages

4.2.2.2 *The statistical parameters extracted and description*

The randomised complete block model was used and the statistical parameters viz. Repeatability (H^2), coefficient of variation (C.V.), coefficient of determination (R^2), genotypes contribution to total variance, test for significance of genotypes (Pr.> F), least significant difference (LSD) for mean comparisons - as well as the minimum (Min.) and the maximum (Max.) values were extracted.

The first four statistical parameters were inter-related in the sense that they broadly assessed the quality of the data and indicated the overall reliability and precision achievable in the trials. Information pertaining to significance tests and contrasts were important for comparison among the genotypes.

– *Repeatability*

In genetics, repeatability, or heritability in the broad sense (H^2), integrates information on genetic variation and environmental “noise” into one statistic that is very useful in planning breeding programmes. It is the proportion of phenotypic variation in a population that is attributable to genetic variation among individuals. Hence, repeatability gives an indication to what extent the phenotypic differences are related to the genotypic differences. In the above experiments, the classical formula, as shown below, was used for the calculation of repeatability.

$$H^2 = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\text{MS}_{\text{entry}} - \text{MS}_{\text{error}}}{\text{MS}_{\text{entry}} + (r-1)\text{MS}_{\text{error}}}$$

where MS_{entry} is the mean square of genotypes and MS_{error} is the residual mean square.

– *Coefficient of variability (C.V.)*

Variation between units of experiments with different measurements and/or plot sizes can be compared by means of *coefficient of variability (or variance)* where the plot standard deviation is expressed as a percentage of the mean, thus

$$C.V. = \frac{\text{Standard deviation}}{\text{Mean}} \times 100\%$$

Independent of the units of measurement, the C.V. is often used for assessing the quality of data from a trial. For a given trait, the lower the C.V. the more precise the experiment is. For specific crops and traits, the acceptable C.Vs. are known from previous experiments. An unusually high C.V. may indicate that the trial was not well managed leading to unreliable results and wrong conclusions.

However, the C.V. on its own can be a poor indicator of quality. Trials have been under disease, pest or other pressures and, as a consequence, provide important genotype discrimination, often produce high C.Vs. Also, high C.Vs. tend to be associated with low yielding trials and the use of C.Vs. as a criterion for rejecting trials may discard a higher proportion of these trials, leading to bias in estimates (Fox *et al.*, 1997b).

– *Coefficient of determination: R-squared*

R-squared is the proportion of variability in the data set that is accounted for by the statistical model. It provides a measure of how well future outcomes are likely to be predicted by the model. Hence, R-squared is a statistic that will give some information about the goodness of fit of a model. The higher the R value, the more precisely the data fit the model. Broadly, R-squared can be seen to be related to the unexplained variance.

For the RCB model used in the trials, the R-squared formula took into consideration the Sum of Squares (SS) of Block and Genotype over the total SS.

$$R^2 \equiv \frac{SS_{block} + SS_{genotype}}{SS_{total}}$$

The most general definition of the coefficient of determination is

$$R^2 \equiv 1 - \frac{SS_{error}}{SS_{total}}$$

The coefficient of determination could further be partitioned into the proportionate contribution of each source of variation in the ANOVA to the total Sum of Square (SS). Of particular interest was the contribution of genotypes to total. The corresponding formula was basically similar to that of R-squared, except that the numerator included only the SS of genotypes. Thus,

$$\text{Genotype contribution to total variation} \equiv \frac{SS_{genotype}}{SS_{total}}$$

The proportion of variation due to blocking could similarly be calculated, or, in the above case, by the simple difference between R-squared values and those of genotypic contribution to total.

$$\text{Block contribution to total variation} \equiv \frac{SS_{block}}{SS_{total}}$$

– *Comparison of means*

The F-tests and the Least Significant Differences (LSDs) were obtained as per the standard methods described in statistical books. The LSD is one of the various *a-posteriori* unplanned contrasts very frequently used in statistics to compare between any two treatment means from an experiment. LSD at 5% level of probability can be determined as $LSD_{0.05} = t_{(0.05)} \times SED$, where $t_{(0.05)}$ is the value from the t-table for error degrees of freedom, and the given level of significance. SED is the standard error of difference between two means. For a RCBD, this is given by $\sqrt{(2s^2/r)}$ where s^2 is the error mean square in the ANOVA and r is the number of replications. If any two means differ by more than the LSD, it is considered to be significantly different.

4.2.3 Results

Tables 4.4 and 4.5 in the following pages summarise the basic statistics of the 29 traits, averaged over the five sub-trials, at plant cane and first ratoon crops respectively.

Table 4.4: Basic statistics of each trait, averaged over 5 sub-trials and standard deviations, for plant cane

	Repeatability	C.V.	R-square	Genotype contribution to total variation	Genotype significance (Pr. > F)	LSD	Min	Max
Cane quality characters measured at pre-harvest season (April) – 1st sampling date								
Brix % (FW)*	0.83 ± 0.09	7 ± 1	0.89 ± 0.06	0.87 ± 0.07	0.00 ± 0.0	0.96 ± 0.17	5.52 ± 1.85	11.38 ± 0.3
Pol. % (FW)	0.78 ± 0.08	12 ± 2	0.86 ± 0.05	0.83 ± 0.07	0.00 ± 0.0	1.14 ± 0.17	2.78 ± 1.29	8.87 ± 0.39
Fibre % (FW)	0.87 ± 0.08	9 ± 2	0.92 ± 0.04	0.86 ± 0.11	0.00 ± 0.0	1.97 ± 0.42	7.77 ± 0.62	21.85 ± 2.81
Drymatter % cane	0.76 ± 0.05	6 ± 1	0.86 ± 0.03	0.78 ± 0.09	0.00 ± 0.0	2.34 ± 0.31	16.49 ± 1.1	29.23 ± 1.98
Brix % (DW)*	0.91 ± 0.08	6 ± 2	0.94 ± 0.05	0.90 ± 0.09	0.00 ± 0.0	3.89 ± 1.1	21.99 ± 8.23	55.04 ± 2.71
Pol. % (DW)	0.86 ± 0.10	9 ± 2	0.91 ± 0.06	0.89 ± 0.07	0.00 ± 0.0	4.12 ± 0.85	13.19 ± 7.34	39.72 ± 1.94
Fibre % (DW)	0.91 ± 0.08	4 ± 1	0.94 ± 0.05	0.90 ± 0.09	0.00 ± 0.0	3.89 ± 1.1	44.96 ± 2.71	78.01 ± 8.23
Cane quality characters measured at early harvest season (July) – 2nd sampling date								
Brix % (FW)	0.89 ± 0.06	7 ± 2	0.93 ± 0.04	0.92 ± 0.04	0.00 ± 0.0	1.38 ± 0.37	6.09 ± 2.12	16.51 ± 0.37
Pol. % (FW)	0.90 ± 0.06	9 ± 3	0.93 ± 0.04	0.92 ± 0.04	0.00 ± 0.0	1.45 ± 0.41	3.90 ± 2.17	14.69 ± 0.36
Fibre % (FW)	0.86 ± 0.06	8 ± 1	0.91 ± 0.04	0.89 ± 0.04	0.00 ± 0.0	2.10 ± 0.19	9.93 ± 0.32	24.23 ± 3.05
Drymatter % cane	0.55 ± 0.19	5 ± 1	0.71 ± 0.12	0.68 ± 0.13	0.01 ± 0.03	2.51 ± 0.62	22.38 ± 2.24	32.89 ± 1.14
Brix % (DW)	0.93 ± 0.02	6 ± 1	0.95 ± 0.02	0.94 ± 0.02	0.00 ± 0.0	4.31 ± 0.35	23.2 ± 7.57	60.27 ± 1.13
Pol. % (DW)	0.93 ± 0.02	7 ± 1	0.96 ± 0.02	0.95 ± 0.02	0.00 ± 0.0	4.25 ± 0.66	14.87 ± 7.73	52.85 ± 0.84
Fibre % (DW)	0.93 ± 0.02	5 ± 1	0.95 ± 0.02	0.94 ± 0.02	0.00 ± 0.0	4.31 ± 0.35	39.73 ± 1.13	76.8 ± 7.57
Morphological characters								
Cane diameter (mm)	0.87 ± 0.04	7 ± 1	0.91 ± 0.02	0.9 ± 0.03	0.00 ± 0.0	2.92 ± 0.48	15.28 ± 1.45	34.96 ± 1.93
Cane Height (cm)	0.69 ± 0.13	7 ± 1	0.81 ± 0.08	0.73 ± 0.08	0.00 ± 0.0	29.97 ± 2.42	165 ± 15	325 ± 26
Stalk number/ha (x1000)	0.73 ± 0.13	16 ± 3	0.82 ± 0.09	0.80 ± 0.10	0.00 ± 0.0	23.29 ± 4.8	46.7 ± 5.6	170.3 ± 12.5
Biomass characters (tha⁻¹)								
Cane yield (FW)	0.67 ± 0.1	16 ± 5	0.8 ± 0.07	0.73 ± 0.06	0.00 ± 0.0	21.96 ± 5.91	33.5 ± 11.5	137.2 ± 14.5
CTL yield (FW)**	0.27 ± 0.22	33 ± 14	0.64 ± 0.11	0.61 ± 0.14	0.24 ± 0.26	16.33 ± 7.82	11.0 ± 1.7	44.6 ± 5.5
CTL yield (DW)**	0.48 ± 0.08	25 ± 3	0.76 ± 0.04	0.70 ± 0.08	0.03 ± 0.03	3.27 ± 0.34	2.8 ± 0.5	12.6 ± 1.1
Total biomass (FW)**	0.60 ± 0.21	16 ± 3	0.81 ± 0.10	0.77 ± 0.13	0.03 ± 0.04	35.16 ± 7.41	47.8 ± 14.1	163.3 ± 22.6
Derived biomass characters (tha⁻¹) from 1st sampling date								
Sugar yield	0.74 ± 0.08	20 ± 3	0.83 ± 0.05	0.81 ± 0.05	0.00 ± 0.0	1.48 ± 0.09	1.2 ± 0.6	8.6 ± 0.9
Fibre yield	0.68 ± 0.09	20 ± 3	0.79 ± 0.06	0.78 ± 0.06	0.00 ± 0.0	3.76 ± 0.87	4.4 ± 1.5	23.2 ± 9.4
Cane yield (DW)	0.64 ± 0.08	18 ± 4	0.77 ± 0.05	0.75 ± 0.05	0.00 ± 0.0	5.45 ± 1.16	7.5 ± 2.7	31.9 ± 8.5
Total biomass (DW)	0.59 ± 0.23	17 ± 4	0.80 ± 0.11	0.78 ± 0.13	0.03 ± 0.05	10.28 ± 4.8	11.5 ± 3.4	40.9 ± 8.9
Derived biomass characters (tha⁻¹) from 2nd sampling date								
Sugar yield	0.82 ± 0.07	18 ± 6	0.89 ± 0.05	0.85 ± 0.04	0.00 ± 0.0	2.52 ± 0.63	1.8 ± 1.2	17.0 ± 1.1
Fibre yield	0.67 ± 0.11	18 ± 4	0.79 ± 0.07	0.75 ± 0.08	0.00 ± 0.0	3.9 ± 1.23	5.2 ± 1.9	24.9 ± 7.3
Cane yield (DW)	0.65 ± 0.11	17 ± 4	0.78 ± 0.07	0.72 ± 0.09	0.00 ± 0.0	6.38 ± 1.84	8.7 ± 3.2	39.4 ± 6.8
Total biomass (DW)	0.61 ± 0.22	16 ± 6	0.82 ± 0.11	0.76 ± 0.16	0.02 ± 0.04	12.08 ± 6.67	13.2 ± 4.0	46.6 ± 7.4

* : In brackets: FW = fresh weights; DW = dry weights; **: Traits measured in 2 replicates only

Table 4.5: Basic statistics of each trait, averaged over 5 sub-trials and standard deviations, for first ratoon

	Repeatability	C.V.	R-square	Genotype contribution to total variation	Genotype significance (Pr. > F)	LSD	Min	Max
Cane quality characters measured at pre-harvest season (April) – 1st sampling date								
Brix % (FW)*	0.81 ± 0.10	7 ± 1	0.87 ± 0.06	0.86 ± 0.07	0.00 ± 0.0	1.04 ± 0.09	6.44 ± 1.2	12.56 ± 0.39
Pol. % (FW)	0.76 ± 0.10	12 ± 1	0.84 ± 0.07	0.81 ± 0.06	0.00 ± 0.0	1.24 ± 0.15	2.88 ± 0.88	9.6 ± 0.4
Fibre % (FW)	0.77 ± 0.10	10 ± 2	0.85 ± 0.07	0.84 ± 0.07	0.00 ± 0.0	2.31 ± 0.25	8.77 ± 1.13	21.18 ± 1.47
Drymatter % cane	0.65 ± 0.12	7 ± 1	0.77 ± 0.07	0.75 ± 0.1	0.00 ± 0.0	2.70 ± 0.29	17.7 ± 1.47	29.27 ± 1.83
Brix % (DW)	0.87 ± 0.05	6 ± 1	0.91 ± 0.03	0.91 ± 0.04	0.00 ± 0.0	4.31 ± 0.66	26.95 ± 6.99	55.7 ± 2.31
Pol. % (DW)	0.81 ± 0.07	10 ± 1	0.88 ± 0.05	0.86 ± 0.04	0.00 ± 0.0	4.60 ± 0.54	13.31 ± 4.5	41.47 ± 2.1
Fibre % (DW)	0.87 ± 0.05	4 ± 1	0.91 ± 0.03	0.91 ± 0.04	0.00 ± 0.0	4.31 ± 0.66	44.3 ± 2.31	73.05 ± 6.99
Cane quality characters measured at early harvest season (July) – 2nd sampling date								
Brix % (FW)	0.85 ± 0.08	8 ± 1	0.90 ± 0.05	0.89 ± 0.07	0.00 ± 0.0	1.71 ± 0.2	6.75 ± 2.14	17.3 ± 0.35
Pol. % (FW)	0.84 ± 0.08	10 ± 1	0.90 ± 0.05	0.88 ± 0.07	0.00 ± 0.0	1.87 ± 0.31	4.32 ± 2.29	15.59 ± 0.39
Fibre % (FW)	0.67 ± 0.22	10 ± 4	0.79 ± 0.21	0.76 ± 0.15	0.02 ± 0.11	3.00 ± 1.3	10.31 ± 0.78	22.55 ± 1.78
Drymatter % cane	0.49 ± 0.19	7 ± 2	0.68 ± 0.12	0.64 ± 0.12	0.02 ± 0.05	3.44 ± 1.03	21.34 ± 1.55	34.45 ± 2.07
Brix % (DW)	0.83 ± 0.16	8 ± 2	0.89 ± 0.10	0.88 ± 0.12	0.00 ± 0.0	6.09 ± 1.5	25.93 ± 7.81	60.04 ± 3.24
Pol. % (DW)	0.85 ± 0.11	10 ± 2	0.90 ± 0.07	0.89 ± 0.09	0.00 ± 0.0	5.92 ± 0.77	17.14 ± 8.45	52.56 ± 2.92
Fibre % (DW)	0.83 ± 0.16	6 ± 1	0.89 ± 0.10	0.88 ± 0.12	0.00 ± 0.0	6.09 ± 1.5	39.96 ± 3.24	74.07 ± 7.81
Morphological characters								
Cane diameter (mm)	0.78 ± 0.08	7 ± 1	0.86 ± 0.06	0.85 ± 0.05	0.00 ± 0.0	3.02 ± 0.54	15.92 ± 1.8	32.24 ± 0.54
Cane Height (cm)	0.67 ± 0.11	8 ± 1	0.79 ± 0.07	0.74 ± 0.08	0.00 ± 0.0	26.6 ± 3.6	152.5 ± 10.5	271 ± 23.6
Stalk number/ha (x1000)	0.80 ± 0.10	14 ± 4	0.87 ± 0.07	0.85 ± 0.07	0.00 ± 0.0	16.5 ± 4.5	33.3 ± 6.1	135.6 ± 28.8
Biomass characters (tha⁻¹)								
Cane yield (FW)	0.52 ± 0.2	22 ± 4	0.7 ± 0.13	0.64 ± 0.14	0.01 ± 0.02	16.9 ± 2.4	19.3 ± 6.6	85.7 ± 5.3
CTL yield (FW)**	0.31 ± 0.28	30 ± 9	0.66 ± 0.15	0.63 ± 0.15	0.23 ± 0.28	7.0 ± 1.7	4.3 ± 0.8	21.7 ± 2.1
CTL yield (DW)**	0.35 ± 0.24	30 ± 8	0.69 ± 0.12	0.65 ± 0.12	0.15 ± 0.17	2.6 ± 0.6	1.7 ± 0.4	8.3 ± 0.8
Total biomass (FW)**	0.54 ± 0.25	21 ± 7	0.78 ± 0.13	0.73 ± 0.12	0.07 ± 0.15	26.1 ± 6.6	27.4 ± 9.8	102.5 ± 6.6
Derived biomass characters (tha⁻¹) from 1st sampling date								
Sugar yield	0.64 ± 0.17	24 ± 6	0.76 ± 0.11	0.75 ± 0.11	0.00 ± 0.0	1.1 ± 0.2	0.8 ± 0.3	6.0 ± 0.95
Fibre yield	0.58 ± 0.12	24 ± 3	0.73 ± 0.08	0.70 ± 0.07	0.00 ± 0.0	2.5 ± 0.5	2.4 ± 0.8	13.0 ± 3.3
Cane yield (DW)	0.53 ± 0.16	22 ± 4	0.70 ± 0.10	0.66 ± 0.1	0.00 ± 0.0	4.0 ± 0.6	4.1 ± 1.5	19.4 ± 1.6
Total biomass (DW)	0.49 ± 0.19	23 ± 6	0.75 ± 0.1	0.72 ± 0.09	0.06 ± 0.09	9.7 ± 2.0	6.8 ± 2.5	26.3 ± 2.6
Derived biomass characters (tha⁻¹) from 2nd sampling date								
Sugar yield	0.72 ± 0.19	24 ± 7	0.82 ± 0.12	0.79 ± 0.12	0.00 ± 0.0	2.0 ± 0.4	1.6 ± 0.6	11.7 ± 1.1
Fibre yield	0.50 ± 0.08	25 ± 5	0.68 ± 0.05	0.64 ± 0.06	0.00 ± 0.01	3.1 ± 0.8	3.2 ± 1.3	14.8 ± 2.5
Cane yield (DW)	0.54 ± 0.21	22 ± 5	0.71 ± 0.13	0.66 ± 0.14	0.02 ± 0.03	5.0 ± 1.1	5.6 ± 2.2	24.3 ± 2.1
Total biomass (DW)	0.52 ± 0.24	23 ± 7	0.77 ± 0.12	0.73 ± 0.13	0.07 ± 0.13	11.6 ± 2.9	8.3 ± 3.1	30.6 ± 2.4

*: In brackets: FW = fresh weights; DW = dry weights; **: Traits measured in two replicates only

– *Repeatability estimates*

Figure 4.2 illustrates the variations in repeatability of the various traits for plant cane and first ratoon crops. Unless stated otherwise, overall averages (av.) were worked out over sub-trials, crop cycles, sampling dates and fresh and dry weights. The broad sense heritability values were consistently higher in plant cane crop than at first ratoon for all the different characters, except stalk number per plot. Dry weights had slightly higher repeatability values than their corresponding fresh weights at both sampling dates and crop cycles.

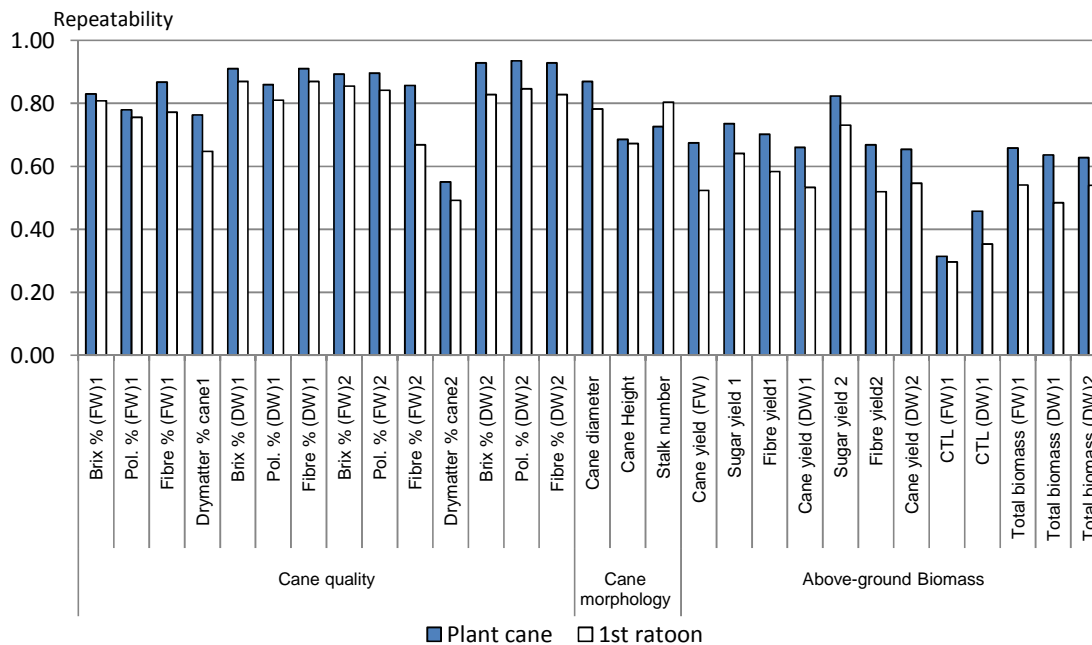


Figure 4.2: Repeatability values, averaged over five sub-trials, of various traits measured at plant cane and first ratoon

FW = fresh weight; DW = Dry weight; suffixes 1 and 2 indicate measurements at the 1st and 2nd sampling dates respectively

Among the cane quality characters, sucrose content (Pol and Brix) had higher repeatability values when evaluated in July (av. = 0.87) than those made in April (av. = 0.79). The higher values at the second sampling date were systematically observed in individual sub-trials and at the two crop cycles. On the other hand, no clear tendencies were observed for fibre content at the two different dates of sampling and the differences were marginal (av. April = 0.85 vs. av. July = 0.82).

Among the cane morphology traits, cane diameter had the highest repeatability values (av. = 0.83), followed by stalk number (av. = 0.76) and average height (av. = 0.68).

Cane yield, fresh and dry weights, had average H^2 -values of 0.6 in the two crop cycles. Among the derived biomass parameters, sugar yield maintained the highest repeatability values in both years (av. = 0.73). Fibre yields, calculated from cane stalks, had estimates close to their corresponding cane yield values (av. = 0.62). The total aboveground biomass yields showed similar tendencies (av. = 0.59). CTL expressed the lowest H^2 -values (av. = 0.36).

