Family evaluation for quality traits in sugarcane

By

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Submitted in fulfilment of the requirements in respect of the degree of Magister Scientiae Agriculturae in the Department of Plant Sciences (Plant Breeding) in the Faculty of Natural and Agricultural Sciences at the University of the Free State
Bloemfontein

January 2019

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Declaration

I, Tondani Mishasha, declare that the Master's Degree research dissertation that I herewith submit for the Master's Degree qualification Magister Scientiae Agriculturae at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

Signed …… Date ……..

Tondani Mishasha

Acknowledgements

I would like to express my sincerest gratitude to the following individuals and organisations for their contribution towards the success of my Masters' Degree dissertation.

First and foremost, I would like to give praise to God Almighty for giving me strength, wisdom, knowledge and ability to undertake this research study.

I would like to thank my supervisors, Prof Marvellous Zhou and Dr Rouxléne van der Merwe, for their patience, guidance, encouragement and continued support during the course of this study.

The South African Sugarcane Research Institute (SASRI), National Research Foundation (NRF) and University of the Free State (UFS) for giving this opportunity and funds to further my studies. SASRI for giving me access to their data and research materials for the purpose of this research.

SASRI and plant breeding staff for managing trials and data collection.

Dr Sumita Ramgareeb, resource manager at SASRI of Breeding and Field Resource Unit, for assisting with travel arrangements for data collection, workshops and conferences.

Mrs Sadie Geldenhuys, secretary for Plant Breeding at UFS for her assistance with registration and encouragement.

My family and friends for their love and continued support during the course of this study. More especially my grandmother (Mrs Anna Mushasha), Aunt (Ms Idah Mushasha), Uncle (Mr Phophi Mushasha), brothers (Thendo and Rotshidzwa Matshisevhe) and sisters (Mukovhe and Nthabiseng Mushasha) for keeping me going when challenges arose.

Dedication

This dissertation is dedicated to the memory of my late mother (Mrs Jane Tshifulufhelwi Matshisevhe) who always inspired me and believed in me. You were gone before I could make you proud but your belief in me contributed to the success of this study and continues to inspire me.

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List of abbreviations and SI units

BLUP	Best linear unbiased prediction
BP	Bi-parental
CV	Coefficient of variation
^{o}C	Degrees Celsius
DF	Degrees of freedom
DAFF	Department of Agricultural Forestry and Fisheries
DM	Dry matter
ERC	Estimated recoverable crystal
F	Family
FML	Irrigated mini-line
g	Genotype
g	Gram
UML	Coastal short cycle average potential mini-line
Gs	Predicted selection gains
H	Broad-sense heritability
HTV	High trait value families
HVP	High value parents
IG	Individual genotype
kg	Kilogram
K	Potassium
KZN	KwaZulu-Natal
m	Meter
ml	Millilitre
MO	Males only
MP	Melting pot
N	Nitrogen
P	Phosphorus
Pol	Polarisation
r	Correlation coefficient
R^2	Coefficient of determination

RCBD	Randomised complete block design
SAS	Statistical analysis system
SASA	South African Sugar Association
SASRI	South African Sugarcane Research Institute
SD	Root mean square error/ Standard deviation
SE	Standard error
SML	Sandy soil mini-lines
spp	Species
TML	Coastal short cycle high potential mini-line
USA	United States of America
%Gs	Percentage predicted selection gains
σ^2_F	Family variance
σ^2_{FR}	Family by replication variance
$\sigma^2_{G(F)}$	Genotype nested within family variance
$\sigma^2_{G(FR)}$	Residual variance

Abstract

Family evaluation and selection has the potential to increase the selection efficiency, thereby improving breeding for quality traits at early stages of sugarcane breeding. Data from family evaluation trials can also be useful for quantifying the performance of parental genotypes that were used to produce families. The objectives of this study were to compare family to individual genotype evaluation and determine optimum sample size for estimating sucrose content; to determine family evaluation breeding parameters and phenotypic correlations for quality traits; to determine the proportions of families and parents producing significantly higher trait values compared to the trial mean for the coastal short cycle mini-line breeding programme. Data were collected from 20 genotypes within each family plot for hand held refractometer Brix %cane data (surrogate for sucrose content), estimated recoverable crystal (ERC) %cane, polarisation (Pol) %cane, Brix %cane, Brix dry matter (DM) %cane, purity %, fibre %cane and DM %cane. Data were analysed using statistical analysis system software (SAS). Both family and individual genotype variances were significant ($P<0.01$) suggesting selection for individual genotypes within selected superior families will increase efficiency of selection. Broad-sense heritability (H) values ranged from 0.74 to 0.77 for families and from 0.14 to 0.17 for individual genotypes. Families (7.06 to 12.72%) produced higher predicted selection gains (%Gs) than individual genotypes (2.42 to 5.47%). The higher H and %Gs indicated superiority of family selection compared to individual genotype evaluation. Response surface graphs showed four replications and 10 genotypes produced H values of 0.77 to 0.80 compared to 0.74 to 0.77 with sample size of 20 genotypes in three replications. Reducing the sample size from 20 to 10 genotypes will reduce data collection costs. ERC %cane, Pol %cane and fibre %cane had the largest H and %Gs values, suggesting that family evaluation was effective for these traits. Results showed strong associations among sucrose related traits (ERC %cane, Pol %cane and Brix %cane) with correlation coefficient values ranging from 0.80 to 0.99. The significant ($P<0.05$) negative correlation between sucrose related traits and fibre %cane ($r>0.07$) showed a decrease in sucrose content with an increase in fibre content. Results showed that Brix %cane had a high H, high %Gs and significant positive correlations with both ERC %cane and Pol %cane, suggesting that Brix %cane is a suitable trait for family evaluation. Brix %cane can be measured easily in the field using a hand held refractometer, providing data to quantify within family variability in early stages of sugarcane breeding. Results showed highly significant family, female and male variances, highlighting the existence of genetic differences and control among families. Results showed a lower proportion of families producing significantly higher best

linear unbiased prediction (BLUP) values compared to the population mean. Results suggested the need to use breeding values to guide parent selection for breeding programmes.

Key words: Sugarcane, family and parent evaluation, broad-sense heritability, predicted selection gains

Chapter 1

General introduction

Sugarcane is a tall perennial grass belonging to the *Saccharum* genus in the Poaceae family. It grows in the tropical and sub-tropical regions (Verheye, 2010). The *Saccharum* genus is comprised of six species; *Saccharum spontaneum*, *Saccharum robustum*, *Saccharum officinarum*, *Saccharum barberi*, *Saccharum sinense* and *Saccharum edule* (D'Hont et al., 1998). The *Saccharum* genus along with its closely related taxa (*Erianthus, Sclerostachya, Narenga* and *Miscunthus*) are generally known as the *Saccharum* complex (Besse et al., 1998).

Sugarcane is one of the world's most economically important crops (SASA, 2015). Brazil is the world's top producer of sugar followed by India (OECD-FAO, 2018; Food and Agriculture Organization of the United Nations, 2018). More than 70% of the world's total sugar is produced from sugarcane (Zhang et al., 2014). Although sugarcane is cultivated primarily for sugar production (Verheye, 2010) the crop has recently gained interest for biofuel production (Martinelli and Filoso, 2008; You et al., 2013). Sugarcane production contributes significantly to the South African economy through agricultural and industrial investments and through providing employment. In South Africa, sugarcane is produced in Eastern Cape, KwaZulu-Natal (KZN) and Mpumalanga provinces. The total sugar produced for the 2018 season was 2155556 tons in South Africa (SASA, 2019). The South African sugar industry provides direct employment in cane production and processing, and indirect employment in a number of support industries in the provinces where sugarcane is grown (SASA, 2015). The South African Sugarcane Research Institute (SASRI), a division of the South African Sugar Association (SASA), was established in 1925 to conduct sugarcane research. The initial objective of SASRI was to import, test and recommend varieties (Zhou, 2013).

High sugar yield is the ultimate aim of sugarcane breeding. Cane yield and cane quality are the components of sugar yield (Khan et al., 2001), thus making cane yield and cane quality important sugarcane breeding objectives. Millers assess cane quality based on the amount of recoverable sugar per ton on cane crushed. High sucrose, high purity, low fibre and low non-sugars are some of the most important quality characteristics that contribute to high recoverable sugar (Meyer and Wood, 2001). Millers are interested in high sucrose content varieties since low sucrose varieties often lead to poor sugar recovery at processing. Sugarcane is made up of

fibre and juice. The juice in sugarcane is composed of water and soluble solids. Soluble solids consist of sucrose and non-sucrose components (reducing sugars and salts). There is a need to develop high sucrose accumulating varieties for the South African subtropical growing conditions. Therefore, efforts are needed to increase gains from selection for quality traits (Zhou and Lichakane, 2012).

Selection is an important process of plant breeding and is practised across all stages of sugarcane breeding. Selection focuses on improving cane yield, quality, sugar yield, ratooning ability and disease and pest resistance (Zhou, 2004). The breeding programme starts with parent selection and the selected parents are planted in the glasshouse and photoperiod house to produce flowers for crossing. Parents are selected based on high cane yield, high sucrose content, high sucrose yield, good ratooning ability, and disease and pest resistance (Zhou, 2013). Selection is done to identify varieties suited to the major agro-climatic regions of the sugar industry. Even though sugarcane is commercially grown as a clone, seedlings are raised from true seed. Crosses are made specifically for each breeding programme and the clones are adapted to the particular agro-ecological region.

Currently SASRI operates seven regional breeding programmes; two breeding programmes (B and S) for the Midlands region, four breeding programmes (K, G, U and T) for the coastal region and one breeding programme (F) for the irrigated region. In the Midlands, the harvesting period ranges from 20 to 24 months, in the coastal region the harvesting cycle ranges from 12 to 18 months and at the irrigated region harvesting is done at 12 months. These programmes differ in region, climate, soil, pests and diseases. Varieties are developed to adapt to the conditions in their specific breeding programme.

Family evaluation involves the selection or rejection of whole families of seedlings based on data derived from family plots (Kimbeng and Cox, 2003). Family selection research for sugarcane traits was first introduced in Australia in 1970 (Hogarth, 1971; Hogarth et al., 1990; Jackson et al., 1995; Cox and Stringer, 1998; Kimbeng et al., 2000; Stringer et al., 2011). Previous studies have shown that family selection was adopted in many breeding programmes in Brazil (De Resende and Barbosa, 2006; Pedrozo et al., 2011), India (Shanthi et al., 2008; Babu et al., 2009), USA (Milligan and Legendre, 1990; Chang and Milligan, 1992) and South Africa (Bond, 1977; 1989; Zhou and Lichakane, 2012; Zhou, 2014; Zhou and Mokwele, 2015). In South Africa, family evaluation was implemented for quality traits in 1999 (Zhou and

Lichakane, 2012), for yield in 2011 (Zhou, 2014) and for *Eldana saccharina* in 2014 (Zhou and Mokwele, 2015). The information derived from progeny performance during family evaluation can also be used for parent evaluation (Kimbeng and Cox, 2003).

Family evaluation and selection has proved to produce larger gains than individual genotype selection for sugarcane yield and sucrose content (Stringer et al., 2011; Zhou, 2014). Family selection, followed by individual genotype selection, has proved to be superior in terms of producing larger genetic gains than either family selection or individual selection alone (Kimbeng and Cox, 2003). Family evaluation was found to be useful because families can be replicated over years and locations, thus improving estimates of family values and effects. Elite families are identified and superior genotypes are selected within the best families, thereby increasing the breeding efficiency from family selection.

1.1 Justification

An increase in sucrose content of sugarcane increases sugar yields, thereby making increase in selection gains for sucrose content economically beneficial (Jackson, 2005). Therefore, improved sucrose content is an important sugarcane breeding objective. Comparisons between family and individual genotype evaluation will validate the use of family evaluation over individual genotype evaluation. There is a need to reduce costs associated with transport, data collection and mill room analysis. Thus resource allocation will optimally determine design parameters and sample size. Knowledge of phenotypic correlations is important to predict traits that are expensive or difficult to measure based on performance of traits that are easier to measure. Although studies have shown the potential of family evaluation for quality traits (Zhou and Lichakane, 2012; Zhou et al., 2013), further research is still required to quantify the benefits of family evaluation for different breeding programmes. Determining the proportion of elite families and parents will guide family selection. Families and parents, producing significantly lower trait values, can be identified and discarded early, whilst those with high trait values can be crossed more frequently to generate more progenies. Family effects and breeding parameters (variance components, broad-sense heritability, predicted selection gains and breeding values) will be established and used to determine potential increase in breeding efficiency from family evaluation. Phenotypic correlations will aid in determining the interrelationships between two or more traits. Phenotypic correlations will also be useful in predicting the response of traits based on the performance of correlated traits.

1.2 Aim and objectives

The aims of this study were to determine the magnitudes of benefits of family evaluation for different breeding programmes and maximise the benefits of family selection. Knowledge of magnitudes of heritability and selection gains over time will provide information to improve the precision of family evaluation.

The objectives of this study were:

1. To compare family to individual genotype selection and determine sample size for estimating sucrose content using a hand held refractometer in breeding trials.
2. To determine family evaluation breeding parameters for quality traits, phenotypic correlations and their implications on sugarcane breeding.
3. To determine the proportions of elite families and parents for the coastal short cycle breeding programme.

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Chapter 2

Literature review

2.1 Introduction

2.1.1 Origin, history and distribution of sugarcane

Sugarcane is grown in tropical and sub-tropical regions of the world for sucrose in the stalks (Dillon et al., 2007; Verheye, 2010). Sugarcane is believed to have originated in Polynesia (Daniels and Roach, 1987; Zhang et al., 2014) and New Guinea (Barnes, 1974; Verheye 2010) where it was used mainly for chewing. The *Saccharum* genus is presumed to have originated before the continents moved to their current shapes and locations (Cheavegatti-Gianotto et al., 2011). The *Saccharum* spp. is believed to have two centres of diversity: the Old World (Asia and Africa) and the New World (North, Central and South America). The noble cane *S. officinarum* was domesticated from *S. robustum* in the New Guinea region and was then dispersed to Asia and Pacific and Indian sub-continent through human migration. In Asia, *S. officinarum* hybridized with local *S. spontaneum* giving rise to the North Indian and Chinese cultivars (Daniels and Roach, 1987; Grivet et al., 2004). Because of the unique capability of sugarcane to store sucrose (Tew and Cobill, 2008), it became one of the most famous crops in the world and many countries adopted the crop. It is cultivated in more than 90 countries worldwide (Da Costa et al., 2011).

2.1.2 History of sugarcane production in South Africa

The earliest cultivation of sugarcane dates back to 1635 when Portuguese mariners found cane growing near Umzimkhulu (McMartin, 1948; Antwerpen et al., 2005). However Edmund Morewood planted the first commercial cane in South Africa on the north coast of KwaZulu-Natal (KZN) province in 1848 and established a small sugar mill in the Compensation area (Schrire, 1983). The first sugar cultivars were imported from Mauritius in 1848 and proved to be so successful that the first mill was built on the Compensation flats in 1850. According to Lewis (1990), the sugar industry was established in the old “Natal” province in the 1840s. In South Africa, sugarcane is grown in KZN, Mpumalanga and Eastern Cape provinces (DAFF, 2017).

2.2 Economic importance

Sugarcane produces 70% of the total sugar produced in the world while 30% is produced from sugar beet (Zhang et al., 2014) and is a cash crop in several countries. Brazil is the largest producer of sugarcane followed by India and China. Brazil produced 36.1 million metric tons, while India produced 27.3 million metric tons and China 17.2 million metric tons for the 2016 season (Taylor, 2017). In Brazil, the economic interest of sugarcane has grown significantly due to the increased demand for sustainable energy production worldwide (Cheavegatti-Gianotto et al., 2011). South Africa is among the top 15 sugar producing countries worldwide (SASA, 2015). South Africa produced 2155156 tons sugar for the 2018 season (SASA, 2019). In South Africa sugarcane contributes to the national economy through agricultural and industrial investments, foreign exchange earnings as well as employment (SA sugar industry, 2015). It is a major contributor to the rural economic activity in the sugarcane growing areas of KZN, Mpumalanga and the Eastern Cape generating approximately R20 billion in annual revenues (Maloa, 2001). The sugar industry in South Africa directly employs 79000 people and supports 350000 jobs in support services and downstream industries (DAFF, 2017).

2.3 Sugarcane taxonomy

Sugarcane belongs to the genus *Saccharum* L., in the *Andropogoneae* tribe of the *Poaceae* family also known as the grass family and found abundantly in tropical and subtropical places. Sugarcane shares common characteristics with four other closely related genera which together make up the *Saccharum* complex. The *Saccharum* complex is comprised of the genus *Saccharum*, *Erianthus*, *Miscanthus*, *Narenga* and *Sclerostachya* (Daniels and Roach, 1987). Species of sugarcane include *S. officinarum* L. (noble cane), S. *spontaneum* (wild cane), *S. robustum* (wild cane), *S. edule*, *S. barberi* and *S. sinense*. *S. officinarum* is native to New Guinea and is cultivated for its high sugar and low fibre. *S. spontaneum* is native to south Asia, Africa and several parts of the world, where it grows in a wide variety of environments ranging from deserts to swamps. It is characterised by very low or no sugar content and high fibre. *S. barberi* is native to north-eastern India and its characteristics include long thin stalks, early maturity and low to medium sugar content. *S. sinense* is a species cultivated in China and has long, thin stalks, early maturity, low to medium sugar content and prominent nodes. *S. robustum* is a wild species of *Saccharum* that is the closest to *S. officinarum* (Cheavegatti-Gianotto, 2011). *S. edule* is found only in New Guinea and involves a small group of clones. It is closely related to *S. robustum* and is thought to have evolved from *S. robustum.* Modern sugarcane (*Saccharum* spp.) cultivars are interspecific hybrids, derived from a hybridisation of

S. officinarum (noble cane) and *S. spontaneum* (wild cane) and followed by a series of backcrosses to the noble parent (Daniels and Roach 1987; Dillon et al., 2007).

2.4 Sugarcane genetics

Sugarcane has a very large and complex genome with high levels of polyploidy. The level of polyploidy and genome size differ among hybrids of the *Saccharum* complex (Zhang et al., 2014). The basic chromosome number of *S. spontaneum* is x = 8 with a chromosome number of 2n = 40 to 128 (Sreenivasan et al., 1987). *S. robustum* has 2n = 60 to 80 with a basic chromosome number of x = 10. *S. officinarum* has a basic chromosome number x = 10 and 2n = 80 (Piperidis et al., 2001). *S. sinense* (2n = 80 to 124) and *S. barberi* (2n = 111 to 120) are interspecific hybrids from crosses between *S. officinarum* and *S. spontaneum*. *S. edule*, believed to be an intergeneric hybrid of either *S. officinarum* or *S. robustum* with other species has 2n = 60 to 80. Modern cultivars' chromosome numbers range from 2n = 100 to 130. About 70 to 80% chromosomes of modern cultivars are derived from *S. officinarum*, 10 to 20% from *S. spontaneum* (Piperidis et al., 2001; Zhang et al., 2014) and the 10% recombinants.

2.5 Sugarcane breeding

2.5.1 History of sugarcane breeding in South Africa

In South Africa, the first sugar was produced in 1852 from varieties of noble cane (*S. officinarum*). Initially, the South African sugar industry cultivated imported varieties (McMartin, 1948; Zhou, 2013). However, the majority of imported varieties were susceptible to pests and diseases and were not adapted to local growing conditions (Zhou, 2013). In 1925, SASRI was established with the aim of importing, testing and releasing varieties with desirable agronomic traits, including resistance to pests and diseases. Majority of the imported varieties were not adapted to South Africa. The low temperatures in winter when sugarcane flowered, resulted in lack of pollen viability. Crosses imported from India produced only two successful varieties, NCo310 and NCo376. In the 1940s research showed that viable pollen and crossing could be achieved when temperature was maintained above 20ºC. Glasshouse and photoperiod facilities were constructed in 1966 and 1971, respectively to facilitate flowering, thus overcoming pollen infertility (Zhou, 2013).