– *Coefficient of variances (C.V.)*

The cane quality and cane morphology characters had highly appreciable C.V. values that fluctuated between 5-12% in both crop years. Those for the biomass characters were around 21%. In general, the C.Vs. were lower in plant cane than in first ratoon (Figure 4.3). Dry weight derivatives of the cane quality traits had slightly lower C.Vs.

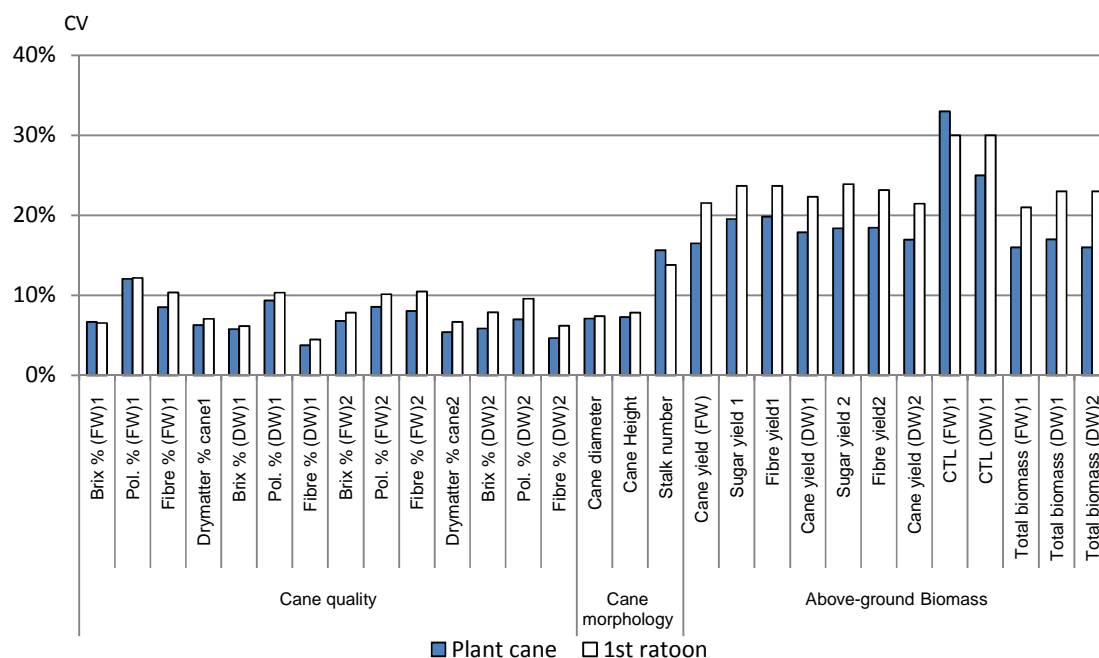


Figure 4.3: Coefficient of variations, averaged over five sub-trials, of traits measured at plant cane and first ratoon crops

FW = fresh weight; DW = Dry weight; suffixes 1 and 2 indicate measurements at the 1st and 2nd sampling dates respectively

The plot size adopted in this study is commonly used at the third clonal stage of selection (stage 4) at the MSIRI breeding programme. With this layout, the C.V. for cane yield roughly ranges between 10% and 35%, subject to the region and crop stand (analysis from past five years data). At Pamplémousses experimental station, where the trials were implemented, the C.V. of cane yield

ranged between 15-30%. The values of 15-20% obtained from this study were, in consequence, indicative of good precision achievable at both plant cane and first ratoon crops.

The C.Vs. of CTL fresh and dry weights ranged between 25-33% and were the highest among all measured traits. No past data were available to validate them. Cane tops and leaves are not weighed under the routine selection programme. The C.Vs. of the total aboveground biomass characters reflected those of cane yield at plant cane and first ratoon crops respectively.

– *Coefficient of determination: R-squared (R^2)*

The R-squared values, partitioned into genotype and block components of variation, for the plant cane and first ratoon crops are illustrated in Figures 4.4 and 4.5. The data overall fitted the RCBD model very well with a mere 18% of the total variation due to environmental noise. The cane quality parameters, once again, showed high reliability (av. $R^2 = 88\%$) with very little unexplained variance. This was followed by cane morphology characters, (av. $R^2 = 84\%$) and biomass characters (av. $R^2 = 76\%$). Traits measured on dry weights had relatively higher values than their corresponding fresh weights. Comparisons between the two crop cycles once again showed a higher prediction power from the plant cane results.

Brix, Pol and fibre contents had R^2 -values around 90%. Dry matter % cane averaged to 75% and was lowest at the second sampling date in both years. Among the biomass characters, sugar yield expressed the highest prediction power (av. $R^2 = 83\%$) with the model, more so at the second sampling date (av. $R^2 = 86\%$). Cane yield averaged to 75%. The lowest fits of the model were obtained with CTL yields fresh weight at both crops (av. $R^2 = 65\%$).

The majority of the variations were due to genotypes (av. = 80%) while blocking (av. = 3%) had little impact in general. Genotype contribution to total variation was consistently higher at the plant cane crop and with dry weight measurements.

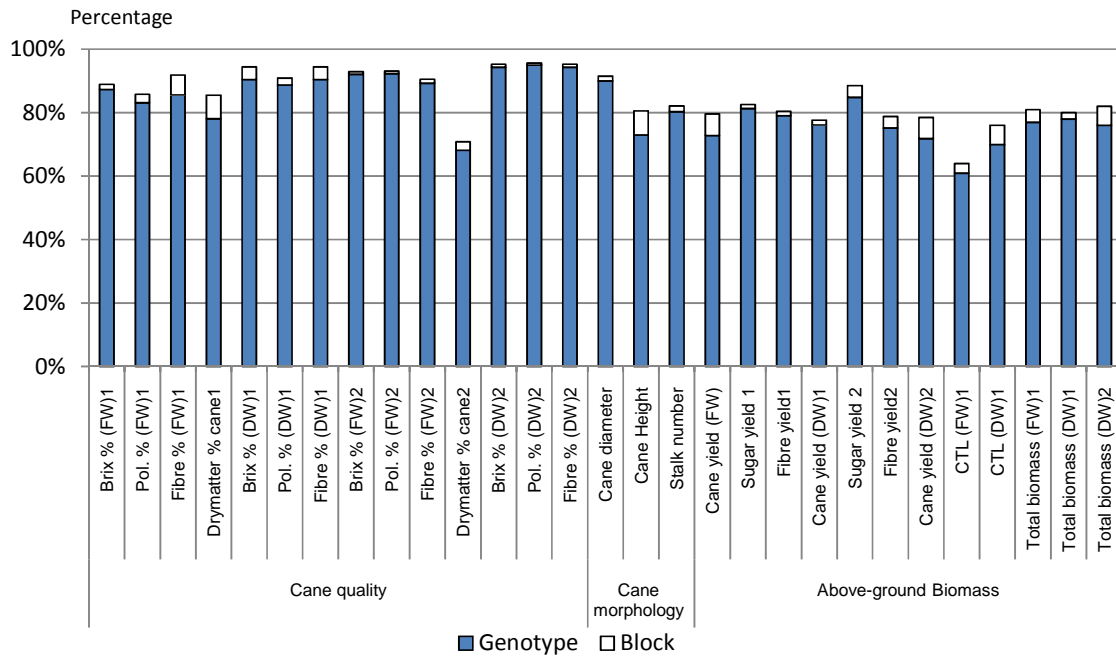


Figure 4.4: Coefficient of determinations (partitioned into genotype and block components) averaged over five sub-trials, for plant cane

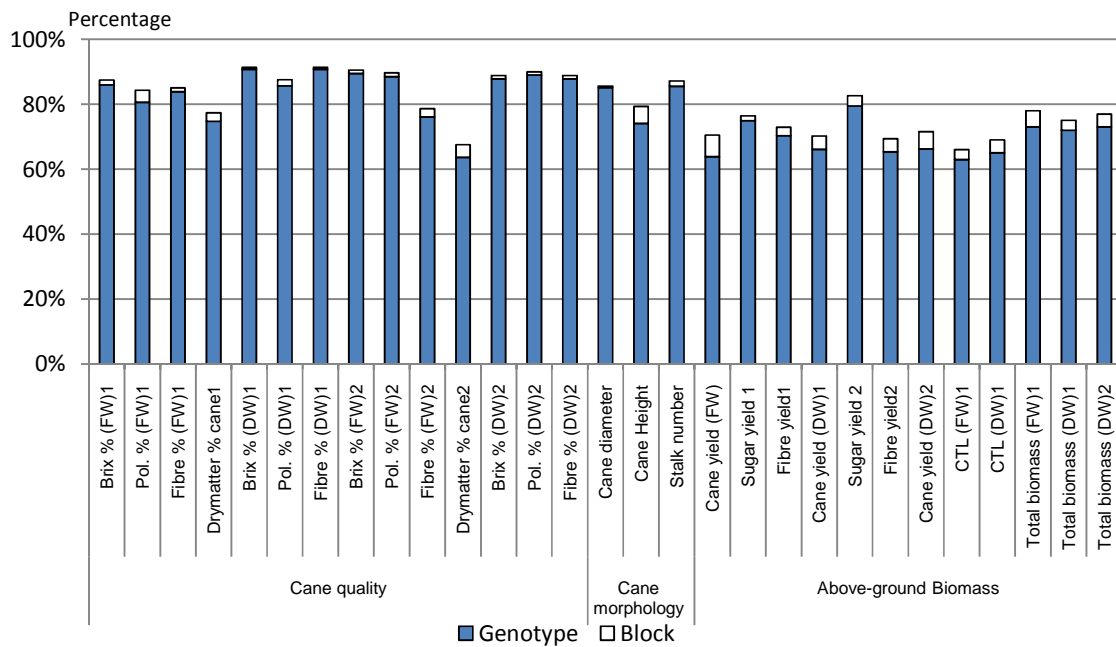


Figure 4.5: Coefficient of determinations (partitioned into genotype and block components) averaged over five sub-trials, for first ratoon

FW = fresh weight; DW = Dry weight; suffixes 1 and 2 indicate measurements at the 1st and 2nd sampling dates respectively

At both plant cane and first ratoon crops, the cane quality parameters showed the highest genotypic contribution to total SS (av. = 86%). For sucrose content estimates (Pol % cane; Brix % cane), the values were steadily higher with samples taken in July (av. = 90%) than those taken in April (av. = 82%). No clear trends were observed for fibre content.

Among the cane morphology characters, variations due to genotype were highest with stalk diameter (av. = 88%) followed by stalk number per plot (av. 84%) and least in cane height (av. = 74%). The contribution of blocks to total variation in the three traits followed the reverse trend: 1% in cane diameter, 2% in cane number and 6% in cane height on average.

The aboveground biomass traits showed an overall genetic contribution of 72% to total variation. Blocking contributed to another 3%. Sugar yield had the highest genetic contribution (av. = 80%) to total variation. On the other hand, CTL genetic contribution was lowest (av. = 66%) among the aboveground biomass characters.

– *Significance tests of genotypes*

The significance of variations due to genotype together with the LSDs in the individual trials have been summarised in Tables 4.4 and 4.5. Of the 290 different ANOVA runs made, blocks were found to be non-significant in 76% of cases. Variations due to genotypes were non-significant in merely 7% of total analyses and these were predominantly observed in CTL parameters.

Highly significant differences were principally observed in the cane quality and cane morphological characters. Cane yield and all derived parameters of economic importance (sugar, fibre and dry matter yields) were significant to highly significant in all the sub-trials, except in sub-trial 5 in the first ratoon, where cane yields, fresh and dry weights, were non-significant.

The variances due to CTL fresh weights were non-significant in sub-trials 1, 2 and 5 at both plant cane and first ratoon crops. Their corresponding dry weight measurements were non-significant in sub-trials 1 and 5 at plant cane and 2 and 5 at first ratoon. This high frequency of non-significance was predictable from the extremely low repeatability estimates, high C.Vs. and low coefficient of determinations observed previously with the CTL traits.

4.2.4 Discussion

– *General observations*

The results presented were averages of five sub-trials which obviously masked the quality of data in individual sub-trials. Nevertheless, the global analyses threw some light on the overall status of the experiment and the traits measured. It became clear that very high precision could be achieved in cane quality characters. The morphological and cane biomass parameters were also of good quality. One exception was with the CTL trait: All the data quality parameters indicated the necessity for higher precision in its measurement, most probably by increasing the sample size, to efficiently discriminate between genotypes for the trait.

The results also showed that, in the majority of analyses, the F-tests for genotypes were significant to highly significant. There were thus high variations among genotypes allowing selection to operate effectively. Moreover, in those trials, paired comparisons between genotypes were statistically valid. The LSD and individual contrasts could be reported with the means in a table.

– *Fresh weight versus dry weight measurements*

Overall, dry weight estimates showed slightly higher precision than their corresponding fresh weights. These results were in agreement with Nayamuth *et al.* (2005) who justified the use of dry weights instead of fresh weights for studies on sucrose accumulation. The precision gained, however, will have to be further established with actual selection simulations made with both fresh and dry weights. Any methodology adopted should be in light of the relative importance of the approach and the associated costs involved. This part of the analysis will be fully developed in chapter seven.

– *Sampling date: pre-harvest (April) versus Early-harvest (July) sampling*

Higher variability and precision in sucrose content assessment were observed in samples taken in July than in April. This tendency was systematically observed in all the five sub-trials and at both crop cycles. There was, however, no clear trend for fibre content between the two dates. Both Pol and fibre contents are obtained from the same sample. It could be reliably formulated that in inter-specific populations, if data collection is to be done only once for evaluation of both characters, then the second sampling date (July) was more reliable for most efficient selection.

– *Plant cane versus first ratoon crops*

Furthermore, it was also evident that, in the current experiment, precision in selection would be higher in plant cane crop than at first ratoon. This was most probably due to the healthier crop stand observed at plant cane than in first ratoon, essentially due to a more favourable climatic condition prevailing in the first year. The grand mean of cane yield fresh weight at plant cane was at 80 t ha^{-1} while at first

ratoon it was around 48 t ha^{-1} . The tendency would naturally be to select in plant cane. However, sugarcane is a crop that is planted once and harvested over several ratoons. In sugarcane breeding, many genotypes have been observed to perform very well in plant cane but their performance decline very rapidly in subsequent crop cycles (personal experience). Hence, the need to analyse test genotype performance, at least, in one ratoon is mostly desirable. In consequence, for the above analyses, both plant cane and first ratoon results had to be given due importance.

4.3 *Calculation of means and adjustments for sub-trial effects*

4.3.1 Introduction

At the MSIRI, the number of genotypes per trial seldom goes beyond 25 and RCB or lattice designs are frequently used with three replicates. In a given location, there exist several such small sub-trials, each bearing the same set of commercial control cultivars, adapted to the region and harvest date. The test genotypes are compared with the average of, or specific, commercial varieties within the sub-trials and each sub-trial is treated independently of the other.

Under such circumstances, thus, there is no need to combine several trials. Selections from individual trials represent the best performing varieties for further evaluation. In this study, however, interest was to obtain comparable means of all the 60 test genotypes that were randomly distributed in five sub-trials. The latter were laid contiguously in the same field with four commercial varieties common in each sub-trial (see section 3.3). Within trial comparisons of any two treatment means were straight forward. However, two genotypes planted in two different trials could not be directly compared as the variations among sub-trials had to be considered. In consequence, to obtain a single list of means of the 64 clones (60 test genotypes and four controls), an adjustment for sub-trial effect was felt necessary. The objective of this study was, in consequence, to devise a methodology to obtain the adjusted means of genotypes from different sub-trials within the same site, using the common commercial varieties as regulators.

4.3.2 Methodology

The methodology basically involved a stepwise approach that could be listed as follows:

- a) Calculation of means of genotypes from individual sub-trials as per the designs adopted
- b) Calculation and testing for significance of variations across sub-trials using the common commercial varieties, and
- c) Adjustment of individual genotype means for sub-trial effects

Figure 4.6 illustrates the procedure using arbitrary values for cane yield (tha^{-1}). Correction of the effects would thus adjust for fertility trends/variations in the field across sub-trials. It was assumed that, within the same field:

- The genotype by sub-trial interaction was negligible,
- The trial effect was additive and
- The test genotypes responded similarly as the control varieties to variations in the field.

The individual genotypes means, in consequence, would be adjusted using an approach similar to that adopted in augmented designs (Federer and Raghavarao, 1975; Lin and Poushinsky, 1983), with sub-trials acting as super-blocks.

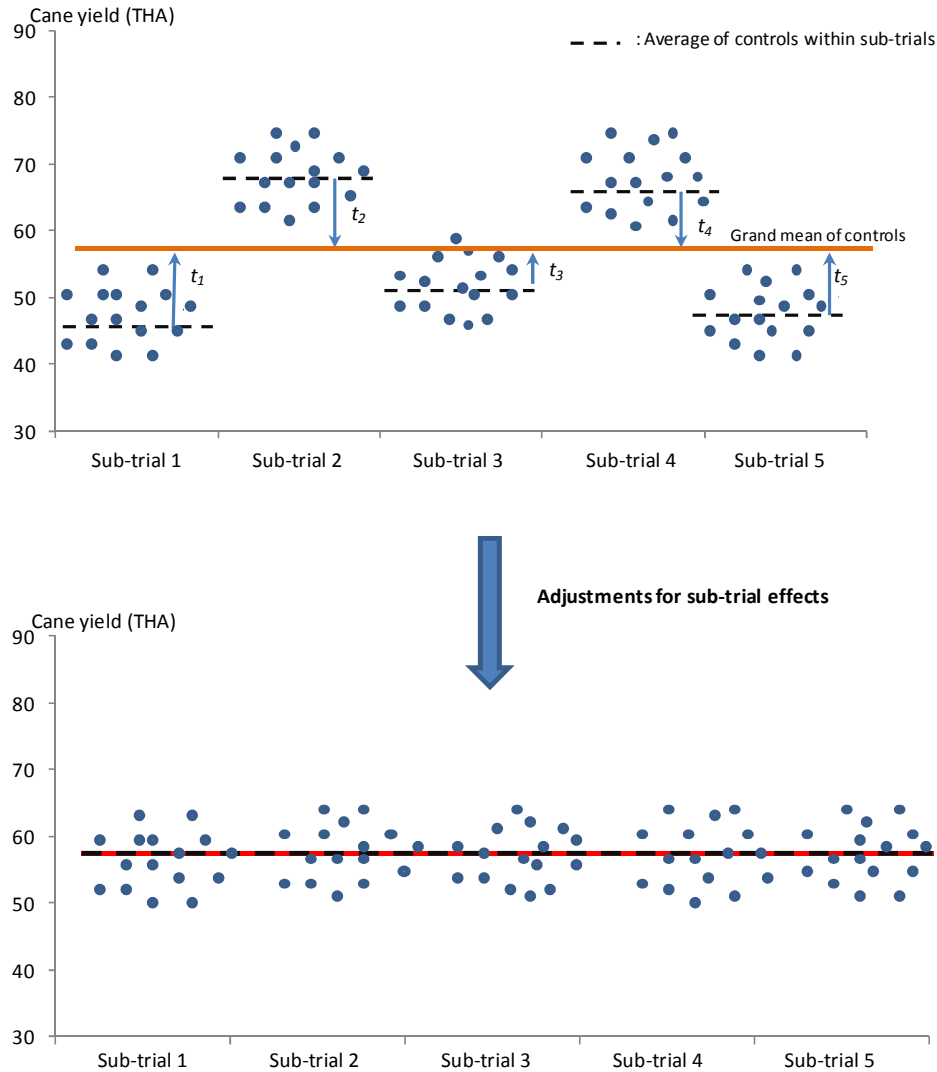


Figure 4.6: A model for cane yield (tha^{-1}) adjustment for sub-trial effects.

Dots represent means of individual genotypes.

Broken horizontal lines: Means of control varieties in individual sub-trials

Continuous horizontal line: Grand mean obtained from control varieties in the five sub-trials

t_i = real effect of i -th sub-trial

– *Calculation of means from individual sub-trials as per the designs adopted*

The means of each genotype were obtained from the analysis of variances as described in the previous section. Since the RCB model was used, the means were represented by the simple averages of the genotypes in the three replicates.

– *Calculation and testing for significance of variations across sub-trials*

The sub-trial effects were obtainable from the common commercial controls in the five sub-trials. Each sub-trial had four commercial varieties, M1400/86, M1176/77, M695/69 and R570, included in the three replicates.

- The mean values of each control variety per sub-trial were calculated.
- The process was repeated in each sub-trial which resulted in four varieties in five sub-trials, the latter now acting as five complete blocks in an RCB design.
- Significance of the sub-trials, as super-blocks, was determined from the analysis of the new trial. A significant F-value for block effect indicated that variation between two sub-trials was considerable and adjustment for trial effect was highly desirable. It was assumed that a non-significant F value meant that variation among sub-trials was negligible and the five sub-trials could be safely merged without any adjustment in the genotype mean values.
- The sub-trial effects were worked out using the method for calculation of block effects. Hence,

$$t_i = \bar{T}_i - \bar{G}$$

Where t_i = real effect of i -th sub-trial,

\bar{T}_i = i -th sub-trial (as block) mean and

\bar{G} = the grand mean of the four commercial controls in five sub-trials

– *Adjustment of individual genotype means for sub-trial effects*

Means of the 64 genotypes altogether were adjusted for sub-trial effects corresponding to the sub-trials in which they appeared and subject to significance tests of the 'super-blocks'. The adjusted means were thus equal to the relative difference between each genotype mean with the average of controls in its respective sub-trial added to the grand mean of the controls. Thus for each genotype in i -th sub-trial:

$$\text{Adjusted mean} = \text{Grand mean of controls} + (\text{genotype mean} - \text{mean of controls in } i\text{-th sub-trial})$$

As a consequence, after adjustments, all the five sub-trials had the same average of controls. Furthermore, the relative distance between each genotype mean with the average of controls, or between any two genotype means within a sub-trial, was unaffected. The difference between any two genotype means from any two different sub-trials could thus be reliably appreciated.