2.5.2 South African Sugarcane Research Institute breeding programmes

SASRI operates seven regional programmes within three major agro-climatic regions (Figure 2.1). Varieties are developed for the main agro-ecological sugarcane growing conditions including the irrigated, coastal (Indian Ocean coastline) and Midlands (Table 2.1) regions. The irrigated breeding programme is based at Pongola research station which is characterised by low rainfall and therefore the sugarcane is grown under irrigation and harvested at 12 months. The coastal long cycle high potential breeding programme is based at Kearsney research station and is characterised by high and well distributed rainfall and the crop is harvested at 14 to 16 months.

The coastal long cycle average potential breeding programme, based at Gingindlovu research station, represents low and poorly distributed rainfall, shallow soils and the crop is harvested at 18 months. The coastal short cycle average potential breeding programme is based at Gingindlovu research station and it represents a 12 month harvest cycle before *E. saccharina* damage causes significant yield loss. The coastal short cycle high potential breeding programme, based at Empangeni research station, was established to develop sugarcane cultivars with high cane yield, high sucrose content and maturity at 12 months. The area is characterised by high rainfall and temperatures with deep and rich soils. The Midlands breeding programme aims to develop cultivars for a climate with long cold winters and shorter summers with humic (Bruyns Hill research station) and sandy (Glenside research station) soils, respectively.

2.5.3 Stages of sugarcane breeding

2.5.3.1 Crossing

A sugarcane breeding programme starts with parent selection. Parent genotypes for each breeding programme are selected based on genetic values (new parents) and breeding values (repeat parents) for the major traits of economic importance. The selected genotypes are planted in the glasshouse and photoperiod facilities where they are subjected to photoperiod treatments to induce flowering. Three mating designs, namely Bi-parental (female x male), Males only (cross-pollination of several genotypes) and Melting pot (female x several males) are used.

Figure 2.1. Map showing sugarcane growing areas and SASRI research stations

Table 2.1. South African Sugarcane Research Institute (SASRI) regional breeding programmes

Region	Programme	Location	Age (months)
Irrigated	Irrigated	Pongola (F)	12
Coastal	Coastal long cycle high potential	Kearsney (K)	16 to 18
	Coastal long cycle average potential	Gingindlovu (G)	18
	Coastal short cycle average potential	Gingindlovu (U)	12 to 15
	Coastal short cycle high potential	Empangeni (T)	12
Midlands	Sandy soils	Glenside (S)	20 to 24
	Humic soils	Bruyns Hill (B)	20 to 24

2.5.3.2 Selection stages

Selection is carried out in five selection stages to identify genotypes with the best combination of traits for further testing (Table 2.2). The first stage consists of evaluation of seedlings in terraces where 250000 genotypes are raised from true seed. The second stage is mini-lines where family evaluation and selection is followed by visual selection within the best families. Stage three (single-lines), is based on yield estimates of individual genotype row plots, disease and pest data and visual evaluation of genotypes in the field. The fourth stage (observation) is the first replicated stage where data collected from plant and first ratoon crops are used to select genotypes for advancement. Stage five (advanced variety trials) is the final evaluation of genotypes before recommendation for commercial release. The breeding cycle takes 10 to 24 years to release a variety, depending on age of crop at harvest.

Table 2.2. SASRI selection stages in a breeding programme

Stage	Clones	Design	Reps	Crops and ratoons	Rate (%)	Criteria
Stage 1 seedlings	50000 × 5 250000	Replications of families	Family	1	70	Family values, visual assessment and freedom from diseases
Stage 2 Mini-lines	35000 × 5 175000	Replications of families	Family	1	11	Family values for yield, sucrose and pest and disease resistance
Stage 3 Single lines	4000 × 5 20000	Replications of families	Family	1	10	Sucrose content, sucrose yield, pests and disease resistance
Stage 4 Observation	400 × 5 2000	Lattice, 2 × 8 m	3	2	10	Combined analysis for high yield, sucrose, pests and disease
Stage 5 Advanced variety	40 × 5 150	Lattice, 5 × 8 m 5 trials	3	3		Combined analysis across sites and crops
Release	1 to 2					

Adapted from Zhou (2013)

2.6 Sugarcane quality traits

The main objective of a sugarcane breeding programme is to produce higher sugar yielding genotypes. However sugar yield is a product of both cane yield and sucrose content. This makes higher cane yield and sucrose content primary objectives during sugarcane breeding. This study focused on cane quality traits which include: estimated recoverable crystal (ERC) %cane, polarisation (Pol) %cane, Brix %cane, Brix dry matter (DM) %cane, purity %, fibre %cane and DM %cane. ERC %cane is the estimated amount of sucrose in the juice that can be recovered as sugar measured as a percentage. ERC %cane can be calculated using a formula:

$$\text{ERC \%cane} = a \cdot S - b \cdot N - c \cdot F \quad \text{Equation 2.1}$$

Where, S is the sucrose %cane, N is the non-sucrose %cane (calculated as Brix %cane minus sucrose %cane), F is the fibre %cane and a, b and c are constant parameters related to the sucrose losses within the factory (Peacock and Schorn 2002).

Pol %cane is the total sucrose content in juice expressed as a percentage. It is the apparent sucrose in the sugarcane juice. High sucrose content contributes to high recoverable sugar thus selection for Pol %cane will be economically beneficial (Jackson, 2005). This makes increased sucrose content an important sugarcane breeding objective. There is a need to increase gains from selection for sucrose content due to the subtropical growing conditions in South Africa (Zhou and Lichakane, 2012). Brix %cane is the total soluble solids in sugarcane juice expressed as a percentage (Yusof et al., 2000). Brix %cane includes sucrose and non-sucrose (glucose and fructose). Brix %cane can either be measured in the field with a hand refractometer (hand refractometer Brix) or at the sugarcane laboratory. Hand refractometer Brix is quicker and inexpensive to measure (Zhou and Lichakane, 2012) compared to measuring Brix %cane in the sugarcane laboratory.

Purity % is defined as the percentage total sucrose present in the total soluble solids content in juice. It is a ratio of Pol %cane to Brix %cane. It is also known as an index of maturity. A higher Purity % indicates higher sucrose content (Acland, 1973; Wagih et al., 2004). Fibre %cane is the total water insoluble matter of the cane and bagasse (SASTA laboratory manual, 2005). Fibre %cane is associated with low sugar recovery at processing. Fibre reduces extraction of sucrose from cane thus the fibre %cane to Pol %cane ration must be as low as possible. However Fibre %cane has been associated with a degree of resistance to *E. Saccharina* (Nuss and

Atkinson 1983; Zhou and Lichakane, 2012). Dry matter %cane is the sum total soluble and non-soluble solids. It is determined using the formula:

DM %cane = Sucrose % + Non-sucrose……..…. Equation 2.2

2.7 Family evaluation

2.7.1 History of family evaluation

In the early stages of sugarcane breeding mass selection was used to select genotypes based on visual assessment for cane yield and Brix %cane (measured with a hand refractometer) (Kimbeng and Cox, 2003). Mass selection (individual plant selection) involves identifying the best plants for advancement based on the phenotype (Oliveira et al., 2013; Brasileiro et al., 2016). The efficiency of mass selection is low due to effects of genotype by environment interaction (Barbosa et al., 2001; Stringer et al., 2011; Mbuma et al., 2018).

Prior to family and parent evaluation, the proven cross and parent system was used (Bond, 1977, Heinz and Tew, 1987) to increase the quality of selection populations (Skinner, 1971). The proven cross system was widely practiced in several breeding programmes, including South Africa (Heinz and Tew, 1987; Skinner et al., 1987). The proven cross system used a number of progenies advanced through the breeding stages as an indicator of cross performance (Skinner, 1971, Skinner et al., 1987; Ming et al., 2006). Crosses and parents with a larger number of progenies advancing through the breeding stages were considered valuable, thereby creating a potential bias against new crosses that had fewer progenies tested and advanced. It took a longer time to obtain and quantify values of a cross because of longer breeding cycles (Milligan and Legendre, 1990; Kimbeng and Cox, 2003). The proven cross system used limited statistical analysis (Zhou, 2009), therefore, there is need to explore statistical methods to further optimise parents and family evaluation.

Family evaluation is the quantification of family or cross performance using data collected from progeny plots (Falconer and MacKay, 1996). After family evaluation, progenies from families producing higher trait values are planted in larger numbers and individual genotype selection is focused on these families. Family evaluation and selection is superior to individual genotype selection, particularly for quantitative traits such as cane yield that are controlled by several additive genes with low heritability (Stringer et al., 2011). Family evaluation has been widely practiced in many breeding programmes for different crops such as maize (Crossa and Gardner,

1989; Viana et al., 2010; Ajala et al., 2012; Noor et al., 2013), potato (Diniz et al., 2006), wheat (Thakare and Qualset, 1978; Crossa et al., 2014) and sugarcane. Family evaluation in sugarcane breeding was first described in Australia by Hogarth (1971), which showed higher genetic gains from family selection compared to mass selection (individual genotype selection). Australia adopted family evaluation later when harvesters with automatic weighing machines became available (Hogarth and Mullins, 1989).

2.7.2 Advantages of family evaluation

The main advantage of family evaluation is that families can be replicated and planted across locations, thus providing more accurate estimates of breeding parameters and effects of genotype by environment interaction. In contrast, individual genotypes cannot be replicated because of insufficient planting material. Individual genotypes are more prone to effects of genotype by environment interaction caused by field variability as well as inter-plot competition. Family selection is suitable for traits with low heritability, such as cane yield, that are susceptible to genotype by environment interaction (Hogarth, 1971). In recent years, family evaluation data have been used to estimate breeding values of genotypes used for crossing.

2.7.3 Family evaluation in South Africa

Prior to family evaluation, the proven cross system was used to evaluate crosses (Bond, 1977). The proven cross system uses the proportion of advanced genotypes across stages to determine the quality of a cross (Skinner et al., 1987). Crosses with higher numbers of progenies advanced to the next stage, were considered of higher value. The proven cross system used no statistical comparisons and required several years to evaluate families (Milligan and Legendre, 1990).

In South Africa, family evaluation research started in the 1970s (Bond, 1989). Bond (1977) investigated the possibility of using family evaluation, before planting single lines, with the aim to eliminate crosses that have a low probability of producing varieties with commercial potential. The study showed a positive correlation ($r = 0.69$) between number of progenies selected from a family and the family mean yield, indicating the potential of using progeny yield to predict family performance. Another study (Bond, 1989) validated these results. Family evaluation for quality traits started in 1996 (Zhou and Lichakane, 2012; Zhou et al., 2013), followed by yield traits in 2011 (Zhou, 2014) and *E. saccharina* damage in 2013 (Zhou and Mokwele, 2015).

2.8 Resource allocation

Resource allocation is used to optimally determine design parameters and sample size in plant breeding trials. A larger sample size, more replications and locations are expected to accurately estimate breeding parameters (Lorenz, 2013). Leite et al. (2009) investigated the minimum sample size needed to efficiently estimate genetic and phenotypic parameters of yield related traits while studies by Kimbeng et al. (2009) and Zhou et al. (2012) used resource allocation to determine optimum replications in sugarcane variety trials. Studies by Zhou (2014) and Zhou and Mokwele (2015) determined optimum sample size for family evaluation for yield and quality traits. However, no studies have reported optimum sample size for quality traits.

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Chapter 3

Comparing family to individual genotype evaluation and determining sample size for estimating sucrose content (Brix %cane) using a hand held refractometer

3.1 Abstract

Family evaluation quantifies the performance of families using data collected from progeny plots. Selection of individuals is focused on the selected elite families. Determining sample size is required to optimise resource allocation in plant breeding trials. The aims of this study were to compare family to individual genotype evaluation and determine the optimum sample size for estimating sucrose content (Brix %cane) using a hand-held refractometer. Each family was planted to three replications. Data on HR Brix % (surrogate for sucrose content) were collected from 20 genotypes within each family plot for the irrigated, coastal short cycle and Midlands sandy soils trials. Data collected were analysed to estimate variance components that were in turn used to calculate broad-sense heritability (H). Simulation values of H with combinations ranging from one to five replications and one to 20 genotypes were plotted using response surface graphs to identify optimum sample size. Family and individual genotype variance were significant ($P<0.01$). H values ranged from 0.74 to 0.77 for families and from 0.14 to 0.17 for individual genotypes, respectively. Families produced higher predicted selection gains (%Gs) (ranging from 7.06 to 12.72%) compared to individual genotypes (ranging from 2.42 to 5.47%). Populations grown in the irrigated region produced higher H and %Gs compared to coastal short cycle and sandy soils populations. Significant family and individual genotype variances suggested that selecting individual genotypes within selected superior families will increase efficiency of selection. The higher H and %Gs indicated superiority of family compared to individual genotype evaluation. Simulations, using response surface graphs, showed that a sample size of 10 genotypes and four replications produced H values of 0.77 to 0.80 compared to 0.74 to 0.77 with a sample size of 20 genotypes and three replications. Thus, reducing sample size from 20 to 10 genotypes will reduce data collection costs while increasing accuracy of family evaluation.

Key words: Family evaluation, broad-sense heritability, predicted selection gains, sample size

3.2 Introduction

Family evaluation quantifies breeding parameters of families using data collected from progeny plots (Falconer and MacKay, 1996) and is used to identify families producing higher trait values. Individual genotype evaluation involves the planting of progenies from families and these progenies are evaluated for their trait values without reference to their families (Oliveira et al., 2013; Brasileiro et al., 2016). After family evaluation, progenies from families producing higher trait values are selected and planted in larger numbers and individual genotype selection is focused on these elite families. The probability of producing superior progenies at later stages is expected to be higher among families with higher trait values. Prior to family evaluation, the proven cross system was used to determine performance of crosses (Heinz and Tew, 1987). The proven cross system used the number of seedlings that were advanced through the stages of breeding as an indicator of cross performance (Skinner et al., 1987). With the proven cross system, some families with larger numbers of advanced progenies are expected to have a higher probability of producing commercial varieties than those with fewer progenies advanced. Using the proven cross system, older crosses that have produced a higher number of progenies were considered superior (Walker, 1963), creating a bias against new crosses.

Family evaluation is widely practised in many breeding programmes for different crops such as maize (Crossa and Gardner, 1989; Viana 2007; Noor et al., 2010; Ajala et al., 2012), potato (Diniz et al., 2006), wheat (Thakare and Qualset, 1978; Crossa et al., 2014) and sugarcane (Kimbeng and Cox, 2003). Family evaluation in sugarcane breeding started with research in Australia (Hogarth, 1971). Results showed potentially higher genetic gains from family selection compared to mass selection (individual genotype selection). The advantage is that families can be replicated, while individual genotypes cannot be replicated due to limited planting material and the large numbers of genotypes involved. Because families can be replicated, breeding parameters can be estimated, providing a statistical basis for selection. Family evaluation was adopted for yield and quality traits in Australia (Hogarth and Mullins, 1989), Brazil (De Resende and Barbosa, 2006; Pedrozo et al., 2011), India (Shanthi et al., 2008; Babu et al., 2009) and South Africa (Bond, 1977; 1989; Zhou and Lichakane, 2012; Zhou, 2014; Zhou and Mokwele, 2015).

In South Africa, family evaluation research started in 1970s (Bond, 1989). Bond (1977) investigated the possibility of using family evaluation before planting single stools with the aim of eliminating crosses with a low probability of producing varieties of commercial standard.

Results showed a positive correlation (r = 0.69) between number of progenies selected from a family and the family mean yield, which indicated the potential of using progeny yield to predict family performance. Zhou and Lichakane (2012) and Zhou et al. (2012) showed presence of family effects and evaluated breeding parameters for quality traits. Further studies on family evaluation for cane yield (Zhou, 2014) and *E. saccharina* (Zhou and Mokwele, 2015; Zhou, 2016) followed.

Despite previous research on family breeding parameters for quality traits (Zhou and Lichakane, 2012), there has been no reported results comparing family to individual genotype selection. To demonstrate the value derived from family evaluation for quality, comparison of breeding parameters between family and individual genotypes is required. Previous studies comparing family to individual evaluation for cane yield showed significant benefits (Zhou, 2014).

Resource allocation is used to optimally determine design parameters and sample size in plant breeding trials. A larger sample size and more replications are expected to accurately estimate breeding parameters (Lorenz, 2013). Leite et al. (2009) investigated the minimum sample size needed to efficiently estimate genetic and phenotypic parameters of yield related traits while studies by Kimbeng et al. (2009) and Zhou et al. (2012) used resource allocation to determine optimum replications in sugarcane variety trials. Brix %cane in juice of progenies measured using a hand held refractometer can be used to predict sucrose content of a genotype (Kimbeng and Cox, 2003). Hand held refractometer Brix %cane can be measured on individual genotypes providing data to quantify progeny variability within families. Brix %cane measurements, using a hand held refractometer, is quick to measure in the field and less expensive. However, there is no knowledge on the optimum number of genotypes (sample size) and replications required to get accurate estimates of breeding parameters in sugarcane breeding for sucrose content. Therefore, the aims of this study were to compare family to individual genotype evaluation and to determine the optimum sample size and replication number, using a hand held refractometer to measure Brix %cane in stage one sugarcane breeding trials.

3.3 Materials and methods

3.3.1 Experimental materials

Data were collected from three trials; FML15 at 12 months, SML15 at 24 months and UML15 at 15 months. The number of families, male and female parents in the different trials are shown in Table 3.1.

Table 3.1. Trial, number of families, parents (female and male), planting and harvesting dates and type of cross for the 2015 Brix %cane trials

Trial	Families	Females	Males	Planted	Harvested	MO/MP	BP
UML15	40	22	14	2015	2016	25	15
FML15	49	34	7	2015	2016	42	7
SML15	50	29	18	2015	2017	31	19

UML – Short average potential mini-line, FML – Irrigated mini-line, SML – Sandy soil mini-line, MO – Males only crosses, MP – Melting pot crosses, BP – Bi-parental crosses

3.3.2 Experimental design

Trials were laid out in a randomised complete block design (RCBD) with three replications per family. After germination, the number of seedlings (genotypes) from each family were counted and divided into three sets. Each set was randomly assigned to one of the three replications. The seedlings were raised in airbricks on concrete slabs for 10 months and the same design was carried over to field planting. The growing conditions in the airbricks are presumed to be uniform due to similar and uniformly prepared soil media and uniform irrigation, thus no carry over effects were expected.

3.3.3 Description of research sites

The Gingindlovu (29º1'S, 31º36'E, 93 m above sea level) trial (UML) in the coastal short cycle average potential represents a shorter growing cycle that provides harvest before the *E. saccharina* damage causes significant yield loss. Crops were rainfed and harvesting was done between 12 to 15 months. The irrigated region (27º24'S, 31º35'E, 308 m above sea level) at Pongola (FML) is characterised by low rainfall, thus sugarcane is grown using irrigation. Harvesting was done at 12 months. The Midlands programmes represent a climate with long cold winters and shorter summers. The trial (SML) planted in Glenside research station (29º20'S, 30º46'E, 997 m above sea level) is characterised by sandy soils that are low in organic matter and nutrients.

3.3.4 Trial establishment and management

Seedlings for the coastal short cycle average potential, irrigated and Midlands sandy soils trials were germinated from true seed in the glasshouse at Mount Edgecombe research station in Durban, KZN. A week after germination, the seedlings in trays were counted to determine the germination percentage. The seedlings were then transferred and grown outside the glasshouse.

When seedlings were five weeks old, they were transported to Gingindlovu and Pongola research stations where they were hardened for two weeks before transplanting into airbricks. Seedlings for the Midlands sandy soils trial were grown at Mount Edgecombe. The airbricks were filled with a mixture of sand, soil and baggase compost in a ratio of 1:1:2. The seedlings were left to grow in airbricks for 10 months until they produced mini-stalks. The seedlings were irrigated three times a day to prevent moisture stress. Fertiliser (N:P:K = 5:1:5) was applied weekly at a rate of 10 kg per hectare to achieve optimum growth and replenish nutrients lost to leaching. The vegetative stalks of each seedling were cut at the base and topped at the natural breaking point during harvesting. The vegetative material from each seedling was planted in the field in a tramline design. Tramline refers to a system where two adjacent rows are planted followed by an unplanted row.

3.3.5 Data collection

Brix %cane data was determined using a hand held refractometer from a sample of the first 20 genotypes per plot. A refractometer determines sugarcane Brix % by measuring the refractive index of light as it passes through the sugarcane juice. The hand held refractometer was calibrated after each reading using distilled water. A stalk was cut from the base of each genotype in the plot. The stalk was then cut at the centre. Sugarcane juice was squeezed from the centre end of the stalk onto the refractometer and a reading obtained.