4.3.3 Results

– Sub-trial effects

Irrespective of significance tests, the sub-trial effects were worked out individually for each variable. Table 4.6 summarises the average absolute adjustment applicable and the overall percentage adjustment for each variable. The percentage adjustment for individual genotype mean was calculated as the magnitude of the corresponding sub-trial effect in which the genotype was tested over its original mean value. Hence,

$$\text{Genotype mean \% adjustment} = \frac{|\text{trial effect}|}{\text{genotype mean}} \times 100$$

Table 4.6: Means of absolute trial effects and percentage changes averaged over individual genotype means

	Plant cane crop		First ratoon crop	
	1st sample	2nd sample	1st sample	2nd sample
Cane quality characters				
Brix % (FW)	0.33 (4%)	0.12 (1%)	0.15 (2%)	0.14 (1%)
Pol. % (FW)	0.37 (7%)	0.14 (2%)	0.11 (2%)	0.11 (1%)
Fibre % (FW)	0.31 (2%)	0.29 (2%)	0.53 (4%)	1.09 (7%)
Dry matter % cane	0.46 (2%)	0.29 (1%)	0.58 (3%)	0.97 (3%)
Brix % (DW)	1.33 (3%)	0.59 (1%)	1.12 (3%)	2.10 (5%)
Pol. % (DW)	1.45 (6%)	0.42 (1%)	0.47 (2%)	1.52 (4%)
Fibre % (DW)	1.33 (2%)	0.59 (1%)	1.12 (2%)	2.10 (4%)
Cane morphology				
Cane diameter (mm)	1.64 (4%)		1.29 (5%)	
Cane Height (cm)	7.07 (3%)		12.31 (6%)	
Stalk number/ha	2858 (3%)		5489 (8%)	
Biomass characters (tha⁻¹)				
Cane yield (FW)	3.09 (4%)		5.46 (13%)	
CTL yield (FW)	1.81 (8%)		1.24 (13%)	
CTL yield (DW)	0.38 (7%)		0.41 (11%)	
Total biomass (FW)	5.11 (5%)		6.88 (13%)	
Cane yield (DW)	0.5 (3%)	0.87 (4%)	1.09 (11%)	1.27 (10%)
Sugar yield	0.30 (8%)	0.47 (8%)	0.46 (20%)	0.75 (19%)
Cane fibre yield	0.37 (4%)	0.41 (4%)	0.43 (8%)	0.45 (6%)
Total biomass (DW)	0.72 (3%)	1.42 (5%)	1.44 (11%)	2.09 (13%)

In general, the corrections for sub-trial effects were marginal among the various traits. The magnitude of adjustments was higher in the first ratoon than at the plant cane crop (overall average: plant cane = 5%; 1st ratoon = 7%). The mean correction for each variable at the plant cane crop remained below 10%. At the first ratoon crop, the highest percentage change required was for sugar yield (20%). The

cane quality characters were the least affected, particularly at the second sampling date at the plant cane crop.

– *Analysis of variances and significance tests of sub-trial effects*

Analysis of variance of the four commercial controls in the five sub-trials was done for each trait. The interest was to verify the significance of variation across sub-trials that would validate the necessity of any adjustment for sub-trial effects.

Table 4.7: Sub-trials significance tests

Traits	Plant cane crop		First ratoon crop	
	1st sample	2nd sample	1st sample	2nd sample
Cane quality characters				
Brix % (FW) [@]	ns	ns	ns	ns
Pol. % (FW)	ns	ns	ns	ns
Fibre % (FW)	ns	ns	**	*
Dry matter % cane	ns	ns	ns	ns
Brix % (DW)	ns	ns	**	ns
Pol. % (DW)	ns	ns	ns	ns
Fibre % (DW)	ns	ns	**	ns
Cane morphology				
Cane diameter (mm)		**		**
Cane Height (cm)		ns		ns
Cane number/ha		ns		ns
Biomass characters (tha⁻¹)				
Cane yield (FW)		ns		ns
CTL yield (FW)		ns		ns
CTL yield (DW)		ns		ns
Total biomass (FW)		ns		ns
Cane yield (DW)	ns	ns	ns	ns
Sugar yield	ns	ns	*	ns
Cane fibre yield	ns	ns	ns	ns
Total biomass (DW)	ns	ns	ns	ns

[@]: FW = characters measured on fresh weight; DW = characters measured on dry weight
 ns = Non-significant; *: Significant ($\alpha = 0.05$); ** = Highly significant ($\alpha = 0.01$)

In general, variations between the sub-trials were non-significant in 51 out of 58 different ANOVA runs made (Table 4.7). At the plant cane crop, merely one trait, cane diameter, showed significant variation across sub-trials. The remaining six were observed at the first ratoon crop and were mainly clustered in the first sampling date data. Cane diameter showed highly significant sub-trial effect in both years.

– *Adjustment of genotype means for sub-trial effects*

Following the above results, fine-tuning of the means were done only in traits where variations due to sub-trials were significant.

4.3.4 Discussion

In individual randomised complete block trials, the averages of genotypes obtained from the different replicates are equivalent to their means. No adjustment is necessary as each genotype is represented in each complete block. Adjustments of means is the foundation of many designs with incomplete blocks, the most prominent being augmented designs (Federer and Raghavarao, 1975), the class of lattice designs (Yates, 1936), including α -designs (Patterson and Williams, 1976) and Row and Column designs (Williams and John, 1989). Fine-tuning of means also arise in more complex situations when there is a need to combine several trials, especially multi-environment trials (METs) where not all varieties are tested in all the environments.

ANOVA has the nice feature that the estimators for the variance components are unbiased, but it also has two significant limitations. First, field observations often yield records from different levels of blocking that cannot be analysed jointly with ANOVA. Second, ANOVA estimates of variance components require that sample sizes be well balanced, with the number of observations of each set of conditions being essentially equal. In field situations, individuals are often lost and even the most highly balanced design can quickly collapse.

With the advent of powerful computers and sophisticated software, the last decade of the millennium has seen major improvements in the options available for the analysis of field trials. Patterson and Thompson (1971) provided the methodology for recovery of inter-block information. This led to the development of the Residual Maximum Likelihood (Reml) technique, which has become the standard method for estimating variance components with more than one level of blocking, without imposing any special demands on the design and balance of data. Various software have been developed to this end, but the most sophisticated one with high predictive power and with all the necessary functions required to predict genetic effects seem to be ASReml (Wei *et al.*, 2007). The software is specifically designed for fitting linear mixed models (LMMs) for large data sets, with high model flexibility and speed.

A mixed model is basically a linear model, in which more than one effect is random, and in addition, there is at least one fixed effect apart from the general mean (Piepho, 2005). The fixed effects can be estimated by Best Linear Unbiased Estimation (BLUE), while random effects are estimated by Best

Linear Unbiased Prediction (BLUP). There is often considerable debate among statisticians as to the classification of variety effects as fixed or random. Smith *et al.* (2005) made an overview of the mixed model approaches in the analysis of crop cultivar breeding and evaluation trials. They believe that variety effects should be assumed to be random since BLUPs are shrunk estimates, the amount of shrinkage depending on heritability. This minimizes selection errors when identifying the best varieties, it provides more realistic predictions of genetic gain and allows a valid analysis of data combined across stages of selection.

Overall, the advantages using LMMs include the ease with which incomplete data can be handled, the ability to use more realistic within-trial models for error variation (e.g. incomplete blocks, spatial, correlation models) and the ability to assume some sets of effects (e.g. variety and/or environment effects) to be random rather than fixed. Moreover, the mixed-model approach allows an analysis of multi-environment-trials relative to mean performance, genetic by environment interaction and genotype stability in a unique framework. Mixed-model prediction uses information from an entire data set to obtain environment-specific inferences, allowing prediction of genotype performance even in environments where the genotype was not tested (Balzarini *et al.*, 2001). The approach has been extensively used for evaluating and predicting the genetic merit in animals and is now widely used in many areas of research. Despite the clear benefits of the general mixed model approach, however, adoption within plant breeding and crop variety evaluation programmes has been very slow (Smith *et al.*, 2005).

In the analysis of field trials, furthermore, traditionally, the principal method of handling spatial variation was through the use of complete and incomplete block designs. These traditional designs consider spatial heterogeneity among blocks and attempt a planned reduction of the experimental error. This approach does not consider the presence interplot competition and of spatial variability within blocks. Following the appealing idea presented by Papadakis (1937) and developed by Wilkinson *et al.* (1983), there has been growing interests in developing techniques to adjust a plot for spatial variability by using information from the immediate neighbours. Important landmarks involve the class of autoregressive-integrated-moving average models (ARIMA) proposed by Gleeson and Cullis (1987), extension of the model in two directions in the field by Cullis and Gleeson (1991), and identification of global, natural and extraneous variations in the experimental trial by Gilmour *et al.* (1997).

As a result, the most advanced method of analysing plant breeding trials in a single frame currently seems to be the removal of spatial trends through the spatial analysis methods, and the use of factor-analytic models for combining the different trials with some genotypes in common. The spatial mixed

linear model is also the base model in which competition effect is taken into account (personal communications – Stringer, 2009). However, running these types of combined analyses requires specialised training in model building and further familiarisation with the latest version of ASReml software, as pulling data across trials, sites and regions, the model gets complex and both difficult and computationally intensive to run. For these reasons, this new technology was not adopted in this study as it was felt necessary to acquire greater knowledge and guidance in using the sophisticated ASReml software.

A simple methodology was devised to obtain comparable means of the 64 genotypes evaluated in this study. There were mainly two levels of blocking in the field: first, the complete replicates within each sub-trial and second, the five sub-trials planted contiguously in the same field with the commercial varieties as the only common factors. The conventional ANOVAs were adopted to calculate means of genotypes within each sub-trial. Inter-plot competition was ignored, although, in the presence of high biomass varieties with fast growth rates, such an effect might be considerable. The genotype means were adjusted for sub-trial effects, subject to the significance of the latter at 95% probability. Adjustments of genotype means for sub-trial effects were, in general, marginal, accounting to some 5% at plant cane and 7% at first ratoon crops with all the traits confounded. The sub-trial effects were also non-significant in the majority of the traits involved. In those cases, the original means of the 64 genotypes, obtained from individual sub-trial analyses, were used for further inferences. Significance was mostly observed in some traits from the first sampling date of the first ratoon crop and with cane diameter. For these traits, the genotype means were accordingly fine-tuned for the sub-trial effect. Unless stated otherwise, the new means were used in the rest of this study.

CHAPTER 5

5 Multivariate analysis approach in the identification of high sucrose and high biomass varieties in a germplasm of *Saccharum* sp. and allied genera

5.1 Introduction

In new breeding experiments, many traits are measured, often more than what are directly required, with the hope that they might lead to the discovery of hitherto unlocked genetic relationships, or become useful at a given point in the analyses. On the whole, this leads to an array of correlated characters that need to be reduced based on their relative importance and contribution to total variation. Most statistical methods described in elementary texts, however, pertain to univariate data analysis because they are only concerned with analysing variation in a single random variable. Multivariate data analysis (MVDA), on the other hand, considers several related random variables simultaneously, each one being considered equally important at the start of the analysis (Manly, 1988).

MVDA essentially models reality where each situation, product, or decision involves more than a single variable. Despite the quantum of data available in a table, the ability to obtain a clear picture of what is going on and make intelligent decisions is a challenge. MVDA can provide a summary, or overview, of the table. It is possible to identify the dominant patterns in the data that can be displayed graphically. The pattern can help analyse groups (or clusters) in the table, how these groups differ, and to which group individuals belong. Furthermore, with MVDA, it is possible to find relationships between different traits and the product quality. The objective is to use one set of variables to predict another, for the purpose of optimisation, and to find out which variables are important in the relationship. According to Becker and Leon (1988), MVDA has three main purposes: to eliminate noise from the data, to summarize the data and to reveal the structure in the data.

The technique has been widely used to measure the diversity in germplasm collections and to assess the relative contributions that various traits make to the total variability in a crop collection (Flores *et al.*, 1997; Pezzotti *et al.*, 1994; Popi *et al.*, 2001; Sapra and Lal, 2003; Yao *et al.*, 2007). Multivariate techniques are also commonly applied in stability analysis to provide further information on real multivariate response of genotypes to environments (Fox *et al.*, 1997a). In sugarcane, the techniques have been used in the classification of parents in germplasm collections, METs and analysis of factors contributing to sugarcane yield (Ferraro *et al.*, 2009; Hemaprabha *et al.*, 2005; Nair *et al.*, 1998; Panhar *et al.*, 2003; Wang *et al.*, 2008).

The objectives of this study were to use the multivariate data analysis techniques to examine the extent of genetic divergence between the genotypes in the experiment, identify selectable clones with high biomass and high sucrose properties and determine the contribution of major traits within specific groups in the collection.

5.2 *Materials and methods*

5.2.1 The data

From the previous analyses, it was found that the second sampling date data (July vs. April) were more precise in the joint determination of sucrose concentration and fibre content. Particularly for sucrose content, broad-sense heritability values and genetic contribution to total variances were systematically higher in all sub-trials and crop cycles when measured in July (see section 4.2). No clear indications were obtained for fibre content in the two crop cycles. Since the multivariate study aimed at selection of different types of canes from the population, use of data giving highest precision, especially from the sampling date point of view, was felt necessary. For this reason, all data collected in April were avoided. As a result, 18 inter-related parametric characters were obtained in each crop cycle. The new mean values obtained from chapter four, averaged over the two crop cycles, were used for the multivariate analyses.

5.2.2 The multivariate analyses

– Principal component analysis (PCA)

The statistical underpinnings of multivariate approaches have been described in many books and summarised by Manly (1988). PCA is designed to reduce the number of variables that need to be considered to a small number of indices that are linear combinations of the original variables. The reduction is achieved by linear transformation of the original variables into new set of uncorrelated variables known as Principal Components (PCs). The first step in PCA is to calculate the eigenvalues, which define the amount of the total variation that is displayed on the PC axes. The first PC summarises most of the variability present in the original data relative to all remaining PCs. The second PC summarises most of the variability not explained by the 1st PC and uncorrelated with the first, and so on. The advantage of using PCA is that it accounts for a large proportion of variability in its first components with subsequent dimensions accounting for diminishing percentage of pattern and increasing percentage of noise. The other main advantage of PCA is that once the patterns have been found, the data can be compressed, by reducing the number of dimensions without much loss of information.

The proportion of variation accounted by each PC is expressed by the eigenvalue divided by the sum of the eigenvalues. The eigenvector defines the relation of the PC axes to the original data axes. PCA can be performed on two types of data matrices: a covariance matrix and a correlation matrix. With characters of different scales, a correlation matrix standardising the original data set is preferred (Manly, 1988).

The PCA methodology was used to reduce the dimension and attempt to find patterns in the data. The PCA was done with the 18 inter-related parametric traits. The eigenvalues and eigenvectors and the principal component scores were obtained using Genstat discovery edition 3 software. Since the data were of different scales, the correlation matrix was chosen. The eigenvalues approaching one or more were extracted.

– *Cluster analysis*

The term *cluster analysis* encompasses a number of different algorithms and methods for grouping objects of similar kind into respective categories. It is an exploratory data analysis tool which aims at sorting different objects into groups in a way that the degree of association between two objects is maximal if they belong to the same group and minimal otherwise.

Many algorithms have been proposed for cluster analysis (Manly, 1988). Clustering methods, such as UPGMA (Unweighted Pair Group Method with Arithmetic mean, also known as average linkage method) is an agglomerative of “*hierarchical clustering*” method used in bioinformatics for the construction of phylogenetic trees. This technique was used to group similar genotypes into classes. The basic inherent properties of the clusters were determined and those of economic importance were identified.

5.3 Results

– *Principal Component Analysis*

PCA condensed the 18 parameters into seven components, which accounted for 100% of the variability existing among the genotypes evaluated. Figure 5.1 illustrates the relative importance of the first five PCs (roots) of total variance. Altogether, they described 97% of the total variation. Only the first four PCs had values greater than one. Patterns were found and the first two PCs explained the majority (79%) of the variances in the data.

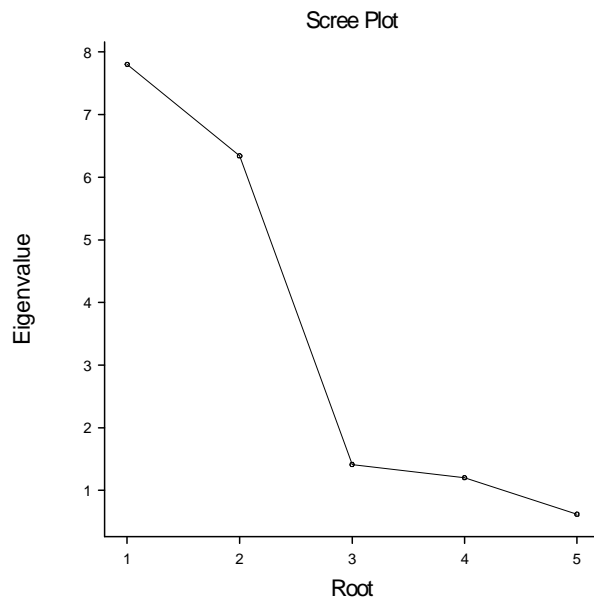


Figure 5.1: Scree plot of first five principal components (roots) and their relative eigenvalues explaining the proportion of variation in the data.

Table 5.1 demonstrates the eigenvectors, eigenvalues of individual traits and percentage of variation explained by the first five PCs. PC1 was related to cane quality characters. PC2 could be explained by variables contributing to the aboveground biomass. Total biomass was associated with cane yield, CTL yield and cane morphology, namely cane diameter, height and cane number. PC3 showed one dimension of the data, not explained by the first two PCs, where cane dry matter percent was positively associated with sucrose content and with stalk number per hectare but negatively associated with stalk diameter. PC4 gave another dimension where dry matter percent cane was positively associated with fibre percent cane but negatively linked to stalk number. PC5 highlighted the contribution of CTL biomass in the total variations and its association with cane diameter.

Table 5.1: Eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first five principal components (PC) for 18 morpho-agronomic traits of sugarcane clones. Loadings with magnitude greater than 2.5 have been highlighted.

	PC 1	PC 2	PC 3	PC 4	PC 5
Cane quality Characters					
Brix % (FW)*	0.28	-0.21	0.24	0.15	-0.01
Pol % (FW)	0.28	-0.21	0.23	0.17	-0.02
Fibre % (FW)	-0.31	0.12	0.12	0.34	0.00
Drymatter % cane	-0.15	-0.03	0.48	0.64	0.00
Brix % (DW)*	0.31	-0.18	0.12	-0.07	-0.01
Pol % (DW)	0.30	-0.21	0.14	0.00	-0.04
Fibre % (DW)	-0.31	0.19	-0.11	0.07	0.01
Cane morphology					
Cane diameter (mm)	0.16	-0.21	-0.47	0.26	-0.32
Cane height (cm)	-0.26	-0.11	-0.15	0.19	-0.10
Stalk number (ha)	-0.22	-0.04	0.45	-0.44	0.31
Cane Biomass characters					
Cane yield (FW)	-0.14	-0.35	-0.17	-0.06	0.23
Cane yield (DW)	-0.16	-0.34	-0.07	0.10	0.24
CTL yield (FW)	-0.22	-0.22	0.14	-0.21	-0.60
CTL (DW)	-0.26	-0.17	0.25	-0.16	-0.50
Sugar yield	0.12	-0.37	0.03	0.03	0.13
Fibre yield	-0.29	-0.21	-0.10	0.13	0.20
Total biomass (FW)	-0.16	-0.34	-0.14	-0.14	0.06
Total biomass (DW)	-0.17	-0.34	-0.02	0.01	0.09
Eigen values	7.8	6.339	1.412	1.202	0.619
Individual percentages	43.33	35.22	7.84	6.68	3.44
Cumulative percentages	43.33	78.55	86.39	93.07	96.51

*: in brackets:- FW: Fresh weight; DW: Dry weight

Salient features observed from the first PC were the negative associations between sucrose content estimates (Brix and Pol) and fibre percent, between cane diameter and cane height, and between cane diameter and stalk number. The latter was also visible in PC3, PC4 and PC5. Furthermore, the loadings were higher with dry weight measurements than their corresponding fresh weight counterparts. This indicated a higher variation with dry weight measurements.

These observations were confirmed by the actual phenotypic correlation coefficients obtained between the different traits (Table 5.2). Laboratory Brix was very highly correlated with Pol (r -value = 1.0**) indicating that any of the two characters could be interchangeably used for the estimation of sucrose content. The latter, in turn, was very highly negatively correlated with fibre content (r values < -0.7**). The total negative correlation (r = -1.00**) observed between Brix and fibre percent dry

weights was natural as only the two traits were involved in the determination of total dry matter content from the cane samples.

Sucrose content association with cane diameter was significantly positive (r-values $> 0.5^{**}$) but negative with cane height (r-values $< -0.4^{**}$) and stalk number (r-values $< -0.35^{**}$). Conversely, high fibre varieties tended to have thin cane stalks (r-values $< -0.5^{**}$), tall (r-values $> 0.5^{**}$) and dense canes (r-values $> 0.35^{**}$). Furthermore, the fresh and dry weight measurements were significantly and very highly correlated (r-values $> 0.90^{**}$). This was more applicable to aboveground biomass characters, where the correlation coefficients were around 0.97^{**} .

Table 5.2: Correlation coefficients between different quantitative traits

	Brix % (FW)	Pol. % (FW)	Fibre % (FW)	Dry matter % cane	Brix % (DW)	Pol. % (DW)	Fibre % (DW)	Cane diameter	Cane height	Stalk no.	Cane yield (FW)	Cane yield (DW)	CTL yield (FW)	CTL yield (DW)	Fibre yield	Sugar yield	Total biomass (FW)
Cane quality characters																	
Pol. % (FW)	1.00**																
Fibre % (FW)	-0.73**	-0.72**															
Dry matter % cane	-0.01 ^{ns}	0.00 ^{ns}	0.68**														
Brix % (DW)	0.94**	0.94**	-0.90**	-0.30*													
Pol. % (DW)	0.97**	0.97**	-0.85**	-0.21 ^{ns}	0.99**												
Fibre % (DW)	-0.95**	-0.94**	0.90**	0.31*	-1.00**	-0.99**											
Cane morphology																	
Cane diameter	0.51**	0.52**	-0.51**	-0.26*	0.53**	0.55**	-0.54**										
Cane height	-0.44**	-0.42**	0.55**	0.34**	-0.51**	-0.45**	0.50**	-0.09 ^{ns}									
Stalk number	-0.35**	-0.36**	0.38**	0.24 ^{ns}	-0.36**	-0.36**	0.36**	-0.68**	0.28*								
Cane biomass characters																	
Cane yield (FW)	0.09 ^{ns}	0.11 ^{ns}	-0.01 ^{ns}	0.05 ^{ns}	0.06 ^{ns}	0.11 ^{ns}	-0.08 ^{ns}	0.34**	0.52**	0.29*							
Cane yield (DW)	0.08 ^{ns}	0.10 ^{ns}	0.15 ^{ns}	0.28*	-0.02 ^{ns}	0.05 ^{ns}	0.00 ^{ns}	0.27*	0.56**	0.32*	0.97**						
CTL [@] yield (FW)	-0.18 ^{ns}	-0.16 ^{ns}	0.29*	0.23 ^{ns}	-0.23 ^{ns}	-0.17 ^{ns}	0.21 ^{ns}	-0.03 ^{ns}	0.52**	0.51**	0.61**	0.62**					
CTL yield (DW)	-0.27*	-0.26*	0.45**	0.36**	-0.36**	-0.30*	0.34**	-0.21 ^{ns}	0.57**	0.63**	0.54**	0.58**	0.93**				
Fibre yield	-0.37**	-0.35**	0.56**	0.40**	-0.48**	-0.41**	0.46**	-0.01 ^{ns}	0.72**	0.45**	0.81**	0.88**	0.65**	0.68**			
Sugar yield	0.75**	0.76**	-0.55**	-0.03 ^{ns}	0.71**	0.76**	-0.73**	0.59**	0.01 ^{ns}	-0.08 ^{ns}	0.70**	0.66**	0.26*	0.13 ^{ns}	0.22 ^{ns}		
Total biomass (FW)	0.02 ^{ns}	0.03 ^{ns}	0.05 ^{ns}	0.05 ^{ns}	-0.01 ^{ns}	0.04 ^{ns}	-0.01 ^{ns}	0.27*	0.54**	0.36**	0.97**	0.94**	0.73**	0.65**	0.81**	0.63**	
Total biomass (DW)	0.07 ^{ns}	0.08 ^{ns}	0.15 ^{ns}	0.25 ^{ns}	-0.03 ^{ns}	0.04 ^{ns}	0.01 ^{ns}	0.24 ^{ns}	0.55**	0.38**	0.94**	0.97**	0.72**	0.69**	0.84**	0.65**	0.97**

In brackets: FW = Fresh weight; DW = Dry weight

*: $P \leq 0.05$; **: $P \leq 0.01$; ns: Non-significant

@: CTL = Cane Tops and Leaves

The whole data set could safely be compressed into the first two PCs without much loss of information. Figure 5.2 illustrates the two dimensional distribution of genotypes based on their Euclidean distances. High sucrose genotypes clustered to the right of the biplot. Because sucrose concentration was negatively correlated with fibre content (Table 5.2), high fibre clones with low sugar content were observed further to the left. The *Erianthus* 'wild' relatives came together in the fourth quadrant. Commercial control varieties (M1400/86, M1176/77, M695/69 and R570) clustered to the extreme right in the second quadrant. The upper part of the biplot was represented by high biomass yielding clones while the poor yielders grouped further down in the graph.