3.3.6 Data analysis

Data were analysed using linear mixed models in the statistical analysis system (SAS) software (SAS, 2014) for variance components. The following linear mixed model was used for analysis of variance for family effects of Brix %cane:

$$Y_{ijk} = R_i + F_j + RF_{ij} + G(RF)_{ijk} \quad \text{Equation 3.1}$$

Where Y_{ijk} is the observation for the *kth* genotype in the *jth* family of the *ith* replication, R_i is the random effect of the ith replication, F_j is the random effect of, the *jth* family, RF_{ij} is the random interaction effect of the *ith* replication by the *jth* family and is the error term for testing the fixed effects of the family effect and $G(RF)_{ijk}$ is the random effect of the *kth* genotype nested within the random interaction effect of the ith replication by the *jth* family and is the residual error term. This equation was also used to generate variance components for families. The data

was analysed using the mixed procedure of SAS. The estimates of variance components and their probability tests were calculated using the COVTEST option in the model statement.

The following linear mixed model was used for analysis of variance for individual genotypes for the hand refractometer Brix %cane data:

$$Y_{ijk} = G(F)_{k(j)} + G(FR)_{k(ij)} \quad \text{Equation 3.2}$$

Where Y_{ijk} is the observation for genotypes, $G(F)_{k(j)}$ is the random effect of the genotype nested within a family and $G(FR)_{k(ij)}$ is the random effect of the *kth* genotype nested within the random interaction of the *ith* replication by the *jth* family. The estimates of broad-sense heritability for family were calculated as follows:

$$H_F = \sigma^2_F / (\sigma^2_F + \sigma^2_{RF/r} + \sigma^2_{G(RF)/rg}) \quad \text{Equation 3.3}$$

Where H_F is the family broad-sense heritability, r is the number of replications and g is the number of genotypes, σ^2_F is the family effects variance component, $\sigma^2_{RF/r}$ is the variance component for the random interaction effects of family and replication and $\sigma^2_{G(RF)/rg}$ is the residual variance component.

The estimates of broad-sense heritability for genotype were calculated as follows:

$$H_G = \sigma^2_{G(F)} / (\sigma^2_{G(F)} + \sigma^2_{G(FR)}) \quad \text{Equation 3.4}$$

Where H_G is the genotype broad-sense heritability, $\sigma^2_{G(F)}$ is the variance components for genotypes nested within family and $\sigma^2_{G(FR)}$) is the residual variance component.

The estimates of standard errors for broad-sense heritability were estimated using Becker (1992):

$$SE = \sqrt{\frac{2(1-H)^2[1+H(q-1)]^2}{q(q-1)(n-1)}} \quad \text{Equation 3.5}$$

Where SE = Standard errors, H is the broad-sense heritability, q is the number of observations per family and n is the number of families in the trials. Predicted selection gains were calculated using the following formula:

$$Gs = H\sigma K \quad \text{Equation 3.6}$$

Where Gs is the predicted gains to selection, H is the broad-sense heritability, σ is the phenotypic standard deviation and K is the selection intensity (30% for families and 10% for individual genotypes). Predicted percent gains to family selection were calculated as follows:

$$\%Gs = Gs/\mu \times 100 \quad \text{Equation 3.7}$$

Where %Gs is the predicted percent gains to family, Gs is the predicted gains to selection and μ is the overall mean. The optimum sample size and number of replications were determined by simulating Broad-sense heritability for sample size ranging from 1 to 20 genotypes and one to five replications per family. Response surface graphs were used to visualise the simulated broad-sense heritability.

3.4 Results

3.4.1 Variance components

The family variance (σ^2_F) was significant (P<0.01), respectively for the UML15 and FML15 populations and highly significant (P<0.001) for the SML15 population (Table 3.2). The family variance was highest in FML15 (1.99) and lowest in SML15 (0.88). Family by replication interaction variance (σ^2_{RF}) was highly significant (P<0.0001) for all three populations. The FML15 population (1.33) produced the largest family by replication interaction variance followed by UML15 (0.85) and SML15 (0.75). Family by replication interaction variance was smaller than family variance for each of the three populations, respectively. The genotype nested within family by replication interaction variance ($\sigma^2_{G(FR)}$ and also the residual variance) was highly significant (P<0.0001) for the UML15, FML15 and SML15 populations, respectively. The residual variance for families was largest for the FML15 population (8.88) and lowest for SML15 (3.31) population. The proportion of family variance to total variance was 14.58% (UML15), 16.28 % (FML15) and 17.79 % (SML15).

Table 3.2. Variance components, broad-sense heritability (H), selection gains (Gs), percent selection gains (%Gs), R-squared, coefficient of variation (CV), root mean square error (SD) and Brix %cane mean for the UML15, FML15 and SML15 populations

Statistic	UML15	FML15	SML15
		Family evaluation	
σ^2_F	1.33±0.40**	1.99±0.57**	0.88±0.26***
σ^2_{FR}	0.85±0.40***	1.33±0.30***	0.75±0.16***
$\sigma^2_{G(FR)}$	6.95±0.21***	8.88±0.27***	3.31±0.10***
R-square	0.30	0.31	0.39
CV %	14.25	17.54	8.19
Mean ± SD	18.51±2.64	16.98±2.98	22.21±1.82
H	0.77±0.04	0.77±0.04	0.74±0.04
Gs	2.35	2.66	1.57
%Gs	12.72	15.68	7.06
		Individual evaluation	
$\sigma^2_{G(F)}$	1.32±0.23 ***	2.02±0.32***	0.75±0.13***
$\sigma^2_{G(FR)}$	8.02±0.30***	10.10±0.38***	4.41±0.17***
R-square	0.45	0.51	0.51
CV %	15.37	18.71	9.52
Mean ± SD	18.51±2.84	16.98±3.18	22.21±2.11
H	0.14±0.04	0.17±0.04	0.15±0.03
Gs	0.71	0.93	0.54
%Gs	3.82	5.47	2.42

σ^2_F - Family variance, σ^2_{FR} – Family by replication variance, $\sigma^2_{G(FR)}$ – Genotype within family by replication variance, ** Significant at p<0.01, *** Significant at p<0.0001. UML – Coastal short cycle average potential mini-line, FML – Irrigated mini-line, SML – Midlands sandy soils mini-line

In individual evaluation, the genotype nested within family (genotype) variance ($\sigma^2_{G(F)}$) was highly significant (P<0.0001) for the UML15, FML15 and SML15 populations, respectively. The nested within family (genotype) variance was largest for the FML15 population (2.02) followed by UML15 (1.32) and SML15 (0.75). The residual variance ($\sigma^2_{G(FR)}$) was highly significant (P<0.0001) for all three populations. The residual variance was largest for the FML15 population (10.10) followed by UML15 (8.02) and SML15 (4.41). The proportion of genotype variance to the total variance was 14.16 % (UML15), 16.66 % (FML15) and 14.52 % (SML15). The family variance (σ^2_F) was larger than the nested within family (genotype) variance ($\sigma^2_{G(F)}$) for the UML15 and SML15 populations, respectively. The family variance was

1% (UML15) and 15% (SML15) larger than the genotype variance. The genotype variance was 1.71% larger than the family variance for FML15 population. The residual variance for individual genotypes was 13.3% (UML15), 12.09% (FML15) and 24.9% (SML15) higher than the residual variance for families.

In family evaluation, the UML15, FML15 and SML15 populations, respectively, produced R^2 values of 0.30, 0.31 and 0.39. SML15 (0.39) produced the highest R^2 values and UML15 (0.30) population produced the lowest R^2 values for families. The R^2 values for individual genotypes ranged from 0.44 to 0.51 and were higher than the R^2 values for families. The FML15 (0.51) and SML15 (0.51) populations produced the highest R^2 values, followed by UML15 (0.45) for individual genotypes.

The coefficient of variation (CV%) was below 20%, ranging from 8.19 to 17.54% for families across all populations (UML15, FML15 and SML15). The FML15 population (17.54%) had the highest CV% and SML15 (8.19%) the lowest for families. The CV% for individuals were higher than the CV% for families across all populations. The CV% for individuals ranged from 9.52 to 18.71%. The FML15 population (18.71%) had the highest CV % while SML15 (9.52%) had the lowest for individuals.

3.4.2. Broad-sense heritability

Broad-sense heritability (H) values for families ranged from 0.74 to 0.77 for UML15, FML15 and SML15 populations (Table 3.2). The FML15 population (0.77) produced the highest broad-sense heritability while SML15 (0.74) produced the lowest. Individual genotype broad-sense heritability ranged from 0.14 to 0.17 across all populations. The UML15 population (0.14) produced the smallest broad-sense heritability while FML15 (0.17) had the highest broad-sense heritability. The FML15 population produced the highest broad-sense heritability for both families and individuals.

3.4.3. Predicted selection gains

Predicted selection gains (Gs) for families ranged from 1.57 to 2.66 across the three populations (Table 3.2). Percentage predicted selection gains (%Gs) for families ranged from 7.06 to 15.68%. The FML15 population (15.68%) produced the highest percentage predicted selection gains followed by UML15 (12.72%) and SML15 (7.06%). The individual genotype predicted selection gains ranged from 0.54 to 0.93 and the percentage predicted selection gains ranged

from 2.42 to 5.47%. The FML15 population (5.47%) produced the highest predicted selection gains, followed by UML15 (3.82%) and SML15 (2.42%). The family predicted selection gains was higher than the individual predicted selection gains for all populations.

3.4.4 Optimum sample size and replication number

The optimum sample size and number of replications was determined by simulating broad-sense heritability values for combinations ranging from one to five replications and from one to 20 genotypes per family plot. Simulated broad-sense heritability values were plotted against sample size (number of replications and number of genotypes) using response surface graphs to identify the optimum number of replications and genotypes. For the UML15 population, large increases in broad-sense heritability were observed with each additional replication up to four replications (Figure 3.1). After replication four, broad-sense heritability increased marginally, with the increase in broad-sense heritability between 0.03 and 0.06. The simulated broad-sense heritability values increased consistently with each additional genotype sampled up to a sample size of 10 to 12 genotypes. Beyond a sample size of 10 genotypes, the increase in broad-sense heritability was less than 0.01. Sample sizes between 13 and 20 genotypes per plot produced a smaller increase in broad-sense heritability.

The broad-sense heritability values for the FML15 population increased up to replication four (Figure 3.2). From replication four to replication five, the total increase in broad-sense heritability was less than 0.05. The simulated broad-sense heritability values increased with each additional genotype sampled up to a sample size of eight to 13 genotypes. At a sample size of greater than eight genotypes, the total increase in broad-sense heritability was less than 0.01. Sample sizes between 14 and 20 genotypes produced a smaller gains in broad-sense heritability.

For the SML15 population, large increases in broad-sense heritability were observed up to replication four (Figure 3.3). After replication four, broad-sense heritability increased slightly, with an increase of less than 0.06. The simulated broad-sense heritability values increased with each additional genotype sampled up to a sample size of 10 genotypes. Beyond a sample size of 10 genotypes per plot, the increase in broad-sense heritability was less than 0.01. Sample sizes between 11 and 20 genotypes per plot produced a smaller gains in broad-sense heritability.

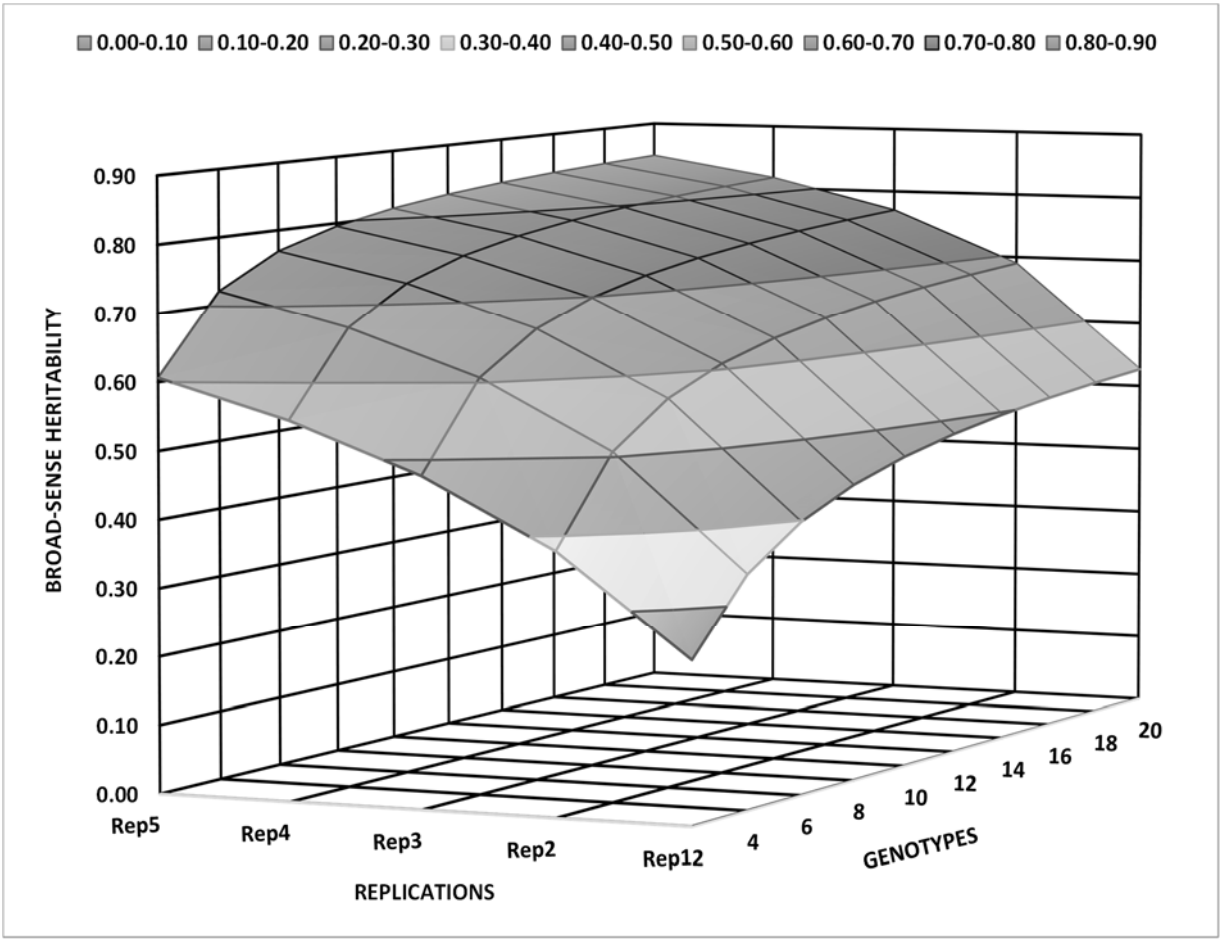

Figure 3.1. The change in broad-sense heritability (H) with an increase in number of replications and sample size (number of genotypes) for the coastal short cycle average potential mini-line (UML15) population

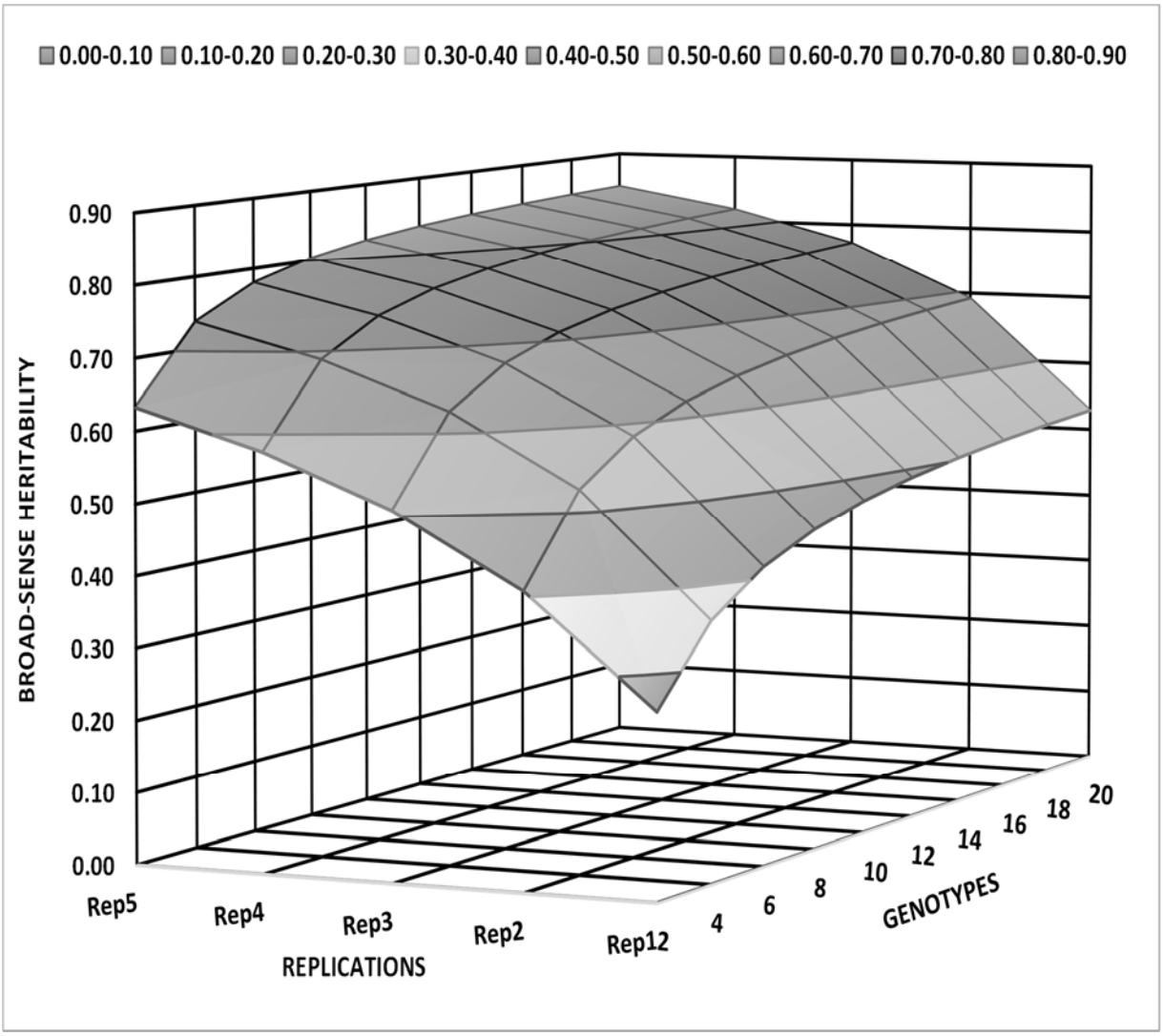

Figure 3.2. The change in broad-sense heritability (H) with an increase in number of replications and sample size (number of genotypes) for the irrigated mini-line (FML15) population