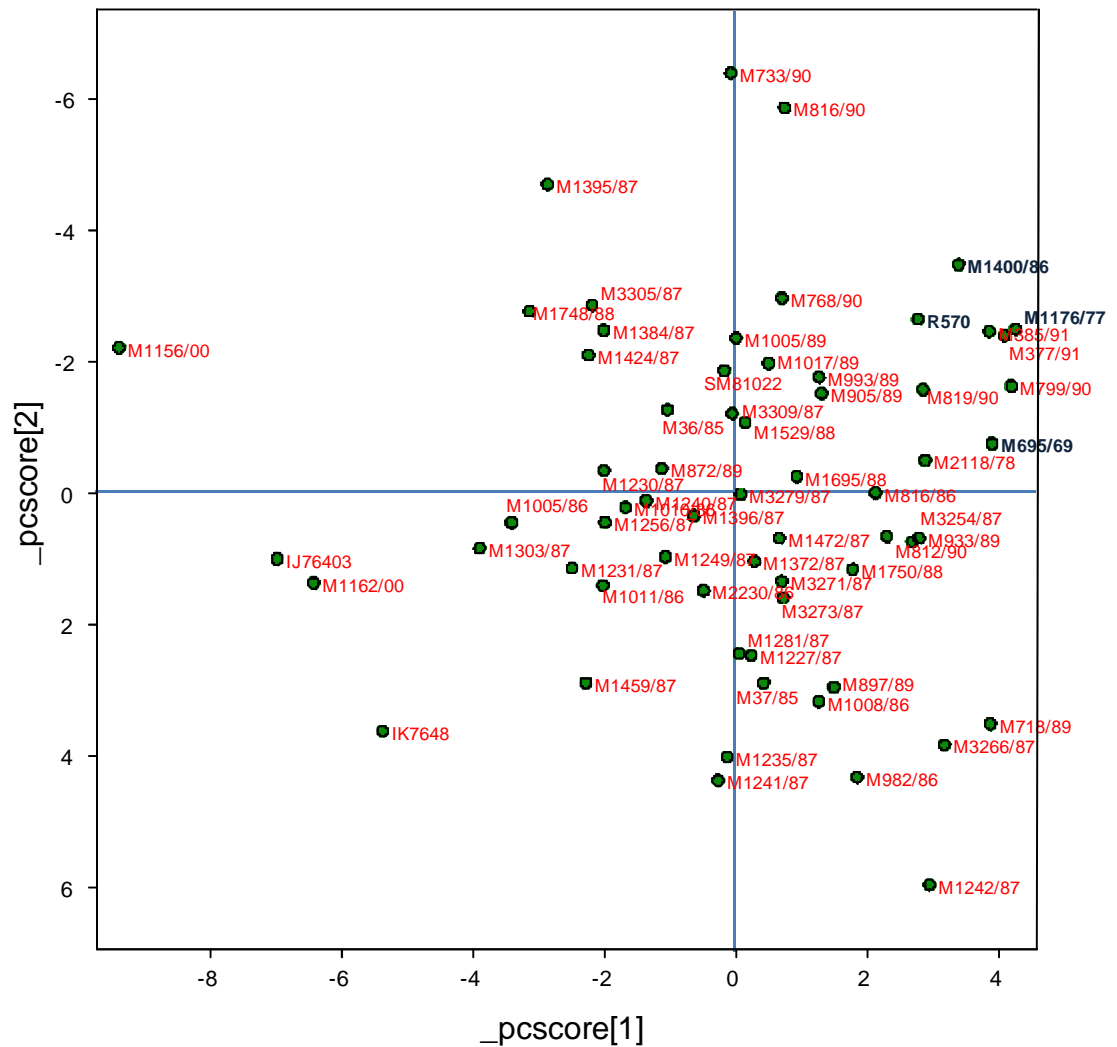


Figure 5.2: A biplot distribution of genotypes displaying cane quality characters (pcscore[1]) against above ground biomass characters (pcscore[2]). In bold: Commercial varieties

Influential values were likely to appear isolated from the body of points in the plot. The ‘wild’ clones (IJ76403 and IK7648) along with M1162/00 were found outlying in the fourth quadrant. To the extreme left, one genotype, M1156/00, stood outstanding in the third quadrant. Further up and more towards the centre, three genotypes, M1395/87, M733/90 and M816/90, could be observed remote from the main cluster of points. In the first quadrant, M1242/87 could be observed as the furthest bottom dot. The main characteristics, measured on fresh weight basis, of these selected clones and a commercial control variety (M1400/86, in bold) are shown in Table 5.3. Their relative ranks over the 64 genotypes evaluated in the experiment are shown in brackets.

Table 5.3: Main features of outlying clones and their ranks (in brackets) among the 64 genotypes evaluated. Measurements made on fresh weight

Genotypes	Type	Brix %	Fibre %	Cane diameter (mm)	Cane height (cm)	Stalk number (x1000)	Cane yield	Leaf yield	Fibre yield	Sugar yield	Total biomass
M1400/86	Control	15 (7)	12 (63)	30 (3)	214 (49)	62 (55)	81 (8)	16 (38)	9 (39)	10 (3)	103 (8)
M1162/00	F1	5 (62)	22 (4)	23 (35)	273 (3)	87 (24)	74 (17)	21 (12)	16 (3)	3 (60)	104 (6)
IJ76403	<i>Erianthus</i>	5 (63)	23 (3)	21 (46)	280 (2)	104 (13)	87 (5)	15 (43)	19 (2)	3 (56)	101 (11)
IK7648	<i>Erianthus</i>	5 (64)	24 (2)	25 (23)	261 (8)	74 (45)	54 (44)	18 (25)	12 (12)	2 (64)	74 (39)
M1156/00	F1	6 (61)	24 (1)	23 (37)	281 (1)	106 (11)	100 (2)	29 (1)	23 (1)	4 (51)	132 (2)
M1395/87	BC1	13 (15)	17 (20)	23 (40)	268 (6)	123 (5)	91 (4)	26 (3)	16 (5)	10 (5)	116 (4)
M733/90	BC2	15 (9)	14 (53)	28 (8)	246 (18)	79 (34)	101 (1)	22 (11)	14 (7)	12 (2)	135 (1)
M816/90	BC2	16 (4)	14 (52)	29 (7)	250 (14)	80 (32)	94 (3)	22 (9)	13 (9)	13 (1)	116 (3)
M1242/87	F1	11 (45)	16 (35)	15 (64)	184 (62)	73 (47)	22 (64)	7 (64)	4 (64)	2 (62)	30 (64)

All the genotypes had some individual or collective features where they were exceptional. The *Erianthus* species and their progenies, M1162/00 and M1156/00, had negligible sucrose content, very high fibre and were relatively tall canes. Their total biomass yields were generally equivalent to the commercial variety. Genotype M1156/00 had the highest CTL biomass and was one of the top total biomass yielders in the experiment. M1395/00 was intermediate between the control variety and the *Erianthus* clones in terms of cane quality characters (sucrose and fibre). It was, however, among the top cane, CTL and total biomass yielders. M733/90 and M816/90 had sucrose content roughly equal to the commercial variety but had slightly higher fibre content. Their stalk diameter was appreciable. They were also among the highest cane, sugar and total biomass yielders. M1242/87 performed very poorly, in general, with relatively low sucrose content and the lowest biomass yield.

Variation among cross categories

In sugarcane interspecific crossing programme, the F1 generation is obtained by crossing noble canes (or hybrids with high sucrose content) with compatible individuals of different species or genus. The latter usually have little or no sugar but are highly vigorous and resistant to major sugarcane pests and diseases. The F1 progenies are usually low in sucrose content but acquire most of the desirable features of the 'wild' clones. Introgression for high sucrose is done in successive generations of backcrossing (BC) with a noble or hybrid cane with high sugar content (see section 2.3.1). Generally, two to three such backcrosses are sufficient to attain appreciable sucrose levels and to dilute the undesirable features of the wild clones.

The present experiment comprised 2 'wild' *Erianthus spp.*, 23 F1, 29 BC1, 6 BC2 and four commercial clones. It was found desirable to see how these different cross categories were distributed with respect to their cane quality and biomass characteristics. Figure 5.3 displays the previous graph with the individuals replaced by their corresponding cross codes. A diagonal from bottom left to top right, intersecting the origin and the mean of the commercial varieties, could be visualised as the success of introgression. There seemed to be a trend retracing the progress due to introgression achieved in interspecific crossing programme. The 'wild' *Erianthus* clones and their progenies (see Table 3.1 for parentage) were at the bottom end. The F1 clones clustered next to them along the diagonal, followed by the BC1 progenies. The BC2 and commercial clones clustered at the top of the line. The figure also depicted that progress with introgression was variable as some BC1 genotypes clustered with the F1 clones whereas some F1 individuals were very close to the commercial hybrids. Similarly, some BC1 clones were very close to the commercial varieties.

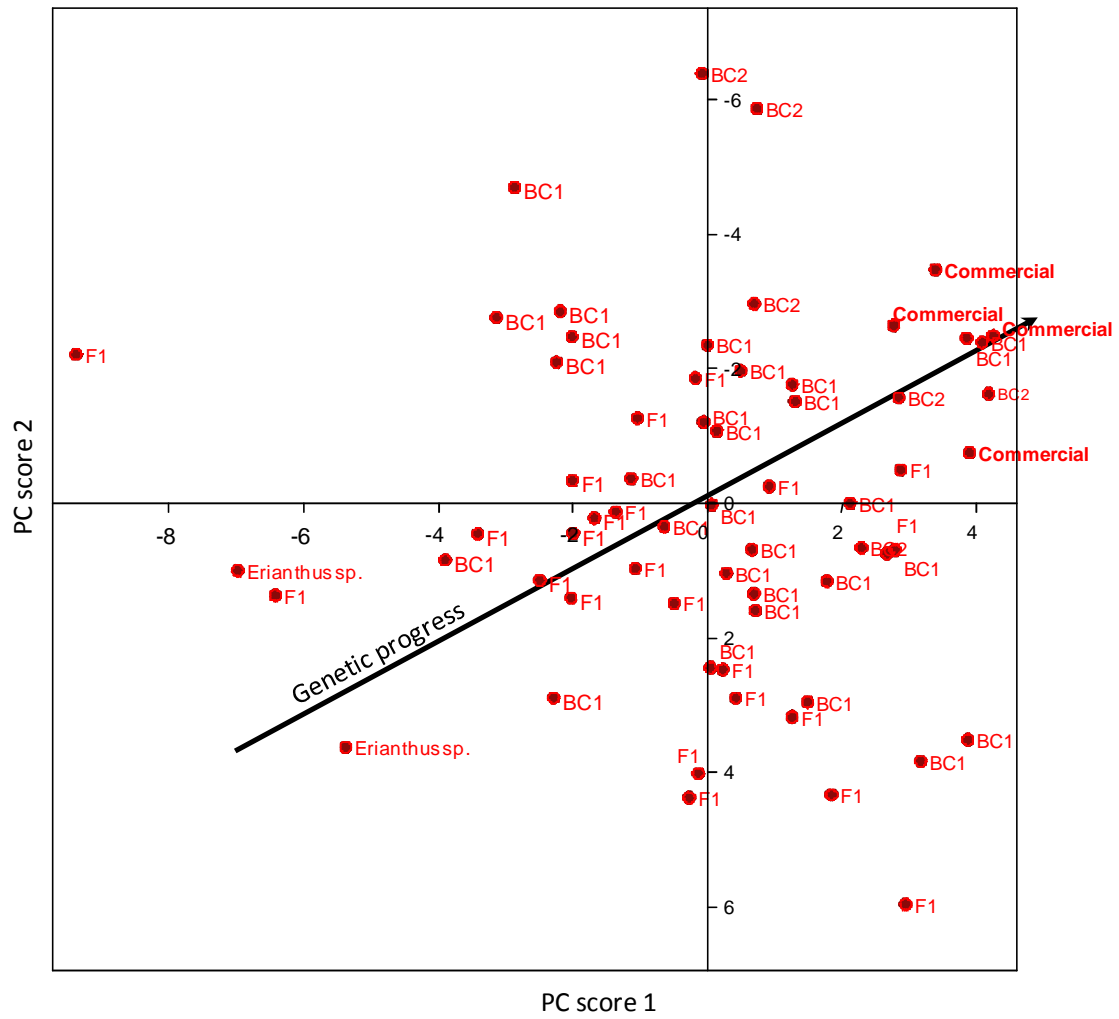


Figure 5.3: A biplot distribution of genotypes of different generations displaying the progress of introgression in interspecific crosses.

– Cluster Analysis

The UPGMA technique of cluster analysis was applied on standardised traits (mean = 0 and variance = 1), which produced a dendrogram of the 64 genotypes based on their Euclidean distances (Figure 5.4). Six major clusters could be identified. The first group was the most distant from the rest. It comprised two *Erianthus* species (IJ76403 and IK7648) and two selfed clones from crosses involving *Erianthus* parents (see Table 3.1 for parentage). The commercial varieties clustered in group five.

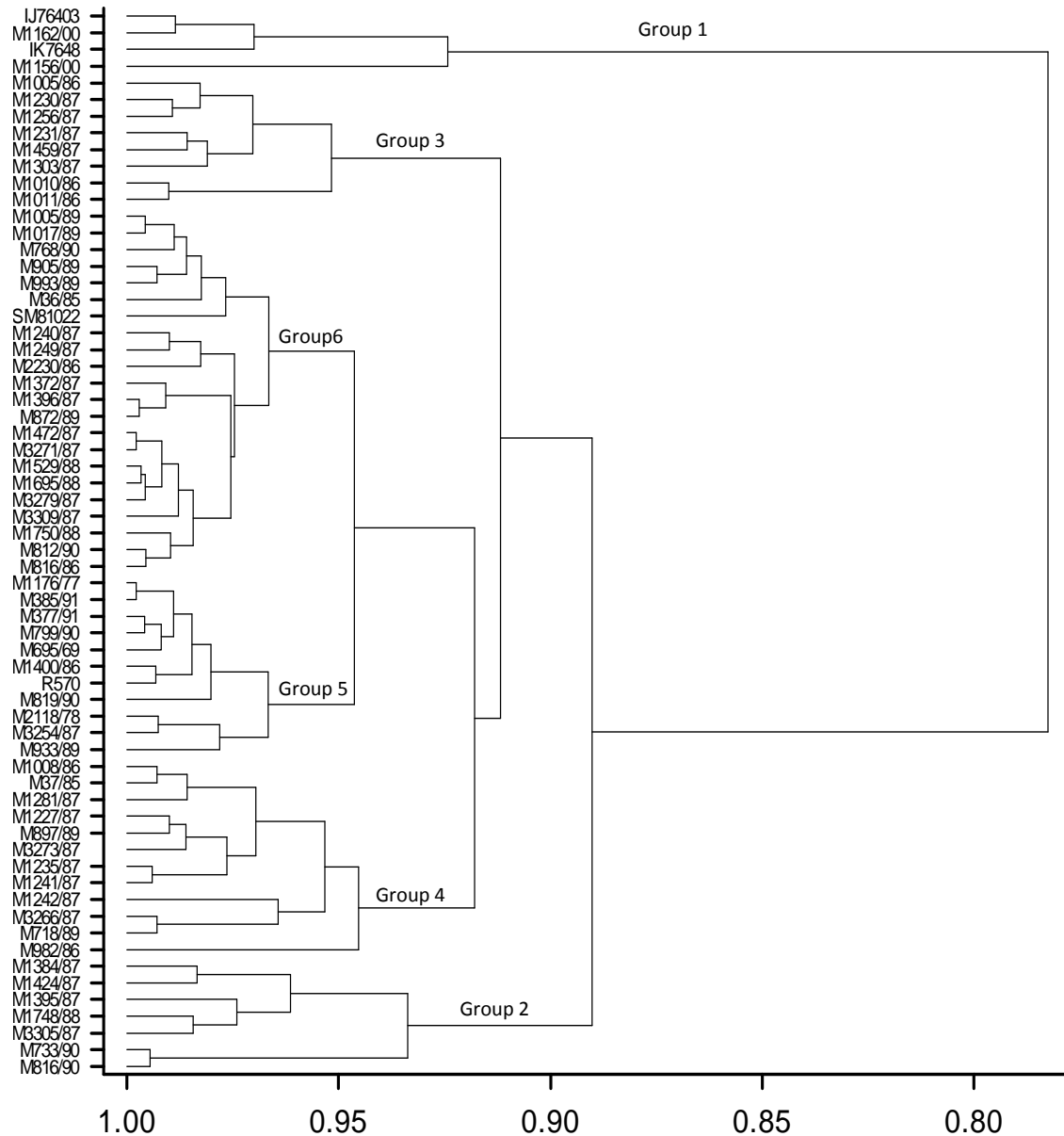


Figure 5.4: Dendrogram obtained from the group average hierarchic UPGMA technique of cluster analysis of 18 parametric data.

Figure 5.5 illustrates the different clusters superimposed on the biplot obtained from the PC analysis. The first five clusters have been encircled. Genotypes in cluster 6 are represented by open dots found near the origin.

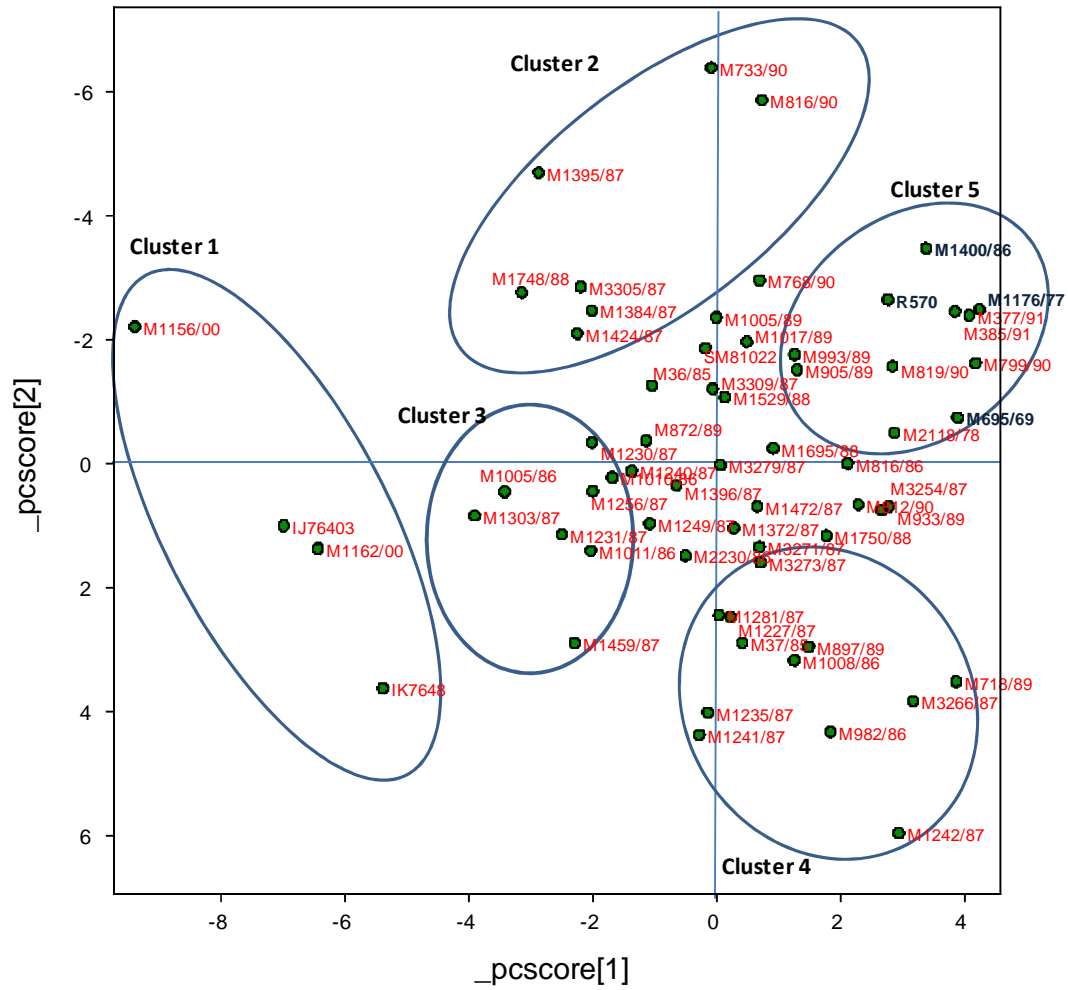


Figure 5.5:- Different clusters superimposed on the biplot obtained from PCA analysis: Clusters 1 to 5 encircled; remaining genotypes formed part of cluster 6.

Important features of the different clusters are summarised in Table 5.4 and are described below. Unless stated otherwise, the comparisons are made with respect to the central tendency.

Cluster 1:

Clones could be characterised as average cane diameter, very tall with high stalk number per unit area. They had very high fibre content and relatively high biomass yield. However, their sucrose concentrations were very low to negligible.

Cluster 2:

Individuals were generally thicker in cane diameter, tall with high stalk number per unit area. They had relatively higher levels of sucrose content than cluster 1, higher fibre content than the control varieties and were high to very high biomass yielders.

Cluster 3:

Genotypes could be characterised as having very thin canes, average in height but very dense in terms of cane number per unit area. Their sucrose contents were second lowest but their fibre contents were high. Their total biomass yields were nearly equal to those of the commercial varieties.

Cluster 4

Clones demarcated themselves as the lowest biomass and sugar yielders with thin canes, and average cane height and stalk number. Their sucrose contents were also low but their fibre contents were relatively higher than the commercial varieties.

Cluster 5

The group was composed of genotypes with relatively thick and short canes and low cane number per unit area. They were rich in sucrose but poor in fibre content. Sugar yield was high but the total biomass was average. The commercial control varieties clustered in the group.

Cluster 6

Individuals in the group were generally average in cane quality traits, cane morphological characters, and biomass yields. They occupied the central position and were near the origin in the body of points.

Table 5.4: Means and standard deviations of variables of genotypes in the different clusters

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cane quality characters						
Brix % (FW)	5 ± 0.2	13 ± 2	10 ± 1.0	11 ± 1.7	15 ± 1.1	12 ± 1.2
Pol. % (FW)	3 ± 0.3	11 ± 2.1	8 ± 1.1	9 ± 1.7	13 ± 1.3	10 ± 1.3
Fibre % (FW)	23 ± 0.8	16 ± 2.1	19 ± 1.9	16 ± 1.4	13 ± 1.1	16 ± 1.2
Dry matter % cane	30 ± 0.8	29 ± 2.1	30 ± 2.2	28 ± 2.4	28 ± 1.2	28 ± 1.4
Brix % (DW)	20 ± 0.6	42 ± 4.7	35 ± 3.0	39 ± 2.6	50 ± 3.3	42 ± 3.1
Pol. % (DW)	12 ± 1.1	37 ± 6.0	28 ± 3.2	31 ± 4.2	45 ± 4.2	36 ± 3.8
Fibre % (DW)	80 ± 0.7	56 ± 5.2	64 ± 3.1	61 ± 3.3	48 ± 3.4	57 ± 3.3
Cane morphology						
Cane diameter (mm)	23 ± 1.3	26 ± 2.6	19 ± 1.8	22 ± 3.1	29 ± 2.3	24 ± 2.4
Cane height (cm)	274 ± 9.3	257 ± 10.4	233 ± 20.1	210 ± 27.3	211 ± 15.3	238 ± 13.1
Stalk number (x1000/ha)	93 ± 15	97 ± 18.8	122 ± 24.8	74 ± 16.2	62 ± 10.9	84 ± 14.3
Biomass characters						
Cane yield (FW)	78 ± 19.8	87 ± 9.2	59 ± 9.7	39 ± 8.4	65 ± 9.1	64 ± 10.1
Cane yield (DW)	23 ± 5.7	25 ± 3.2	17 ± 2.3	11 ± 2.2	18 ± 2.4	18 ± 2.4
CTL yield (FW)	21 ± 6.1	23 ± 3.1	20 ± 3.1	13 ± 3	15 ± 1.9	17 ± 2.8
CTL yield (DW)	6 ± 1.5	7 ± 0.8	7 ± 1.2	4 ± 0.9	4 ± 0.4	5 ± 0.8
Sugar yield	3 ± 0.8	9 ± 2.4	5 ± 0.9	3 ± 0.8	8 ± 1.6	6 ± 1.3
Fibre yield	18 ± 4.7	14 ± 1.7	11 ± 1.5	7 ± 1.4	8 ± 1	10 ± 1.3
Total biomass (FW)	103 ± 23.9	112 ± 12	79 ± 10	52 ± 10.4	80 ± 11.5	82 ± 12.3
Total biomass (DW)	29 ± 5.9	33 ± 4.1	24 ± 2.4	14 ± 2.9	22 ± 3.1	23 ± 3.3
Summary of characters of economic importance classified with respect to central tendency						
Sucrose content	Very low	Average	Low	Low	High	Average
Fibre content	Very high	Average	High	Average	Low	Average
Total biomass	High	Very high	Average	Low	Average	Average

5.4 Discussion

The PCA analysis was effective in the reduction of the number of variables that needed to be considered. The first two PCs showed a definite pattern in the data for cane quality and cane biomass characters combined. For these two major traits, a wide variation among genotypes existed from which different types of cane could be identified. The negative association between sucrose and fibre contents was evident and it was also clear that high fibre canes tended to be taller and thinner. Under

the local environments, similar results were observed by Badaloo and Ramdoyal (2003) in progenies derived from commercial x *S. spontaneum* crosses.

The UPGMA technique of cluster analysis was effective in identifying six different groups of clones. Of these, clusters 1, 2 and 5 were of commercial interest. Clones from cluster 1 could be used for cogeneration. Cluster 5 individuals were comparable with existing commercial varieties where sugar yield is the main focus. Genotypes from cluster 2 could be termed as multipurpose types characterised by slightly lesser sucrose level and relatively higher fibre content than commercial varieties, high sugar yield and high aboveground biomass.

Tew and Cobill (2008) made a world-wide survey on the efforts made by different research institutes in the improvement of sugarcane as an energy crop. They identified three types of sugarcane relative to their composition and use (see Figure 2.13). The first category, termed as 'Sugarcane' (high sucrose, low fibre) involved the traditional breeding strategies where the main objective was sugar. In the case of energy canes, the vegetative biomass was an important product, and this was either a by-product, in the case of 'Type I' energy canes (high fibre and high sucrose content), or the main product, in the case of 'Type II' energy canes (very high fibre, negligible sugar). Clusters 5, 2 and 1 could accordingly be defined as 'Sugarcane', 'Type I' and 'Type II' canes respectively. However, in this study, none of the genotypes showed a fibre percent attaining 30%, the threshold mentioned by Tew and Cobill (2008) for purely high fibre canes. The two *Erianthus* clones, known for their very high fibre content, averaged to 23% in the trials. Their total dry matter content in the cane stem reached 30%.

The multivariate analysis provided an efficient way of summarising the whole data set by compressing the different correlated variables into fewer dimensions and categorising the genotypes into distinct groups. Mean values of 18 inter-related traits were used, each being considered equally important at the beginning of the analysis. The methodology can be used to have a quick overview of the pattern in the data and ultimately as an aid for decision making. However, since mean values were used, the methodology gave no information on the inherent variations existing in individual traits. For one particular variable, it could not prove whether two means were significantly different or not. In the definition of different types of canes, interest is mainly in identifying clones that are significantly better than the cultivated varieties for some specific traits. The whole study is based on standard replicated trials and there is a necessity to test for real differences among genotypes. This part of the study will be taken up in the next chapter.

CHAPTER 6

6 Simultaneous selection of different types of sugarcane varieties from a population of interspecific derived clones

6.1 Introduction

The demands made on a crop variety are always complex in the sense that high levels of expression are required for a considerable number of characters (Simmonds, 1969). Simmonds and Walker (1986) reported that breeders constantly make judgements and take decisions on the general balance of characters displayed by the lines, clones or populations in hand. Skinner *et al.* (1987) similarly reiterated that although breeders speak of selection for yield of cane, or some other important character, it is impossible to select a single character; the entire variety must be selected or rejected. This involves qualitative as well as quantitative traits. Some of them are independent of others, some are positively correlated so that selection for one involves some improvement in the others, and some are negatively correlated making it more difficult to improve both characters. Some characters are influenced much more than others by environmental variation and some are of greater importance than others. All of these factors affect the efficiency of selection.

Various methodologies have been cited in standard books with respect to selection for more than one trait simultaneously (Acquaah, 2007; Bos and Caligari, 2007; Falconer, 1989; Heinz, 1987; Wricke and Weber, 1986). Selection for an abstract trait such as 'general impression' or 'breeder's preference' is characteristic of non-formal way. Formal forms of simultaneous selection include independent culling levels (ICL) selection and index selection.

ICL selection implies truncation selection with regard to each of several traits. Thus for each trait, a threshold phenotypic value is determined. An entry is rejected if it does not attain the critical value for one or more traits, whatever its quality for all other traits. One major disadvantage with ICL selection is that it does not account for genotype by environment interaction. A variety may satisfy all the different thresholds in one particular 'environment', but then fail to do so in another. An 'environment' includes all non-genetic factors affecting the overall performance of the genotype. One method of counteracting this problem is to use long term check cultivars to establish minimum values of acceptability for important traits in different environments.

In contrast to ICL selection, index selection allows mutual compensation of favourable and unfavourable expressions for different traits. With index selection, some index value is assigned to

each candidate. This index value indicates the aggregate value of each candidate across several traits. Maximum gains from selection are achieved by a selection index involving multiple regressions, the index being constructed to allow for heritability, correlations, and economic importance of characters (Acquaah, 2007; Bos and Caligari, 2007; Skinner *et al.*, 1987; Wricke and Weber, 1986). Substantial resources are, however, involved in collection of data and computation, and it is often necessary to make decisions quickly so that the selections can be planted out. Skinner *et al.* (1987) further mentioned that due to inadequacy of the estimates of phenotypic and genotypic variances and covariances the progress of indirect selection by means of index selection may be negligible. Thus results may be obtained that are no better than those obtained by applying visual selection for general impression.

In the absence of mathematical selection indices, the principle of simultaneous selection is achievable economically by basing selection on a grading system. The grade is a selection index that may be completely intuitive, such as 'Breeder's preference', or an effort may be made to emphasize each character in a proportion to its economic importance, broad-sense heritability, consideration also being given to its correlations with other characters (Skinner *et al.*, 1987).

This study aimed at devising a grading system for the precise identification of different types of sugarcane varieties simultaneously from the population studied. The most appropriate threshold levels for specific traits, namely sucrose concentration, fibre content and biomass yield, had first to be established. Hence, a preceding analysis of the existing variation among the individuals with respect to the above traits was vital. The model for selection to be developed should ideally encompass all the different types of canes of economic importance obtainable from a sugarcane breeding programme.

6.2 *Materials and Methods*

The same data used for the multivariate analyses (chapter five) were utilised to construct an algorithm for the identification of different types of sugarcane genotypes. Hence, the first sampling date records were ignored for reasons described in section 5.2 and the adjusted means, corrected for outliers, were used. The algorithm basically involved categorisation of the clones based on significance tests of test genotypes with the commercial control varieties for appropriate traits, supplemented by additional culling levels for sucrose and fibre contents.

In the above experiment, the set objective was to compare individual test genotypes with the average of the four commercial controls included in each sub-trial. While the LSD could be used to make any paired comparisons, the methodology for this planned comparison was to work out an *a-priori*

contrast as described by Sokal and Rohlf (2000). Individual sub-trials data, averaged over crop cycles, were used to test for significance. The procedure involved the calculation of the sum-of-squares (SS's) of a given genotype contrast with the four commercial controls. The SS's of the contrasts were calculated using the “*Golden Rule of Sum of Squares*”. The SS was divided by the residual mean square from the sub-trial to obtain the appropriate F value. The latter was then compared with the tabular F and significance was established.

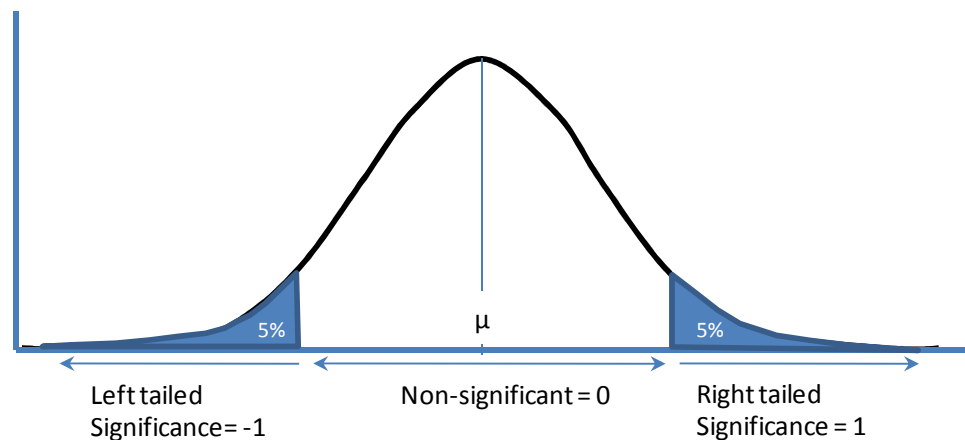


Figure 6.1: Three possibilities of significance testing (F-tests) at $\alpha = 0.05$

Generally, the thresholds in the model were set at 95% probability, a level commonly used in agriculture for significance testing. Three scenarios were possible (Figure 6.1). Performance of a genotype for a particular trait could either be significant left-tailed, non-significant, or significant right-tailed as compared to the average of commercial controls. This approach catered for the variations among traits and also ensured adjustments for genotype x environment interactions. Additional thresholds for sucrose and fibre levels were worked out based on variations observed in the data and on presumed physiological limitations of the sugarcane crop. Furthermore, the analyses were done on fresh weights for Pol and fibre. This was found most appropriate as a start as most sugarcane breeders mention fibre content thresholds in terms of fresh weight (de Boer, 2008; Giamalva *et al.*, 1984; Kennedy, 2005; Rao and Kennedy, 2004; Rao *et al.*, 2007; Tew *et al.*, 2009; Tew and Cobill, 2008).

Once the proper model for selection was defined, the data were processed to identify potentially selectable clones. The model was intended to include only the cane quality and biomass yield characters. Ultimately, selection would involve additional evaluation of the selected genotypes for secondary characters, like minimum cane diameter and other non-parametric records such as lodging

of the cane stem, growth rate, ease of trashing and breeder's preference. These parameters were mainly important from crop management and farmers' acceptability points of view.

6.3 Results

Results are arranged in the following order:

- Analysis of variations among genotypes for cane quality and biomass characters
- Definition of different types of cane
- Identification of thresholds
- Construction of a selection algorithm
- Identification of potential genotypes for different end-uses.

6.3.1 Analysis of variations among genotypes

In the following sections, the trial data, that included a range of clones of different generations, or mobilization groups (wild, F1, BC1, BC2 and commercial), were used to analyse the variations in cane quality and cane biomass characters.

6.3.1.1 Variations in cane quality characters

Cane stalk composition

Table 6.1 and Figure 6.2 show the variation in cane quality characters of the 64 genotypes, classified according to their generations. In general, the crop-year effect was marginal (Table 6.1). The cane stalks were roughly composed of 28% of dry matter and 72% of water.

Table 6.1: Average and standard deviation in cane quality characters, fresh weight, among the different nobilization groups

Variable	Genotype categories					Grand Average
	<i>Erianthus</i> clones	F1	BC1	BC2	Commercial varieties	
Plant cane						
Dry matter % Cane	28.0 ± 0.5	28.2 ± 2.1	28.4 ± 2	28.9 ± 0.8	27.9 ± 0.9	28.3 ± 1.9
Brix %	5.2 ± 0.1	10.3 ± 2.2	12.1 ± 1.6	14.7 ± 0.7	14.9 ± 0.5	11.7 ± 2.6
Pol %	3 ± 0.1	8.1 ± 2.2	10.1 ± 1.7	12.9 ± 0.8	13 ± 0.4	9.6 ± 2.6
Fibre %	23.5 ± 2	17.6 ± 2.6	16 ± 2.3	14.5 ± 0.8	12.3 ± 1.1	16.5 ± 2.9
PF index	0.1 ± 0	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.6 ± 0.2
Purity	0.6 ± 0	0.8 ± 0.1	0.8 ± 0	0.9 ± 0	0.9 ± 0	0.8 ± 0.1
First ratoon						
Dry matter % Cane	30.2 ± 0.5	28.6 ± 2.1	28.8 ± 2	29.2 ± 0.8	28.3 ± 0.9	28.7 ± 1.9
Brix %	5.4 ± 0	10.7 ± 2.2	12.4 ± 1.6	15.2 ± 0.6	15.2 ± 0.8	12 ± 2.6
Pol %	3.2 ± 0.1	8.5 ± 2.2	10.3 ± 1.6	13.4 ± 0.7	13.5 ± 0.6	9.9 ± 2.7
Fibre %	22.4 ± 0.5	16.9 ± 2.3	16.4 ± 2.2	14.7 ± 1.4	13.7 ± 1.3	16.4 ± 2.5
PF index	0.1 ± 0	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.6 ± 0.2
Purity index	0.6 ± 0	0.8 ± 0.1	0.8 ± 0	0.9 ± 0	0.9 ± 0	0.8 ± 0.1
Plant cane and first ratoon pooled						
Dry matter % Cane	30 ± 0.5	28.4 ± 2.1	28.6 ± 2	29 ± 0.8	28.1 ± 0.9	28.5 ± 1.9
Brix %	5.3 ± 0.1	10.5 ± 2.2	12.3 ± 1.6	14.9 ± 0.7	15 ± 0.7	11.8 ± 2.5
Pol %	3.2 ± 0	8.3 ± 2.2	10.2 ± 1.6	13.1 ± 0.7	13.3 ± 0.5	9.8 ± 2.6
Fibre %	23.1 ± 0.8	17.3 ± 2.4	16.2 ± 2.1	14.6 ± 1	13.0 ± 1.1	16.4 ± 2.6
PF index	0.1 ± 0	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.6 ± 0.2
Purity index	0.6 ± 0	0.8 ± 0.1	0.8 ± 0	0.9 ± 0	0.9 ± 0	0.8 ± 0.1

PF index = Pol to fibre ratio; Purity index = Pol to Brix ratio

The total dry matter also included soluble solids other than sucrose, commonly termed as impurities. They are generally undesirable as they do not contribute to sugar production. They find their way in scum, molasses and bagasse during cane milling and sugar extraction. Juice 'purity' is a technical term usually calculated as the proportion of Pol to Brix (MSIRI, 1968). In Figure 6.2, the gap between the bars and the line of cane dry matter gives an indication of the proportion of impurities among the different clones. The purity index was lowest (0.6) among the *Erianthus* clones and improved progressively across the different nobilization groups to reach its highest level (0.9) among the commercial varieties.

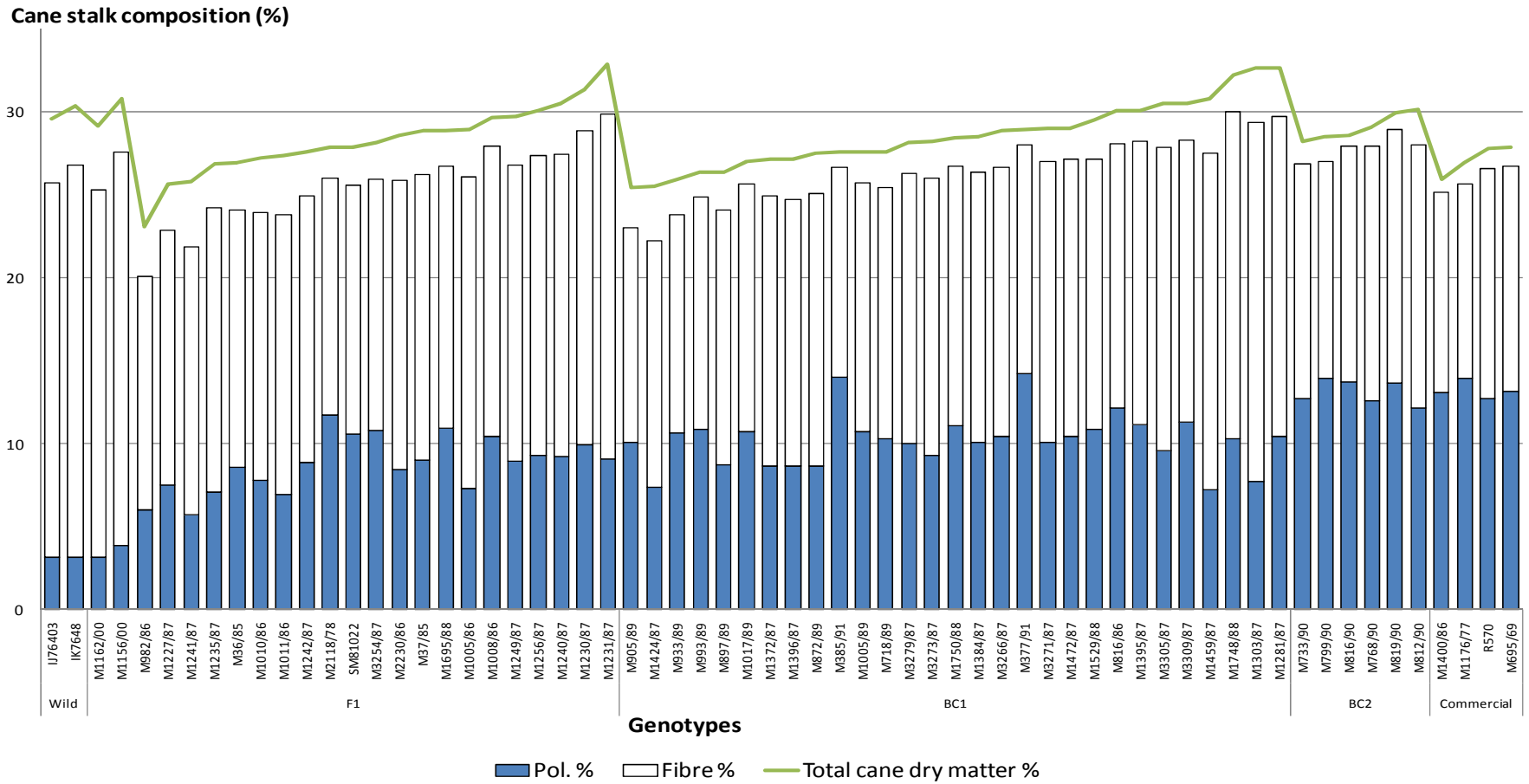


Figure 6.2: Composition, in percentage, of cane stalk dry matter of 64 genotypes classified according to their nobilization status: Adjusted mean values of pooled plant cane and 1st ratoon results

Sucrose and fibre compositions

Sucrose and fibre contents showed a wide variation among the different clones examined. While there was a continuous progress in Pol from wild clones (Pol = 3%) to the commercial varieties (Pol = 13%), variations among individuals were considerable. Two genotypes (3% of total) could be identified with Pol values higher than the best performing commercial variety (M 1176/77) and three were more or less equal to the checks. The converse was true for fibre content: 55 out of the 60 genotypes (92%) had fibre content higher than the commercial varieties. The variations in fibre contents were generally in relation to Pol: the higher the Pol the lower the fibre and vice versa. In the previous chapter, the two characters were found negatively correlated, with an r-value of -0.72^{**} (Table 5.2) which was highly significant.