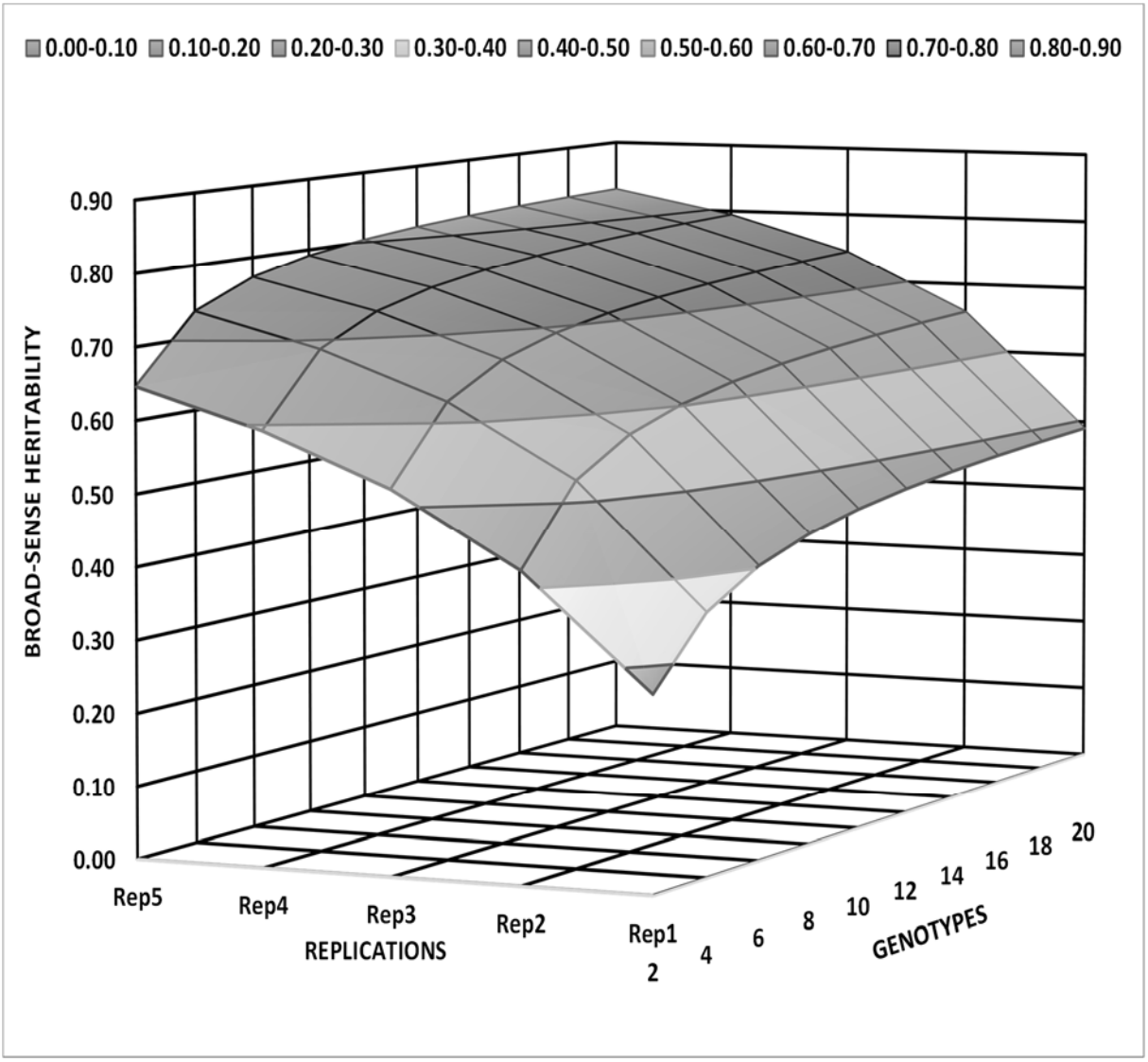

Figure 3.3. The change in broad-sense heritability (H) with an increase in number of replications and sample size (number of genotypes) for the Midlands sandy soil mini-line (SML15) population

3.5 Discussion

Families generally produced higher variances than individual genotypes, suggesting more genetic variability among families than genotypes within plots. This result suggests that Brix %cane would benefit from family evaluation in sugarcane breeding. However, the magnitudes of variance among families compared to genotypes were lower than those reported for cane yield (Zhou, 2014), suggesting that the benefit of family selection for sucrose content would be lower than for cane yield. However, the broad-sense heritability of families was at least four times higher than that of genotypes, suggesting higher precision of selection using families than individual genotypes. Broad-sense heritability is described as the proportion of the genetic

variance to total variance in a population (Zhou and Joshi, 2012). This is because the differences among genotypes would be high and larger than the effect of the environment on the phenotype (Wolie et al., 2013). Selection in vegetatively propagated crops such as sugarcane takes advantage of broad-sense heritability since recombination events are limited to segregation that occurs only at crossing (Zhou and Joshi, 2012). Predicted genetic gains from families were at least three times larger than those for individual genotypes, suggesting that genetic gains for sucrose content will be accelerated by using family evaluation.

Results showed significant variances for both families and individual genotypes. The purpose of family evaluation is to identify families with higher trait values from where individual genotype selection will be done. Results suggested that using family evaluation followed by individual genotype selection would be more efficient for increasing selection accuracy of high sucrose genotypes. Further, families had higher broad-sense heritability values, indicating that the primary selection of families will be more accurate followed, by a relatively low accuracy in identifying genotypes with high sucrose content. Results highlighted the value of family evaluation and selection followed by individual selection in accelerating genetic gains for sucrose content.

Broad-sense heritability for genotypes ranged from 0.14 to 0.17, indicating that environment contributed more to the total variability than genotypes. This result indicates that selection of genotypes would be less efficient compared to selecting families. According to Zhou and Joshi (2012) the magnitudes of broad-sense heritability determines the magnitudes of selection gains. The low magnitudes in broad-sense heritability for genotypes implies that low selection gains can be expected from selection of genotypes. Results suggested lower precision of identifying and selecting superior genotypes using individual genotype evaluation. Zhou (2014) and Zhou and Mokwele (2015) also reported low genotype broad-sense heritability for cane yield (0.03 to 0.19) and *E. saccharina* (0.17). Magnitudes of genotype broad-sense heritability for Brix %cane were higher compared to cane yield and *E. saccharina*.

Family evaluation for hand held refractometer Brix %cane produced higher broad-sense heritability than individual genotype evaluation, indicating the superiority of family evaluation to individual genotype evaluation. This result indicated there was justification for using family evaluation for sucrose content measured as Brix %cane by using a hand held refractometer. Previous studies compared family to individual genotype evaluation for cane yield (Zhou, 2014)

and *E. saccharina* resistance (Zhou and Mokwele, 2015) and found family evaluation to be superior to individual genotype evaluation. In this study, family broad-sense heritability was higher for Brix %cane compared to broad-sense heritability values respectively reported by Zhou (2014) for cane yield (0.55 and 61) and Zhou and Mokwele (2015) for *E. saccharina* (0.56), indicating higher precision of selecting for Brix %cane using family evaluation.

Predicted selection gains provide a tool for evaluating the potential of breeding programmes to produce superior varieties (Zhou and Lichakane, 2012). Families produced higher predicted selection gains values than individuals, further highlighting the superiority and efficiency of family evaluation over individual genotype evaluation. Predicted selection gains percentage gives an indication of the potential genetic gains that can be expected from family or individual genotype selection. Predicted selection gains percentages were higher for families (ranging from 7.06 to 15.68%) compared to individual genotypes (ranging from 2.42 to 5.47%). Results from this study suggested that larger genetic gains can be expected from family evaluation compared to individual genotype evaluation. Results from this study are in agreement with results reported for cane yield (Zhou, 2014) and *E. saccharina* (Zhou and Mokwele, 2015) which showed family evaluation was superior to individual genotype evaluation. However, predicted selection gains percentages for Brix %cane (7.06 to 15.68 %) were lower compared to cane yield (13.02 and 15.2%) and *E. saccharina* (36.52%) for families, highlighting the lower efficiency of family evaluation for Brix %cane. Higher predicted selection gains percentages across all populations indicated that family evaluation has the potential to accelerate the improvement of sucrose content measured indirectly as Brix %cane for different populations. Zhou and Lichakane (2012) reported increasing predicted selection gains over time, which highlighted the potential increase of future genetic gains from family selection.

Determining family evaluation parameters in sugarcane breeding programmes allows for the efficient identification and selection of elite families and genotypes. By using family evaluation, poor performing families that have a low probability of producing varieties of commercial standard can be identified and discarded early. Thereby focusing breeding costs, time and resources on evaluating superior individuals within elite families. Cox and Hogarth (1993) and Cox et al. (1996) showed that selection of superior genotypes within elite families was the most efficient method of family evaluation. Genetic variance describes the amount of genetic variation present for a particular trait. The highly significant genetic variance observed in this study indicated the presence of large variability among the sugarcane families and genotypes

across populations. The existence of large variability among sugarcane families and genotypes implied superior families and individuals can be identified and selected efficiently. The highly significant genetic variability further highlighted the possibility of genetic improvement for sucrose content. Previous studies (Zhou and Lichakane, 2012; Zhou et al., 2013) also reported significant genetic variance for quality traits using family evaluation. Zhou (2014) and Zhou and Mokwele (2015) reported larger family variance in comparison to genotype variance for cane yield and *E. saccharina*, indicating larger genetic variation for families compared to individual genotypes.

The genotype nested within family by replication (residual) variance was larger for individuals than for families. The higher individual genotypes residual variability could be due to inter-plot competition since genotypes are not replicated due to limited planting material and the large numbers of genotypes involved. The smaller family residual variance along with significant family variance highlighted the efficiency of family evaluation compared to individual genotypes. Families are replicated, thus using family evaluation would reduce the effect of random environmental error. Previous studies reported highly significant family genetic variability and lower family residual variability compared to genotypes for cane yield (Zhou, 2014) and *E. saccharina* (Zhou and Mokwele, 2015), indicating the effectiveness of family evaluation.

Family residual variance was highly significant and was the largest variance component for Brix %cane across all populations, indicating that a large proportion of the variability in the trials was not accounted for by the experimental design. The high residual variability was associated with lower R^2 values (ranging from 0.30 to 0.39). The R^2 value, also known as the coefficient of determination, is the ratio of explained variation by a linear model to the total variation in the trial (Hamilton et al., 2015). An R^2 of 1.0 indicates that all of the variability in the trial can be explained by the linear model. Results suggested that most the variability in these trials cannot be explained by the experimental design. A RCBD with three replications and 20 genotypes was used in these trials. The efficiency of the experimental design could be improved by implementing incomplete block designs that can account for within block variability and by using an optimum sample size (number of genotypes) and number of replications. Several studies reported larger residual variability over genetic variability for cane yield (Zhou, 2014), *E. saccharina* (Zhou and Mokwele, 2015) and quality traits (Zhou and Lichakane, 2012; Zhou et al., 2012).

The coastal short cycle, irrigated and Midlands sandy soil populations showed differences in breeding parameters for family and individual genotype evaluation. The irrigated mini-line population, respectively, produced the highest broad-sense heritability and predicted selection gains percentage, followed by the coastal short cycle and the Midlands sandy soil populations. The high broad-sense heritability and predicted selection gains percentage obtained for hand held refractometer Brix %cane was associated with larger genetic variability for the irrigated mini-line population. Zhou and Joshi (2012) reported high heritability for the irrigated breeding programme which indicated the potential for high selection gains. Zhou and Lichakane (2012) reported increasing predicted selection gains for both Brix %cane and Pol %cane for the irrigated region, further highlighting the reason for the high selection gains. According to Zhou and Joshi (2012) a number of varieties that produce high sucrose content have been released from the irrigated region.