When Pol and fibre contents were considered together (without the impurities), the dry matter content averaged to 26% in each group, including the clones of the *Erianthus* genera and commercial varieties. In general, the variation ranged between 20% and 30%, the commercial varieties occupying the central position. The Pol to fibre ratio, commonly termed as PF index, was a good indicator of partitioning of photosynthates into sugar and fibre. The index gradually increased from 0.1 in the wild clones to 1.0 in the commercial varieties. The latter value signified a more or less equal proportion of Pol and fibre, which roughly averaged to 13% for the two traits in the commercial cultivars. Merely four test genotypes attained that level and they were from the BC1 and BC2 generations.

6.3.1.2 Variations in cane biomass characters

Total cane biomass composition

The total cane biomass measured in the trials was composed of clean cane stalks and CTL that combined non-millable cane tops, green and clinging dry leaves. Fallen dry leaves were not considered in the assessment. Table 6.2 and Figure 6.3 show the variations in aboveground biomass characters of the 64 genotypes, classified from low to high yield in each of the different nobilization groups. High variations of total biomass yield (28 - 133 tha^{-1}) were observed in general as compared to the commercial control varieties (70 - 100 tha^{-1}). In the pooled crop-year results, seven out of 60 test genotypes (11%) out-yielded the best commercial variety (M1400/86). Compared to the average of controls, maximum percentage increase was of the order of 51%.

Table 6.2: Average and standard deviation in cane biomass characters (tha^{-1} fresh weight) among the different nobilization groups

Variable	Genotypes categories					Commercial varieties	Grand Average
	Wild	F1	BC1	BC2			
Plant cane							
Cane yield	83 ± 32	72 ± 19	79 ± 18	93 ± 23	89 ± 13	78 ± 20	
CTL yield	22 ± 2	23 ± 5	23 ± 4	23 ± 4	21 ± 1	23 ± 4	
% CTL to total biomass	21%	24%	23%	20%	19%	23%	
Total biomass	106 ± 19	94 ± 22	102 ± 19	116 ± 28	109 ± 13	101 ± 21	
Fibre yield	19 ± 8	13 ± 5	13 ± 3	14 ± 3	11 ± 2	13 ± 4	
Sugar yield	3 ± 1	6 ± 2	8 ± 2	12 ± 3	11 ± 1	8 ± 3	
First ratoon							
Cane yield	57 ± 15	42 ± 15	45 ± 14	56 ± 19	54 ± 6	46 ± 15	
CTL yield	11 ± 2	11 ± 5	12 ± 4	12 ± 4	9 ± 1	11 ± 4	
% CTL to total biomass	16%	21%	21%	18%	14%	19%	
Total biomass	68 ± 19	54 ± 22	57 ± 19	68 ± 28	64 ± 13	58 ± 21	
Fibre yield	13 ± 2	8 ± 3	7 ± 2	8 ± 2	7 ± 1	8 ± 3	
Sugar yield	2 ± 0	3 ± 1	5 ± 2	7 ± 3	7 ± 1	5 ± 2	
Plant cane and first ratoon pooled							
Cane yield	70 ± 24	57 ± 17	62 ± 16	74 ± 21	72 ± 9	62 ± 17	
CTL yield	17 ± 2	17 ± 5	18 ± 4	17 ± 4	15 ± 1	17 ± 4	
% leaf to total biomass	20%	23%	23%	19%	17%	22%	
Total biomass	87 ± 19	74 ± 22	80 ± 19	92 ± 28	87 ± 13	79 ± 21	
Fibre yield	16 ± 5	10 ± 4	10 ± 3	11 ± 3	9 ± 1	10 ± 3	
Sugar yield	2 ± 1	5 ± 2	6 ± 2	9 ± 3	9 ± 1	6 ± 2	

Cane tops, green and clinging dry leaves (CTL) component

The percentage of CTL biomass averaged to 23% in plant cane and 19% in the first ratoon crop. Roughly, CTL accounted for 22% of total aboveground biomass with a range of 14-36%. The ratio was high among the low biomass yielding varieties. The commercial varieties had, on average, the lowest CTL proportion (17%) to total biomass.

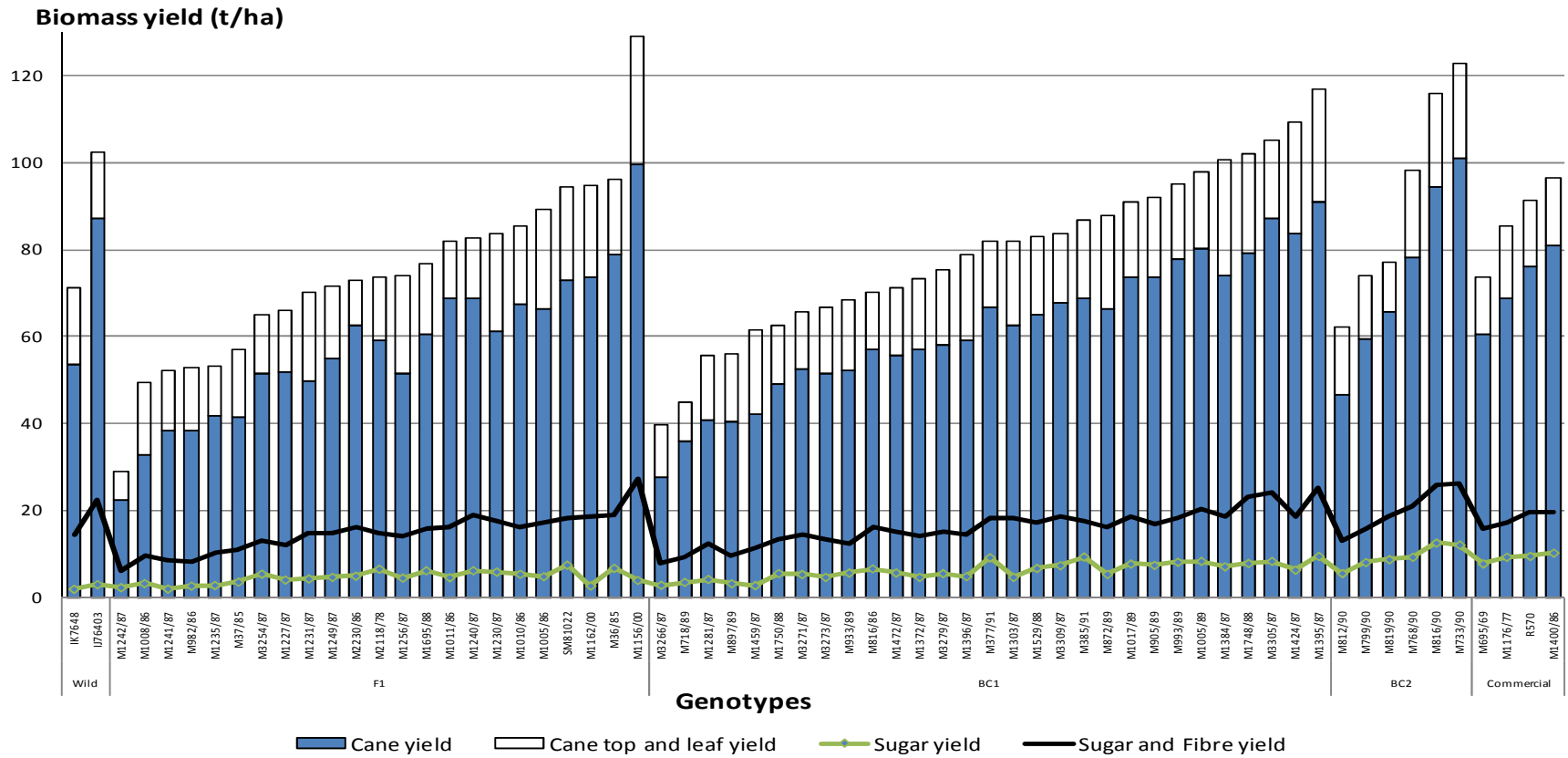


Figure 6.3: Composition of total cane biomass (tha⁻¹), fresh weight, of 64 genotypes classified in increasing order of their biomass yields within each nobilization category: Mean values of pooled plant cane and 1st ratoon results

Cane stalk component

Millable cane stalk yield for the genotypes varied widely. With the two crop cycles combined, average cane yield of the commercial varieties fluctuated between 60 and 80 tha^{-1} . The test genotypes yields varied between 20 to 100 tha^{-1} . Fourteen genotypes had cane yield above the average of commercial controls. Six of them outyielded the best commercial variety, M1400/86.

Sugar and fibre yields

Sugar yield was highest among the commercial and the BC2 genotypes and lowest among the *Erianthus* and F1 clones. Highest fibre yields were observed among the *Erianthus* clones and their two progenies (see Table 3.1 for parentage). Some BC1 and BC2 clones also ranked among the top fibre yielders. A number of genotypes of the different generations exceeded the commercial varieties in terms of sugar yield, fibre yield and total biomass yield.

6.3.2 Definition of different types of cane

Current commercial varieties are highly selected clones for their relatively high sucrose content and sugar yield, with little importance given to their fibre content. Hence any test genotype reaching their level in Pol and sugar yield could be considered as candidate for further testing in a selection programme. Results from the analysis of biomass characters confirmed that high biomass yield could be expected from any of the different nobilization groups and with variable PF ratio. Given the negative correlation between Pol and fibre, four different types of varieties could be defined by integrating cane quality and high cane biomass characters:

- Type 1: Current conventional sugarcane varieties with high sucrose and low fibre
- Type 2: Varieties with slight increase in fibre without affecting sucrose content
- Type 3: Varieties with moderate increase in fibre with a decrease in sucrose content
- Type 4: Varieties with large increase in fibre with serious impact on sucrose content

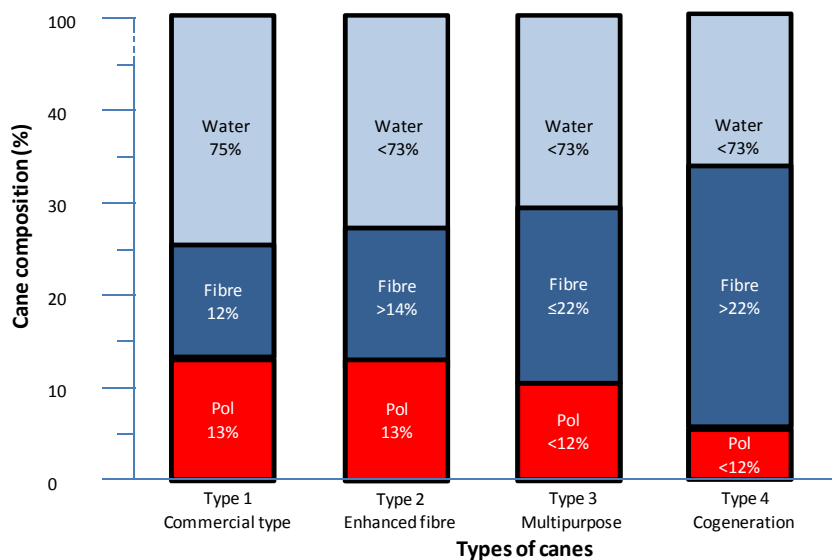


Figure 6.4a: Different scenarios achievable in cane composition

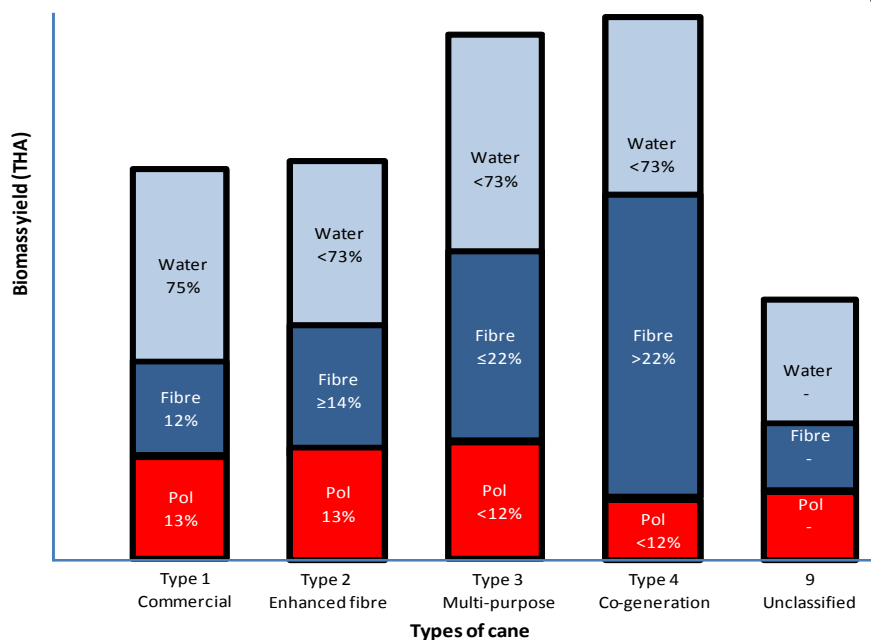


Figure 6.4b: Selection scenarios integrating cane composition and cane biomass characters.

The percentages are indicative, and not absolute, of the different types of canes expected.

Figures 6.4a-b illustrate the different types of canes expected from progenies derived from inter-specific crosses. Traditional varieties have been assigned as Type 1 cane. A Type 2 cane, termed as enhanced fibre type, is essentially one with sucrose concentration and sugar yield comparable with the commercial varieties but with higher fibre content. With Type 1 and Type 2 canes, Pol and sugar yield are not compromised. Type 3 and Type 4 canes represent situations where concessions are made for

sucrose content to the advantage of fibre content. With Type 3 canes, the compromise is moderate, while with Type 4 canes, the concession is severe. A fifth type, numbered 9, includes all those that are not able to achieve the minimum set thresholds (especially biomass yield) and hence, are mostly of undesirable type.

6.3.3 Identification of thresholds for cane quality and biomass characters

The percentages in cane composition shown in Figures 6.4a-b were only indicative of the thresholds in the demarcation of the different types of canes. Fixed minimum thresholds for the sucrose and fibre contents could not be defined as the two traits are known to evolve with time. This could be more clearly observed on sucrose accumulation in four commercial varieties during the harvest season (Figure 6.5). To circumvent this problem, statistically significant contrasts of individual genotypes with the average of commercial checks was felt necessary in the determination of dynamic thresholds.

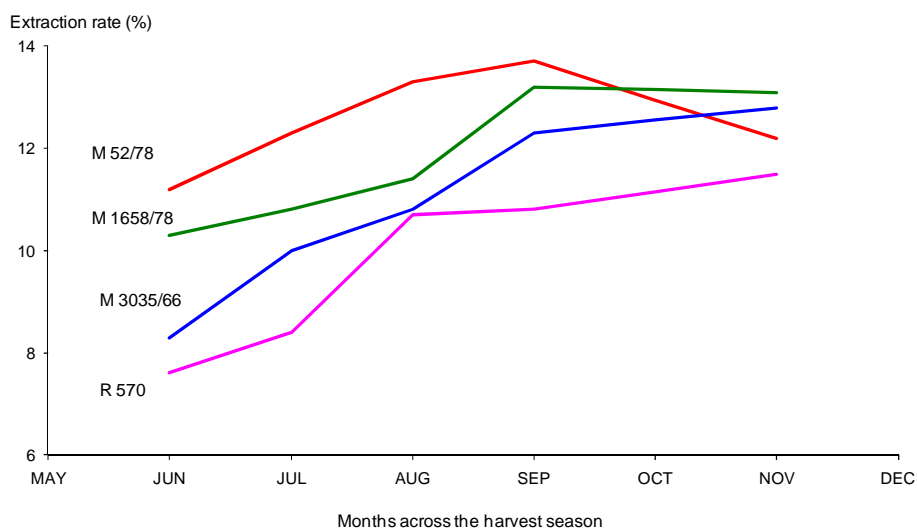


Figure 6.5: Sucrose extraction rate of four commercial varieties across the harvest season

Source: MSIRI final phase trials

In addition, a minimum difference from the average of the control varieties for Pol and fibre was felt necessary. In this study, mean Pol % cane of the control varieties roughly had a 2-unit range (12-14%) and mean fibre % cane a 3-unit range (11-14%). These ranges tended to vary across sub-trials and crop cycles, but the 2- and 3-unit differences were generally maintained. Hence, a ± 1.5 -unit difference for Pol and ± 2 -unit difference for fibre contents from the average of commercial check varieties in each sub-trial was found most appropriate minimum thresholds for the two traits. The differences

were slightly above the specified ranges for the two traits. This was expected to account for comparisons of selectable genotypes with the lowest or best performing control varieties in the trial.

Overall, thus, the minimum threshold value for Pol to be considered as low in the trials was governed by two factors: The value should be significantly lower than the average of standards and the minimum difference should be greater than 1.5 units, whichever gives the lower Pol value. This would differentiate Type 1 and Type 2 canes from Type 3 and Type 4 canes. Similarly, the minimum threshold for fibre content to be considered high was dictated by significance test combined with a 2-unit minimum difference higher than the average of standards, whichever gives the higher fibre value. This truncation would differentiate between Type 1 and Type 2 canes. The threshold to discriminate between Type 3 and Type 4 canes was set at 22% for fibre content. Significance tests would not be helpful in this situation and it was expected that purely high fibre varieties would have fibre content >22% and closer to their wild relatives, like the *Erianthus spp.* Additional prerequisites for selection of the Type 3 and Type 4 canes were that they should have higher biomass yields (sugar and fibre yields confounded) and dry matter percent than the existing commercial varieties. So far sugar remains profitable, the total dry matter yield (sugar + fibre), and not fibre yield on its own, should be compared with the existing commercial varieties for selection. Table 6.3 summarises the different thresholds for the four different types of canes.

Table 6.3: Description of different types of cane with respect to their sucrose, fibre content and biomass yield

Sugarcane type	Description	Significance tests with average of commercial controls and additional thresholds
Type 1	High sucrose %	Pol %: non-significant to significantly high* or $\geq AV_{std} - 1.5$
	Low fibre %	Fibre %: non-significant to significantly low* or $\leq AV_{std} + 2.0$
	High sugar yield	Sugar yield: non-significant to significantly high
Type 2	High sucrose %	Pol %: non-significant to significantly high or $\geq AV_{std} - 1.5$
	Enhanced fibre %	Fibre %: significantly high and $> AV_{std} + 2.0$
	High sugar yield	Sugar yield: non-significant to significantly high
Type 3	Lower sucrose %	Pol %: significantly low and $< AV_{std} - 1.5$
	Higher fibre %	Fibre %: ≤ 22
	High dry matter %	Dry matter %: significantly high
	High dry matter yield	Dry matter yield: significantly high
Type 4	Very low sucrose %	Pol %: significantly low and $< AV_{std} - 1.5$
	Very high fibre %	Fibre %: > 22
	High dry matter %	Dry matter %: significantly high
	High dry matter yield	Dry matter yield: significantly high
9		None of above (unclassified)

*: *Significantly high = right-tailed significance (performance better); Significantly low = Left-tailed significance (performance poorer) at 95% probability*

AV_{std} = *Average of commercial check varieties (standards)*

6.3.4 Construction of selection algorithm

Figure 6.6 shows the algorithm derived from the criteria set above. The population under study is first categorised into two groups with respect to sucrose content: The first group include Type 1 and Type 2 canes and the second group, Type 3 and Type 4 canes. The two groups get differentiated by the thresholds set for sucrose content. The first group is further compared for fibre level and sugar yield. Good performing clones will be categorised as Type 1 or Type 2 canes based on their fibre content. Candidates of the second group (Type 3 and Type 4 canes) are further tested for their dry matter content and biomass yield. The set threshold of 22% for fibre content distinguishes between Type 3 and Type 4 canes.

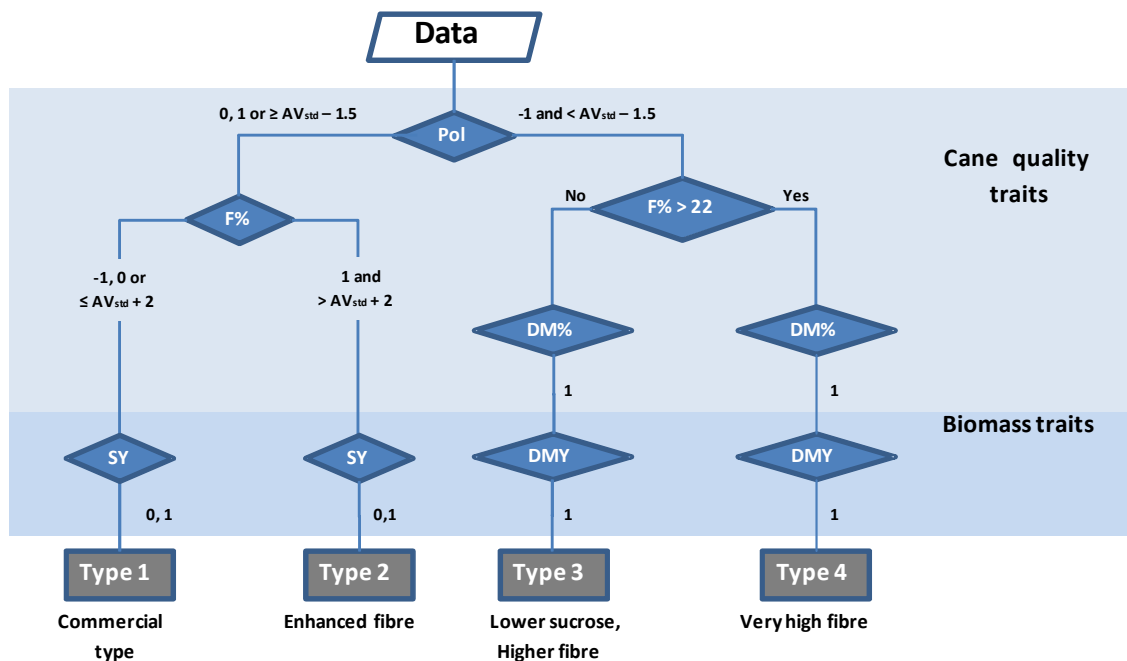


Figure 6.6: Selection index flowchart for different types of canes based on significance tests with the average of commercial controls and appropriate thresholds.

Pol \approx Sucrose content; *F%* = Fibre percent; *DM%* = Dry matter percent cane; *SY* = Sugar yield; *DMY* = Dry matter yield

-1 = significantly low; 0 = non-significant; 1 = significantly high at $\alpha = 0.05$; *AVstd* = Average of commercial varieties

In the flowchart, the numbers along the connectors represent the two-tailed significance tests at 95% probability (details provided in the methodology – Figure 6.1) where -1 = significantly low, 0 = non-significant and 1 = significantly high performance with respect to the average of commercial check varieties. The fifth category, numbered 9 in Table 6.3, has been avoided to keep the schematic presentation simple. Any genotype not satisfying the conditions mentioned in the decision boxes automatically falls into the unclassified category and will not be selected.

6.3.5 Identification of potential genotypes for different end-uses

A selection simulation with the pooled plant cane and first ratoon data assigned 11 genotypes (18% of total) into the four major types of canes. The remaining 49 genotypes did not satisfy, at least, one of the various thresholds set in the model. Details on the classified clones are provided in Table 6.4 and illustrated in Figure 6.7.

Table 6.4: Selection simulation with the pooled plant cane and first ratoon data: Classified varieties only

Genotypes	Group	Mean values					Significance tests*					Cane type
		Pol % cane	Fibre % cane	Dry matter % cane	Sugar yield	Dry matter yield	Pol % cane	Fibre % cane	Dry matter % cane	Sugar yield	Dry matter yield	
Grand mean of controls [@]		13.3	13.0	28.1	9.5	19.8						
M377/91	BC1	14.3	12.9	28.4	9.8	19.5	1	0	0	0	0	Type1
M385/91	BC1	14.1	13.3	28.4	10.0	20.0	1	1	1	0	0	Type1
M799/90	BC2	13.9	13.6	28.4	8.6	17.6	1	0	1	0	0	Type1
M816/90	BC2	13.9	13.5	29.6	12.6	27.0	0	1	1	1	1	Type1
M733/90	BC2	12.9	13.7	28.9	12.9	29.5	0	1	0	1	1	Type1
M768/90	BC2	12.7	15.5	29.3	10.0	23.2	0	1	1	0	0	Type2
M819/90	BC2	13.9	15.2	31.0	8.8	19.7	0	1	1	0	0	Type2
M1395/87	BC1	11.3	16.8	30.7	10.1	28.2	-1	1	1	0	1	Type3
M1748/88	BC1	10.4	20.2	33.0	7.8	25.1	-1	1	1	0	1	Type3
M3305/87	BC1	9.6	18.4	30.3	8.3	26.2	-1	1	1	0	1	Type3
M1156/00	F1	3.6	23.1	29.1	3.4	28.2	-1	1	1	-1	1	Type4

*: Significance tests with average of four commercial varieties at $\alpha = 0.05$ where -1 = significantly low; 0 = non-significant and 1 = significantly high

@: Grand mean of controls obtained from five sub-trials

Five genotypes were of the commercial type (Type1). Two clones, M768/90 and M819/90, were of enhanced fibre type (Type 2). Their sucrose contents were comparable with the commercial varieties and their fibre contents averaged to 15.3%. Three genotypes, namely M1395/90, M1748/88 and M3305/87 were identified as Type 3 canes with Pol values ranging between 9.6 and 11.3 and their fibre content between 16.8% and 20.2%. One F1 clone, M1156/00, was identified as Type 4 cane. Its Pol value was below 5% and the fibre content was at 23%. For the 11 clones selected, the dry matter percent cane broadly had a narrow range of 28% - 30%, with only two clones surpassing the 30% limit (M819/90 at 31% and M1748/88 at 33%).

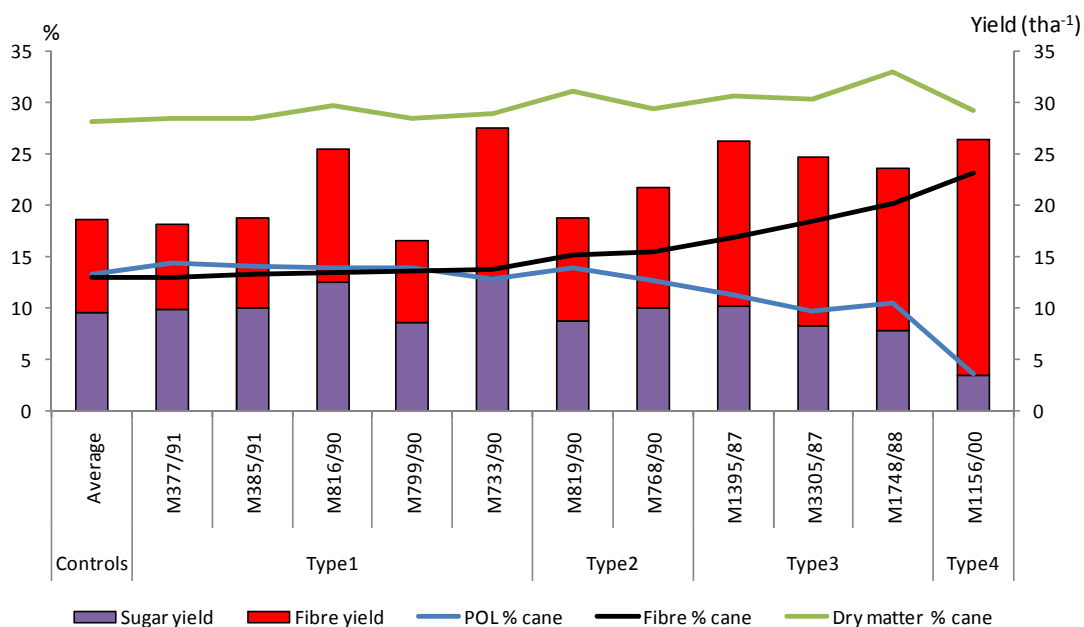


Figure 6.7: Characteristics of the different types of canes categorised for selection. Cane quality characters in percentage of cane stalk; Cane biomass characters scales in tha^{-1} .

In terms of biomass characters of economic interest, the commercial varieties had an average sugar yield of 9.5 tha^{-1} and a fibre yield of 9.1 tha^{-1} . Highest sugar yields ($>12 \text{ tha}^{-1}$) were observed in two genotypes (M816/90 and M733/90) from Type 1 canes. The two clones also had relatively high fibre yields that averaged to 13.7 tha^{-1} . All the Type 3 canes were not only high biomass yielders but also had sugar yield roughly equal to the commercial varieties, despite their lower sucrose contents. The Type 4 clone, M1156/00, demarcated itself by its very high fibre yield (23 tha^{-1}) which was the highest in the population. In terms of total cane dry matter yield (sugar + fibre yield), four genotypes were outstanding ($\sim 28 \text{ tha}^{-1}$) as compared to the commercial controls ($\sim 20 \text{ tha}^{-1}$). They were M816/90 and M733/90 from Type 1 canes, M1395/87 from Type 3 canes and M1156/00 from Type 4 canes.

6.4 Discussion

In this chapter, the objectives were to identify different types of canes obtainable in the population, define the selection thresholds and simultaneously select the best clones from each cane category. Based on the variations observed in sugar and fibre contents and biomass yield potentials, four types of canes were identified. Significance tests with the average of controls were used as dynamic thresholds. Sucrose and fibre content were further supported by additional culling levels. This was felt important in situations where significance tests would fail to ensure a minimum difference from the commercial varieties. A model was constructed involving several traits and 11 genotypes were

screened. All the four cane types were represented. The results showed that although sucrose and fibre contents were negatively correlated, it was possible to obtain varieties with sucrose content equivalent to the commercial ones and with higher fibre content. The population also consisted of genotypes with sugar and dry matter yields much higher than the commercial controls.

For Type 3 and Type 4 canes, there was a choice between any of the four aboveground biomass traits, namely cane yield and total biomass measured on fresh and dry weights. Given that the dry weight was devoid of the confounding effect of water content in the cane stem and it was readily available, its use was felt more appropriate for selection for high biomass varieties. Furthermore, the current approach in the Mauritian sugar industry is to send clean cane stalks, without CTL, to the mill for maximum sugar extraction. With this in mind, the mature cane stalk yield was considered more suitable in the construction of the selection algorithm. CTL was not included, but might well be alternatively considered, should whole cane harvest become economically and physically feasible. Recently, Sweetnam (2009) from NSW, Australia, reported on whole crop harvesting trials where the use of whole sugarcane aboveground biomass was successfully harvested to generate additional fibre for the generation of electricity in cogeneration plants.

Although not all the data available were used for selection, these results concurred very well with the multivariate analyses covered in the previous chapter. With principal component analysis, outstanding varieties of economic importance were found outlying in the second and third quadrants of the biplot (Figure 5.5). Cluster analysis grouped them into three clusters (clusters 1, 2 and 5). The selection index based on significance tests and appropriate thresholds was very effective in identifying the best candidates from each of the three clusters.

CHAPTER 7

7 Determination of the most appropriate traits and time for data collection in a sugarcane population of interspecific derived clones

7.1 Introduction

In an attempt to improve selection efficiency, various studies have been done in sugarcane on the most appropriate trait and stage for data collection. Also, although it is desirable to select directly for important characters, selection is often made on correlated characters on which selection operation is more economical. In this experiment, over and above evaluation of the genotypes at two crop cycles, data on cane quality were collected at two dates (April: pre-harvest and July: early-harvest stages), various characters were measured on both fresh and dry weights and several aboveground biomass characters (cane yield and total aboveground biomass, fresh and dry weight yields) were derived. Various studies done under the local context have found higher genetic variations for sucrose content in March/April than in July (Badaloo *et al.*, 2005; Mamet *et al.*, 1996; Nayamuth *et al.*, 2005). In this inter-specific population studied, however, higher repeatability values and variations for the trait were observed in July than in April. Particularly for cane quality characters, most sugarcane breeders refer to fresh weight scales. Nayamuth *et al.* (2005) vindicated the use of dry weights because of higher variations in the data and higher precision achievable. Although studies in previous chapters gave clear indications on the precision achievable using the different related characters, it was felt necessary to determine the real impact on selection with the various traits. Of particular interests were:

- selection at each crop cycle,
- selection using fresh and dry weights of cane quality characters,
- selection with different cane biomass characters, and
- Selection with first and second sampling date data.

Hence, the main objective of this study was to make selection simulations with the different characters using the selection index developed in chapter six. The aim was to determine the most pragmatic traits and time of data collection for effective selection and could be applied in the routine selection programme.

7.2 Materials and methods

Detailed descriptions of the traits measured were given in chapter three. Five sub-trials were involved and each was treated individually for significance tests (see section 6.2) with the original verified data. The adjusted means (see chapter four) were used so that genotypes in the five sub-trials could reliably be compared. In the previous section, the appropriate model for simultaneous selection for different types of canes was developed (Figure 6.6). The adjusted means (see section 4.3) were used to appreciate the differences between genotypes.

In this study, only significance tests with the average of control varieties were used as thresholds (Figure 7.1). The additional culling levels worked out for sugar content (± 1.5 -unit difference from average of standards) and fibre content (± 2 -unit difference from average of standards and 22%) fresh weights were intentionally removed. They were believed to vary and influence the results with dry weight measurements or with measurements at earlier sampling dates. In consequence, all Type 3 and Type 4 canes were merged into “Type 3/4 canes” with significantly low sucrose content and significantly high dry matter content and biomass yield. Type 1 and Type 2 canes had equal to significantly high sucrose content and high sugar yield with respect to commercial varieties and could only be differentiated from each other by significance tests for fibre content.

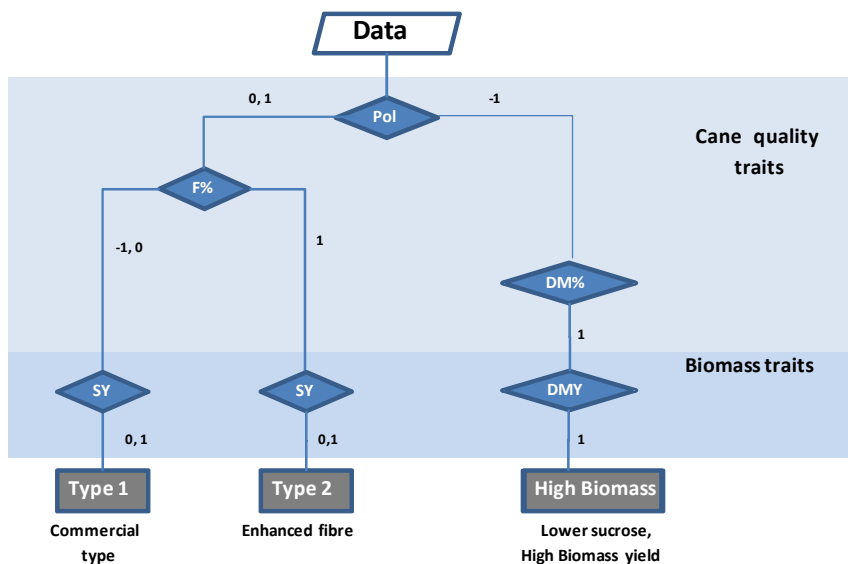


Figure 7.1: Modified selection index based solely on significance tests with the average of commercial controls.

$Pol \approx$ Sucrose content; $F\% =$ Fibre percent; $DM\% =$ Dry matter percent cane; $SY =$ Sugar yield; $DMY =$ Dry matter yield

$1 =$ significantly high; $0 =$ non-significant; $-1 =$ significantly low at $\alpha = 0.05$

A differential-selection analysis was used to show the differences between two selection scenarios. The analysis divides the population into four quadrants depending upon selection or rejection in two different situations. This analysis was similar to the coincidence index (CI) of Mariotti (1980) and Mamet *et al.* (1999). The CI is calculated as the sum of commonly rejected (RR) and commonly selected (SS) quadrant percentages (Table 7.1) by two selection scenarios. Maximum CI achievable was 100% when two approaches commonly rejected and selected the same genotypes from a population.

Table 7.1: Differential selection quadrants for measuring the degree of coincidence between two selection scenarios: Fresh and dry weights taken as an example.

		Selection on dry weight	
		Reject	Select
Selection on fresh weight			
Reject		RR	RS
Select		SR	SS

$$\text{Coincidence index (CI)} = \frac{RR + SS}{\text{Total}} \times 100$$

7.3 Results

7.3.1 Selection simulations at individual crop cycles

Selection simulations were carried out for the individual crop cycles with the data derived from the second sampling date. Of interest in this section was the comparison of selections at each crop cycle and with the crop cycles combined.

In total, 11 genotypes were categorised for selection and 49 were commonly unclassified at plant cane, first ratoon and at combined crop cycles (Table 7.2). Differential selection was observed for the plant cane and for the first ratoon crops. Seven were commonly selected irrespective of their cane types. Three were selected at plant cane but not at first ratoon crop. One genotype was identified at first ratoon but not at plant cane. These values are conveniently presented in selection quadrants as illustrated in Table 7.3. With the plant cane and first ratoon crops, a high CI of 93% was observed. The indices were higher when the individual crop cycles were compared with the average of the two crop cycles.

These results indicated that selection should preferably be done on pooled crop cycles than on plant cane or first ratoon crops.

Table 7.2: Genotypes selected at individual and pooled crop cycles

Genotype	Plant cane	1st ratoon	Pooled
M799/90	Type 1	9	Type 1
M377/91	Type 1	Type 1	Type 1
M385/91	Type 1	Type 1	Type 1
M816/90	Type 1	Type 1	Type 1
M733/90	Type 2	Type 1	Type 1
M768/90	Type 1	Type 2	Type 2
M819/90	Type 2	Type 1	Type 2
M1748/88	9	Type 3/4	Type 3/4
M3305/87	Type 3/4	9	Type 3/4
M1395/87	Type 3/4	Type 1	Type 3/4
M1156/00	Type 3/4	9	Type 3/4
Commonly rejected		49	

Type 1 = commercial type; Type 2 = enhanced fibre type; 9 = reject

Table 7.3: Differential selections at different crop cycles

Plant cane versus 1st ratoon selection

Selection: plant cane	Selection: first ratoon	
	Select	Reject
Select	SS = 7	RS = 3
Reject	SR = 1	RR = 49
CI	93%	

Plant cane versus pooled crop cycles

Selection: plant cane	Selection: pooled crop cycles	
	Select	Reject
Select	SS = 10	RS = 0
Reject	SR = 1	RR = 49
CI	98%	

First ratoon versus pooled crop cycles

Selection: 1 st ratoon	Selection: Pooled crop cycles	
	Select	Reject
Select	SS = 9	RS = 0
Reject	SR = 3	RR = 48
CI	95%	

$$CI = \frac{\text{Commonly selected} + \text{commonly rejected}}{\text{Total}} \times 100$$

7.3.2 Selection simulations with fresh and dry weights of cane quality characters

In the original model, the fresh weights of Pol and fibre contents were used for reasons explained in section 6.2. These two traits were replaced by their corresponding dry weights in the model. The pooled crop cycles data at the second sampling date were used for the comparisons. Results of selection simulations are shown in Tables 7.4 and 7.5. The two selection scenarios identified 12 genotypes, of which 10 were common. The CI was thus at 97%.

Table 7.4: Genotypes selected with fresh and dry weights of cane quality traits

Genotype	Fresh weight	Dry weight
M377/91	Type 1	Type 1
M799/90	Type 1	Type 1
M385/91	Type 2	Type 1
M733/90	Type 2	Type 1
M768/90	Type 2	9
M816/90	Type 2	Type 1
M819/90	Type 2	Type 2
M905/89	9	Type 2
M1156/00	Type 3/4	Type 3/4
M1395/87	Type 3/4	Type 3/4
M1748/88	Type 3/4	Type 3/4
M3305/87	Type 3/4	Type 3/4
Commonly rejected		48

Type 1 = commercial type; Type 2 = enhanced fibre type; 9 = reject

Table 7.5: Differential selections with cane quality traits measured on fresh and dry weights

Selection with fresh weights	Selection with dry weights	
	Select	Reject
Select	SS = 10	RS = 1
Reject	SR = 1	RR = 48
CI	97%	

Thus, no important discrepancies were noted when selections were based on fresh and dry weights for cane quality characters. For practical reasons, selection based on fresh weight could be adopted in the routine selection programme.

7.3.3 Selection simulations with different cane biomass characters

In the trial, the aboveground biomass yield was measured in two parts. Clean cane stalks were weighed to obtain the cane yield. The cane tops, green and clinging dry leaves (CTL) were measured together in a separate operation. The two traits were combined to give the total vegetative aboveground biomass yield. Their corresponding dry weights were also calculated. Selection simulations involved replacing the cane dry matter yield in the original model with each of the four biomass yield alternatives. The pooled crop cycles data of the second sampling date were used for the comparisons. Results are presented in Tables 7.6 and 7.7.

Table 7.6: Selection simulations with different cane biomass characters

Genotypes	Fresh weight		Dry weight	
	Cane yield	Total Biomass	Cane yield	Total Biomass
M377/91	Type 1	Type 1	Type 1	Type 1
M799/90	Type 1	Type 1	Type 1	Type 1
M385/91	Type 2	Type 2	Type 2	Type 2
M768/90	Type 2	Type 2	Type 2	Type 2
M816/90	Type 2	Type 2	Type 2	Type 2
M733/90	Type 2	Type 2	Type 2	Type 2
M819/90	Type 2	Type 2	Type 2	Type 2
M1156/00	Type 3/4	Type 3/4	Type 3/4	Type 3/4
M1395/87	Type 3/4	Type 3/4	Type 3/4	Type 3/4
M1748/88	9	9	Type 3/4	9
M3305/87	9	9	Type 3/4	Type 3/4
Commonly rejected		49		

Type 1 = commercial type; Type 2 = enhanced fibre type; 9 = reject

Table 7.7: Comparison of selection scenarios and coincidence indices

	SS [@]	RR	SR	RS	Total	CI
Cane yield (FW*) with Total biomass (FW)	9	51	0	0	60	100%
Cane yield (DW*) with Total biomass (DW)	10	49	1	0	60	98%
Cane yield (FW) with Cane yield (DW)	9	49	0	2	60	95%
Cane yield (FW) with Total biomass (DW)	9	50	0	1	60	98%
Total biomass (FW) with Cane yield (DW)	9	49	0	2	60	97%
Total biomass (FW) with Total biomass (DW)	9	50	0	1	60	98%

*: FW = fresh weight; DW = Dry weight; @: S = select; R = reject; CI = Coincidence index

A total of 11 clones were identified by at least one of the different selection scenarios and 49 were commonly rejected. Nine were commonly selected by all the biomass traits. The coincidence indices between any two selection scenarios were very high ($\geq 95\%$). Highest coincidence was obtained

between cane yield and total biomass yield fresh weights (CI = 100%). Lowest overlapping of two selection scenarios was obtained with cane yield fresh and dry weights (CI = 95%).

These results indicated that selection based on millable cane yield would be effective in screening the best total aboveground biomass yielders. Both fresh and dry weights of cane yield could be adopted, but for maximum precision, the dry weight alternative was preferable.

7.3.4 Selection simulations with two sampling dates for cane quality characters

Selection simulations with the data collected in April and July were done with the two crop cycles combined and using traits defined in the algorithm described in Figure 7.1. Results are shown in Tables 7.8 and 7.9. Out of the population of 60 test genotypes, an exceptionally high frequency, 30 out of 60 (50%), were categorised for selection with the pre-harvest sampling data. Merely 11 (18%) were graded at the early-harvest season. The coincidence index was as low as 70%. All genotypes categorised in July were also identified in April. The majority of genotypes selected in April had sucrose content relatively equal to or higher than the commercial control varieties. Their fibre contents were significantly higher leading to the majority of the clones graded as Type 2 canes.

There was, thus, a high discrepancy in selection with the two sampling dates' data. Generally, few genotypes were expected to surpass the commercial varieties in terms of sucrose content and sugar yield, more so in the inter-specific derived clones. This tendency was rightly observed with data collected in July but not with those taken in April. More or less similar results were obtained with when Pol and fibre percent canes were replaced by their corresponding dry weights at the two different dates.

Table 7.8: Genotypes selected with 1st and 2nd sampling dates data

Genotypes	April	July
M377/91	Type 2	Type 1
M799/90	Type 1	Type 1
M768/90	Type 2	Type 2
M816/90	Type 2	Type 2
M733/90	Type 2	Type 2
M819/90	Type 2	Type 2
M385/91	Type 1	Type 2
M1748/88	Type 2	Type 3/4
M3305/87	Type 2	Type 3/4
M1156/00	Type 3/4	Type 3/4
M1395/87	Type 3/4	Type 3/4
M2118/78	Type 2	9
M1230/87	Type 2	9
M1281/87	Type 2	9
M993/89	Type 2	9
M1011/86	Type 2	9
M1303/87	Type 2	9
M1384/87	Type 2	9
M1529/88	Type 2	9
M812/90	Type 2	9
SM81022	Type 2	9
M1010/86	Type 2	9
M1424/87	Type 2	9
M905/89	Type 2	9
M1695/88	Type 2	9
M3309/87	Type 2	9
M816/86	Type 2	9
M1240/87	Type 2	9
M1162/00	Type 3/4	9
IJ76403	Type 3/4	9
Commonly rejected	30	

Type 1 = commercial type; Type 2 = enhanced fibre type; 9 = reject

Table 7.9: Differential selections with two sampling dates

Selection with July data	Selection with April data	
	Select	Reject
Select	SS = 12	RS = 0
Reject	SR = 18	RR = 30
CI	70%	

7.4 Discussion

In the various selection simulations, certain genotypes showed a shift from one type of cane to another. This was considered natural with varieties showing significance at the margin, inherent variations in the correlated traits and/or genotype x environment interactions.

For instance, with selection simulations done at individual crop cycles, out of 11 classified clones, only three genotypes maintained their cane category across crop cycles. The remaining eight either changed cane types or were not selected. Under such a condition then, the pooled crop cycle results that showed a more or less intermediate performance were felt most appropriate as a first screening. The study also prompted the need for analysis of individual year data for a more precise evaluation of genotypes.

Moreover, a brief description of a good ratooning variety is one that maintains relatively high yield in subsequent ratoons. Thus, clones categorised at plant cane only would most probably have some weaknesses of ratooning with respect to the commercial varieties in subsequent crop cycles. On the other hand, genotypes categorised at first ratoon crop only would presumably have a better ratooning ability, and, in consequence, would require more favourable attention. In consequence, selection in the second crop cycle should be given more importance than at plant cane in order to promote genotypes with high ratooning ability. Still, testing for ratooning capacity remains an important issue that needs to be further investigated with additional crop cycles.

Selection simulations with fresh and dry weights of sucrose and fibre contents gave very little difference. The very high CI of 97% was indicative of the use of any of the two types of cane quality traits for effective selection. Fresh and dry weight measurements were different only with respect to the water content in the cane stems. For the high biomass yielders, this had a limited range of 5% (68%-73%) in the population studied. This could explain the negligible disparity in selection with the fresh and dry weights of the cane quality characters.

Similarly, the four different cane biomass characters selected more or less the same genotypes, with CI values $\geq 95\%$. Selection based on clean cane stalks automatically screened the best genotypes with high total aboveground biomass yield. This was applicable with both fresh and dry weight measurements. Cane yield dry weight categorised two additional genotypes not identified by cane yield fresh weight. The two genotypes, namely M1748/88 and M3305/87, were among the clones with highest dry matter content: M1748/88 with 33% ranked first while M3305/87 with 30.3% ranked 10th in the population. Hence, use of cane dry matter yield seemed to be the best alternative to select for high biomass varieties with lesser water content.

The high disparity in selection with the two sampling dates (April and July) data and the exceptionally high frequency of genotypes categorised at the first sampling date urged for a closer study of the evolution of sucrose and fibre contents in the population. Theoretically, the commercial controls are relatively rich varieties (in sucrose content) as compared to the interspecific-derived clones. Figures 7.2 and 7.3 show the effects of sampling date on sucrose and fibre contents respectively.

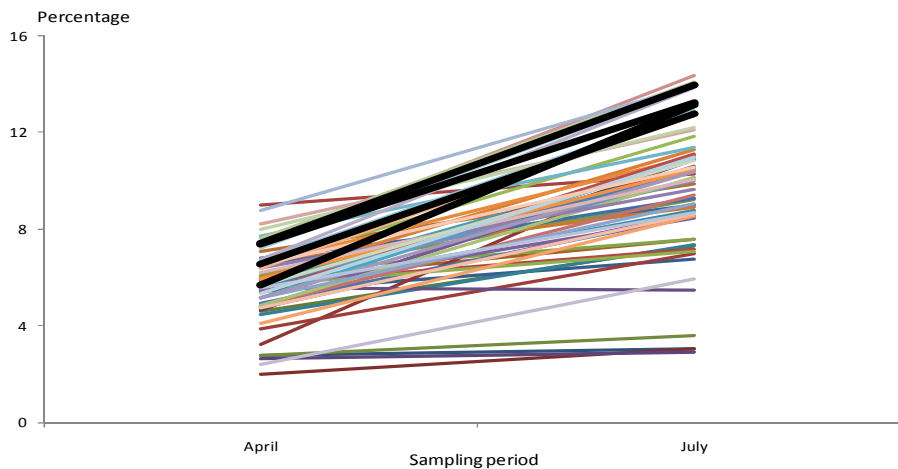


Figure 7.2: Effect of sampling date on sucrose content fresh weight of 64 genotypes averaged over crop cycles.

Dark bold lines represent commercial control varieties

From Figure 7.2, the higher variation of sucrose content in July was clearly visible. Furthermore, a high genotype x sampling date interaction could be observed, as displayed by the crossing-over lines. There was thus a definite change in rank of the genotypes at the two sampling dates. The commercial varieties (in dark bold lines) generally ranked slightly above the central position in April but rapidly improved to be among the 10-best in July. One possible explanation to this phenomenon was that in April, the majority of genotypes, inclusive of the control varieties, had just started accumulating sucrose and the differences among them was not well defined. In July, the genotypes were at or around peak maturity, the commercial varieties demarcating themselves with a generally fast sucrose accumulation rate. The trends were similar at individual crop cycles and in individual sub-trials. This explained the high number of genotypes categorised by the algorithm in April and not in July.

Assuming that the control varieties were test genotypes and a 10% selection rate were applied on Pol % cane in April, then, at least two (M1400/86 and R570) of the four commercial varieties would not have been selected. With sugar yield as the selection criterion, three commercial varieties, M1400/86,

R570 and M695/69, would not have been selected in April. M1400/86 was one of the best sugar yielders (ranked third) in the population in July and is currently one of the best commercial varieties exploited in the island.

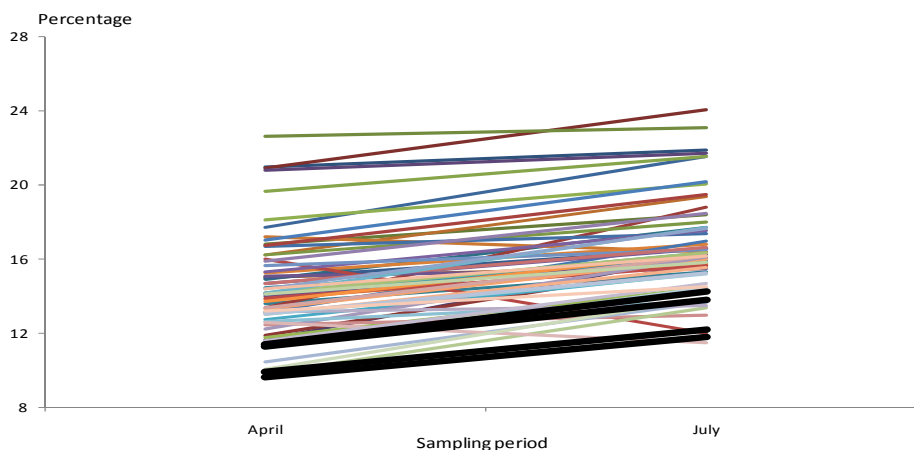


Figure 7.3: Effect of sampling date on fibre content fresh weight of 64 genotypes averaged over crop cycles.

Dark bold lines represent commercial control varieties

Figure 7.3 showed that the variation in fibre content was not much different at the two sampling dates. The commercial varieties ranks did not change much and remained among the lowest fibre content genotypes at both sampling dates.

Hence, selection for fibre content could be applied at any of the two dates. But since both sucrose and fibre contents are determined from the same sample, the data collected in July was felt more applicable for simultaneous selection for different types of canes. This justifies the approach adopted in chapters five and six in using the second sampling date data only for the estimation of potentials of the test genotypes.

CHAPTER 8

8 General discussion

8.1 Selection for high biomass canes

In this study, 60 potentially high biomass genotypes from the inter-specific derived germplasm collection were evaluated in five sub-trials for their cane quality, morphology and biomass characters. As the success of any breeding programme largely depends upon the precision with which selection is carried out, a step-wise procedure was adopted in the identification of high biomass varieties for different end-uses. This involved data validation, analysis of variations within trials and among genotypes, definition of different types of canes, construction of an algorithm for simultaneous selection of high biomass canes and the appraisal of the most appropriate traits for selection and time for data collection.

– Data validation and analysis of variances

The data validation procedure was an important step before running various analyses. A methodology was devised based on residuals that allowed the identification of few genuine outliers. They were corrected using the principle of missing values and all derived characters were updated. A total of 29 parametric traits were analysed individually at plant cane and at first ratoon crop. There was good variation among genotypes allowing selection to operate effectively. In the majority of analyses, the F-tests for genotypes were significant to highly significant. Results pointed to the good management of the trials with C.Vs. within the acceptable limits, good repeatability (H^2) and coefficient of determination (R^2) for the majority of the traits. The cane quality characters were the most repeatable and reliable traits. Cane biomass characters were more variable, but still, good precision was achieved. More precision was felt necessary in the measurement of cane tops and leaves weight. Dry weight measurements seemed to have a slight advantage in precision than their corresponding fresh weights. Particularly for sucrose content, data collection in July (early-harvest period) was found more reliable than those taken in April (pre-harvest period).

– Variances among genotypes

Multivariate data analysis techniques were used to identify dominant patterns in the data and probable groups of genotypes for multiple uses. Principal component analysis provided an efficient way of summarising the whole data set by compressing the different correlated variables into fewer dimensions. The first two PCs explained 79% of total variation. The first PC emphasised on the cane quality traits while the second PC stressed on biomass characters. The biplot with the two PCs was very helpful in visualising the existing variations in the population. There was a continuous variation

among genotypes in terms of sucrose and fibre contents and biomass yields. Sucrose and fibre were also found to be very highly negatively correlated. Isolated genotypes in the distribution were identified and their basic features were described. The biplot also helped evaluate the progress due to introgression for sucrose content among the different generations of backcrosses. Cluster analysis defined six major groups in the population. The characteristics of these clusters were further elaborated. Depending on the way sugarcane is intended to be used, specific genotypes from three different clusters could be exploited commercially.

The clones of different nobilization groups (F1, BC1 and BC2) showed wide variations in terms of sucrose concentration, fibre contents and biomass yields. Highest sucrose concentration (av. = 13.3%) was found in commercial varieties and fibre content (av. = 23.1%) among the *Erianthus* clones. High biomass was achievable from any of the different nobilization groups. Some genotypes produced more than 40% higher biomass yield over the average of commercial varieties. Cane tops and leaves generally constituted 22% of the total aboveground biomass. In general, high fibre varieties tended to have thinner stalk diameter, taller canes and higher stalk number per unit area than the commercial varieties.

– *Definition of different types of canes*

Based on the variations observed in cane quality and cane biomass traits, genotypes were categorised into four types of canes. Fibre percent increased from Type 1 to Type 4 canes. Type 1 canes represented the existing commercial varieties with more or less equal proportion of fibre. Type 2 canes were characterised as enhanced fibre type where sucrose content was not affected by the increase in fibre. Type 3 canes were those where moderate concessions were made on sucrose concentration to the advantage of fibre content. Type 4 canes were represented by genotypes with very high fibre content (>22%), low sugar and high biomass yield. With Type 1 and Type 2 canes, the main end-product remained sugar and fibre was the by-product. Conversely, the main output with Type 3 and Type 4 canes was fibre, and sugar became the by-product. Type 3 canes, however, provided a wider range of possibilities and subject to the sucrose level and cane yield, sugar yield might not be affected. Type 4 canes were essentially meant for cogeneration.

Tew and Cobill (2008) from USDA-ARS Sugarcane Research unit, USA, described three main types of canes: Conventional sugarcane varieties were classified as “*Sugarcane type*”; conventional sugarcane with enhanced fibre and those with lower sucrose content but equivalent sugar yield as existing commercial varieties as “*Type I energy cane*” and purely high fibre varieties as “*Type II energy cane*”. The findings in this study differed from their approach in the sense that their “*Type I energy canes*” was further partitioned into two distinct categories: “Type 2 canes” where fibre content

was increased without concession on **sucrose content** and “Type 3 canes” where **sucrose content** was negatively affected with an increase in fibre concentration. While both Type 2 and Type 3 canes can lead to equivalent sugar yield, Type 3 canes also create additional opportunities of exploiting cultivars for sugar, ethanol and electricity generation. This type of cane is interesting particularly in a socio-economic environment where bioenergy production from sugarcane becomes equally profitable or even more profitable than sugar. Furthermore, given the negative correlation between sucrose and fibre contents, it was more likely to obtain high biomass varieties with lower sucrose content (Type 3 canes) than those where both traits were improved concurrently (Type 2 canes).

– *Construction of an algorithm for simultaneous selection of high biomass canes*

A selection algorithm was developed to categorise high biomass genotypes into the different groups. The model was mainly based on significance tests and, in consequence, catered for variations in individual traits and genetic by environment interactions. Pol and fibre contents were reinforced with additional thresholds to ensure minimum differences from the Type 1 commercial varieties. The model successfully identified 11 high potential genotypes and correctly assigned them to the four different types of canes. Although not all the data available were used for selection, these results agreed very well with the observations made with multivariate analyses. The selection index picked the best candidates from each of the three clusters of interest mentioned above.

– *Appraisal of the most appropriate traits for selection and time for data collection*

In an attempt to identify the most appropriate traits for selection and time for data collection, selection simulations were done with fresh and dry weights, different cane biomass characters and with two sets of data collected at two different dates. The effect on selection was observed by substituting one item in the algorithm constructed for the identification of different types of canes. The degree of overlapping of two selection scenarios was calculated using coincidence indices (CIs), which measured the percentage of commonly selected and rejected clones from the population.

Selection simulations with fresh and dry weights of sucrose and fibre contents did not produce any significant difference in selection (CI = 97%) although heritability values for dry weights were higher. Under such a context then, fresh weights of cane quality characters could be reliably used for selection. The most probable explanation on the findings was the narrow range in dry matter percent cane (Brix % cane + fibre % cane) observed among the high biomass canes. This constituted a major bottleneck in the population studied. Various authors (Rao, 2007; Terajima, 2007; Tew and Cobill, 2008) have reported on high biomass clones with dry matter content of cane reaching 40% while their commercial varieties were around 30% (see Table 2.6). In this population, the wild *Erianthus* clones, known for their high fibre content, had dry matter content around 30% while that of the commercial

varieties averaged to 28.5%. Highest dry matter percent, of the order of 33%, was obtained with one BC1 clone, namely M1748/88.

Little disparity also existed between selection simulations involving different aboveground biomass characters ($CI \geq 95\%$). Selection based on millable cane stalk only was found highly effective in selecting of the best whole crop yielders. But this could also be due to the lack of precision observed in weighing cane tops and leaves. Cane yield, measured on a dry weight basis, was effective in identifying genotypes with higher dry matter content. Although any of the different biomass characters could be used for selection, cane yield dry weight was felt most appropriate in the selection of high biomass varieties with lesser water content.

A wider discrepancy in selection ($CI = 70\%$) was observed with samples taken at pre-harvest and early-harvest seasons. Selections based on early-harvest data (July) were found more effective than those based on pre-harvest data (April) in identifying the best clones. These results concurred well with the repeatability values observed earlier. It was hypothesised that the level of sucrose accumulated in the canes in April was too low and not well defined among the different clones, inclusive of the controls. Various studies done under the local context, however, have found higher genetic variations in the trait in March/April than in July for commercial populations (Badaloo *et al.*, 2005; Mamet *et al.*, 1996; Nayamuth *et al.*, 2005). The population in this study consisted of clones derived from inter-specific crosses that are generally known to have low sucrose and high fibre contents, accompanied with relatively high crop vigour and profuse flowering. These observations point to the need for further investigations on the most appropriate time for cane quality assessment, with additional sampling rounds and in populations of different nobilized groups.

8.2 Further studies

Six out of the 11 high biomass genotypes identified have been selected and planted for further evaluation in several environments in year 2009. Future studies involve evaluations to confirm on their characteristics and type attribution, adaptation, stability and ratooning ability. It will be desirable to sample at different periodic dates across the pre-harvest and harvest seasons to determine the most appropriate time for data collection.

The algorithm developed for selection of different types of canes will be applied in the routine selection programme in search for other high biomass varieties for multiple uses. It will then be possible to further fine-tune the set thresholds in light of the variations observed among genotypes. For instance, in the final phase trials at the MSIRI, elite genotypes are assessed for their sucrose and

fibre contents at three different dates, commonly termed as early-, mid- and late-season sampling, during the 6-month harvest period. A high fibre variety, particularly Type 2 canes, should systematically have superior fibre content than the existing Type 1 commercial varieties throughout the harvest period. Otherwise, it should be relegated to Type 1 canes. Similarly, high biomass Type 3 and Type 4 canes should consistently have significantly lower sucrose content than the commercial varieties at all sampling dates. Otherwise, they should then be considered among the Type 1 or Type 2 canes with specific harvest period. The same philosophy should apply with multi-environment trials.

For the successful exploitation of high biomass canes for multipurpose use, additional studies need to be undertaken both at the field and the factory levels. Higher fibre varieties will certainly have an impact on sugar extraction efficiency. Faced with the EU sugar reform, Barbados attempted a swift shift to large scale cultivation of three high fibre varieties (identified from their germplasm collection) essentially for energy production. Between 2003 and 2007, a total of 260 acres of “purely high fibre varieties” (Type 4 canes under this study) were established, and during the 2007 harvest 216 acres were harvested (de Boer, 2008). The results have been rather disappointing after a few years of experience. The most important findings are listed below:

- The purely high fibre canes were expected to have a rapid growth and a very good ratooning ability. Yet, ratooning was disappointing in the commercial fields.
- Mechanical harvest was not appropriate for the type of cane. Approximately half of the total quantity of biomass was not recovered by reaping with unmodified sugarcane harvesters. Mechanical harvester was presumed to pulverise some cane with the unrecoverable loss of very small fragments of cane and juice from the chopping knives and extractor fans.
- Because high fibre canes had lower moisture content, the stalks were lighter than conventional sugarcane varieties. More than twice the energy input was found necessary to transport the cane biomass to the factory, as the standard tractor-bins weighed less than half its normal tonnage when filled with the high fibre canes.
- With the existing factories, a lower grinding rate (75 instead of 100 tonnes cane per hour) was necessary to process the cane.

Based on the information collected from the harvest and growth pattern, it was concluded that the varieties that were grown were disappointing as far as yields were concerned. CBS consequently found no other alternative than to stop cultivating the new high-fibre varieties and aim at developing “*higher fibre sugar cane varieties*”. This meant the identification of new varieties with higher levels of sucrose and lower levels of fibre (Type 2 and Type 3 canes) than the purely high-fibre cultivars. In Louisiana, on the other hand, three high biomass varieties were released in year 2007 after several

years of field testing and machine harvesting. One of them was of the purely high fibre type. No negative remarks have been reported so far.

The main lessons that could be learnt from the Barbados experience are that:

- Although purely high fibre Type 4 canes hold great promises from the bioenergy point of view, timing may not be right at the moment to exploit such type of cultivars in small countries where land is limited.
- Presently, varieties that can maintain high sugar yield and provide high aboveground dry biomass (Type 2 and Type 3 canes) seem to represent the most plausible alternative for commercial exploitation.
- Selection should be stringent and precise, and should take stock of the various implications of cultivating the different types of varieties.

As energy cane production is fundamentally a reorientation of cane sugar planting and management, a concerted effort integrating agronomic practices, harvesting, transport and processing of the high biomass varieties will be fundamental. The type of varieties to be developed for the future will evidently depend on economic feasibility studies involving the prediction of the price of sugar, fossil fuel, ethanol and gains from power generation. Overall, it should be possible to select sugarcane varieties with properly balanced high sugar recovery and fibre yield, as well as plants with enhanced photosynthetic capacity, ratooning capacity and growth rate that, on the whole, will respond to the national and international scenarios in the near future in relation to both sugar and bioenergy production.

In the short term, in a small country like Mauritius, where land is limited and sugar production is well anchored in the economy, it seems improbable to exploit the purely high fibre Type 4 canes. So far sugar remains profitable, the focus in research should be geared towards the identification of canes where sugar yield is not compromised and fibre yield is maximised. Hence, along with selection for the conventional Type 1 canes, importance needs be given to Type 2 and Type 3 canes, which can be used for both sugar and bioenergy production. In the long term, still, given the high volatility of fossil fuel and the increased premiums given to renewable energy, successful cultivation of Type 4 canes may become a reality. As breeding of a new sugarcane variety takes between 10 to 15 years, efforts should be made now in the identification of all the different types of canes that can ensure high levels of sugar and an important source of low-cost feedstock for bioenergy production for the future.

CHAPTER 9

9 Conclusions

In view of developing varieties with high fibre content that can be used for different purposes, a total of 60 varieties were selected from a collection of genotypes mostly derived from a genetic base-broadening programme. The genotypes were evaluated for 29 parametric traits in five sub-trials together with four conventional commercial varieties. A methodology based on residuals was devised to validate the data which was effective to identify few genuine outliers. The CVs obtained were within acceptable limits and good repeatability values were observed for the majority of the traits. The cane quality characters such as Pol % cane, fibre % cane, dry matter % cane were the most repeatable ones. Cane biomass characters were more variable but good precision was achieved.

The clones varied widely in both fibre and Pol and biomass characters. As expected, on average, the F1 has the highest fibre content (17.3%) and lowest Pol % cane (8.3%), the latter improved in the BC1 (10.2%) and BC2 groups (13.1%) with a corresponding decrease in fibre content. These two characters were negatively correlated ($r=-0.72$) and point to the difficulty of improving both characters simultaneously. Fibre content for the *Erianthus* clones averaged 23% with very little sugar (3.2%) whereas dry matter content varied on average between 28 to 30 % for all groups of clones and seemed to be the main bottleneck for improving both fibre and Pol concurrently.

Multivariate data analyses involving 18 different continuous traits were highly effective in summarising the data and defining groups of similar genotypes. Genotypes with outstanding performances in specific, or a combination of, traits were easily identified from the biplot derived from PCA analysis. Cluster analysis defined six major groups in the population. Depending on the way sugarcane is intended to be used, specific genotypes from three different clusters could be exploited commercially.

Inherent variations in three major traits of interest, namely sucrose content, fibre content and biomass yield, among genotypes in the population were further studied. Based on the findings, four different types of high biomass canes (Type 1 to Type 4 canes) were defined for multiple uses. From Type 1 to Type 4 canes there was a continuous progress in fibre percent. A selection algorithm combining the three major traits was developed that identified 11 high potential genotypes simultaneously. All the four cane types were represented.

In an attempt to define the most appropriate trait for selection and time for data collection, selection simulations were done with different measured characters. It became clear that any of the different

fresh and dry weight measurements could be adopted provided the dry matter content of the high biomass varieties fluctuated within a narrow range. In addition, highest efficiency in selection could be achieved in the inter-specific populations when data are collected at the early harvest stage rather than at pre-harvest stage. These observations, however, differed from various findings done under the local context. It was thus felt necessary to do further analyses with different populations and additional sampling rounds to determine the most appropriate time for data collection.

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