Results from the simulated broad-sense heritability analysis showed that a sample size of four replications and 10 genotypes would provide adequate discriminating ability for estimating family values. Barbosa et al. (2001) and Leite et al. (2009) studied sample size and found that 10 plants per plot were adequate to effectively estimate breeding parameters for Brix %cane. Currently, SASRI uses three replications and 20 genotypes to estimate sugarcane breeding parameters but simulations using response surface graphs showed that four replications and 10 genotypes produced broad-sense heritability values of 0.77 to 0.80 compared to 0.74 to 0.77 produced by three replications and 20 genotypes. Reducing sample size to 10 genotypes and increasing replications to four will reduce data collection costs and time by 33% (40 versus 60 samples). Sucrose content is currently measured in the sugarcane laboratory along with other sugarcane quality traits. However, results from this study suggest the use of a hand held refractometer Brix %cane as a surrogate for sucrose content would reduce costs that are associated with transport and laboratory analysis.

3.6 Conclusions

Family evaluation produced at least double the predicted selection gains achieved through individual genotype evaluation, indicating the potential for increased selection gains using family evaluation for sucrose content in sugarcane breeding. Families and genotypes had significant variances suggesting that selection of superior individuals within selected elite families was more efficient than selecting individual genotypes. Applying individual genotype selection in superior families increases the probability of selecting elite genotypes. Thereby

improving the efficient use of available resources for sugarcane breeding programmes. The irrigated mini-lines and coastal short cycle breeding programmes had larger predicted selection gains percentages, suggesting that parents used in the irrigated mini-lines and coastal short cycle trials could be used for crossing to improve the variability and selection gains among the Midlands sandy soil populations. The study showed that four replications and 10 genotypes would provide adequate discriminating ability for determining family values. A sample size of four replications by 10 genotypes can be used for family evaluation trials using a hand held refractometer to determine Brix %cane. The resources saved by reducing sample size could be used to plant more trials, covering the different regional breeding programmes at SASRI at different research stations in order to identify families that would produce high sucrose content in different breeding programmes. Measuring sucrose content using a hand held refractometer to determine Brix %cane not only provides quick measurements and save data collection costs but also allows for the determination of within family variability.

3.7 References

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Chapter 4

Family breeding parameters and phenotypic correlations among quality traits in sugarcane breeding populations

4.1 Abstract

Family evaluation quantifies trait values of families by analysing data that were collected from progeny plots. Phenotypic correlations provide knowledge on trait interrelationships. The objectives of this study were to determine family breeding parameters, estimate phenotypic correlations and determine their implications on selection for quality traits. Data were measured from the first 20 sub-plots of sugarcane families. Trials were laid out in randomized complete block design with three replications. Data were analysed using statistical analysis software (SAS) for variance components and Pearson's correlations. Variance components were used to calculate broad-sense heritability (H). All quality traits showed highly significant ($P<0.0001$) family variance, suggesting the existence of genetic variability. Estimable recoverable crystal (ERC %cane) (0.64 to 0.77), Pol %cane (0.64 to 0.78), Brix %cane (0.61 to 0.77), Brix dry matter (DM) %cane (0.58 to 0.78), purity % (0.51 to 0.69), Fibre %cane (0.58 to 0.74) and DM %cane (0.65 to 0.75) produced moderate to high H values. ERC %cane (3.61 to 6.27), fibre %cane (4.11 to 5.18), Pol %cane (3.11 to 5.13), Brix %cane (2.58 to 4.00) and Brix DM %cane (2.52 to 3.74) produced the highest predicted selection gains (%Gs). ERC %cane, Pol %cane and fibre %cane had the highest H and %Gs values, suggesting that family evaluation was effective. ERC %cane had significant ($P<0.0001$) correlations with Brix %cane ($r > 0.80$), Pol %cane ($r > 0.98$), Brix DM %cane ($r > 0.41$), purity % ($r > 0.59$) and DM %cane ($r > 0.42$). The significant ($P<0.05$) negative correlation between ERC %cane and fibre %cane ($r > 0.07$) would result in a decrease in sucrose content with increasing fibre content. Brix %cane had a highly significant positive correlation, respectively with ERC %cane and Pol %cane, highlighting that Brix %cane can be used to predict ERC %cane and Pol %cane.

Key words: Family evaluation, heritability, predicted selection gains, phenotypic correlation

4.2 Introduction

Family evaluation research in sugarcane breeding was first described in Australia by Hogarth (1971). It is the quantification of performance of families or crosses using data collected and analysed from progeny plots (Falconer and MacKay, 1996). After family evaluation, progenies

from families producing high trait values are planted in larger numbers and individual genotype selection is focused on these families. Family evaluation and selection proved superior to individual genotype selection, particularly for quantitative traits such as cane yield that are controlled by several additive genes with low heritability (Stringer et al., 2011). During early research, it was recognised that families can be replicated thus providing more accurate estimates of family genetic values when compared to individual genotypes. Family plots are less affected by genotype by environment interaction because they are planted in larger plots.

Before family evaluation, the proven cross system was used to evaluate the potential of crosses (Heinz and Tew 1987; Skinner, 1971). The proven cross system used the number of seedlings that were advanced through the breeding stages as an indicator of cross performance (Skinner et al., 1987). Older crosses produced higher values (Walker, 1963) because they would have been planted more frequently, creating a bias against new crosses. The lack of statistical comparison between old and new crosses increased the bias. It took years to quantify the values of a cross (Milligan and Legendre, 1990). The proven cross system was widely practiced in several breeding programmes, including South Africa (Heinz and Tew, 1987; Skinner et al., 1987).

Kimbeng and Cox (2003) reviewed progress in family evaluation research and found that family selection, followed by within family selection, was a superior and more efficient method of selection. Australian breeding programmes adopted family evaluation when harvesters with automatic weighing machines became available (Hogarth and Mullins, 1989). Several breeding programmes in Brazil (De Resende and Barbosa, 2006; Pedrozo et al., 2011), India (Shanthi et al., 2008; Babu et al., 2009), USA and South Africa (Bond, 1977; 1989; Zhou and Lichakane, 2012; Zhou, 2014; Zhou and Mokwele, 2015) eventually adopted family evaluation.

The success of a variety improvement programme in sugarcane requires inferior varieties to be identified and eliminated from advancing to the next stages of breeding. Knowledge on breeding parameters provides information on how breeding strategies can be used to improve different traits under evaluation. The magnitude of genetic variability in the base population indicates the extent of genetic improvement through selection. Genetic variance is used to estimate broad-sense heritability. Broad-sense heritability is defined as the proportion of the phenotypic variances that is attributable to genetic variance (Falconer, 1989). It is calculated as the proportion of total genetic variance to the total phenotypic variance. Broad-sense heritability

takes into account the total genetic effects (additive, dominance and epistatic genetic variance). In vegetatively propagated crops such as sugarcane, segregation only occurs at crossing, thus selection takes advantage of the variability from initial crossing (Zhou and Joshi, 2012). Broad-sense heritability can be used to determine if the population has adequate genetic variability to identify superior genotypes. Broad-sense heritability of traits can also be used to estimate predicted selection gains. Predicted selections gains refer to the potential genetic gains that can be expected from selection (Zhou and Lichakane, 2012). Genetic variance, broad-sense heritability and predicted selection gains together give a better indication of the efficiency of selection.

Knowledge on trait association is important in plant breeding programmes to determine the possibility of indirect selection and also the impact of selection of one trait on the other (Liu et al., 2007; Tena et al., 2016). Phenotypic correlation analysis is a method used to determine the relationship between two variables. Phenotypic correlations in plant breeding have been studied in a number of crops including cassava (Barandica et al., 2016), oilseed rape (Engqvist and Becker, 1993), soybean (Rodrigues et al., 2015) and maize (Samanci, 1996; Halidu et al., 2015). Phenotype correlations can also be used to determine traits that can be measured at low cost to indirectly select for traits that are expensive to measure.

Knowledge on magnitudes of broad-sense heritability and predicted selection gains will provide the necessary information to improve the precision of family evaluation and identify quality traits that would be ideal for family evaluation. Therefore, the aims of this study were to determine family evaluation breeding parameters (variance, broad-sense heritability, predicted genetic gains), estimate phenotypic correlations among quality traits and to evaluate their implications on breeding for the coastal short cycle high potential mini-line breeding programme.

4.3 Materials and methods

4.3.1 Experimental materials

The crosses included in this study were made in the glasshouse at Mount Edgecombe research station (29.7ºS, 31.03ºE, 96 m above sea level) Durban, KZN using genotypes from SASRI germplasm (Table 4.1). The three mating designs used during crossing were bi-parental, males only (MO) and melting pot (MP). With bi-parental crosses a single female parent is crossed with a single male parent and seed is collected from the female parent only. With males only

crosses, at least two male parents are inter-crossed with each other and seed is collected from both parents. Melting pot involves pollinating a single female parent with several male parents and seed is collected from the female parent. The source of pollen in melting pot crosses is always unknown. The TML10, TML11, TML12 and TML13 trials were planted to 199, 198, 199 and 193 crosses, respectively (Table 4.1). The female parents ranged from 65 to 69, while male parents ranged from 34 to 47.

4.3.2 Description of the research site

The coastal short cycle high potential mini-line breeding programme, based at Empangeni research station (28º43'S, 31º53'E, 102 m above sea level), was established in 1997 to develop sugarcane cultivars with high cane yield, high sucrose content and early maturity at 12 months harvest age. High sucrose content at 12 months requires early maturity and accelerated sucrose accumulation in the sugarcane stalks. The coastal short cycle high potential region is characterised by deep and rich soils with a high rainfall and temperatures. These conditions are conducive to fast sugarcane growing and high cane yields.

Table 4.1. Trial, number of crosses, parents (female and male), planting and harvesting dates and type of cross for the coastal short cycle high potential mini-line breeding programme (Empangeni research station)

Trial	Families	Female	Male	Planted	Harvested	MO/MP	BP
TML10	199	66	47	2010	2011	93	106
TML11	198	65	43	2011	2012	108	90
TML12	199	69	46	2012	2013	126	73
TML13	193	67	34	2013	2014	134	59

TML – Coastal short cycle high potential mini-line, MO – Males only crosses, MP – Melting pot crosses, BP – Bi-parental crosses

4.3.3 Trial establishment and design

Seedlings for the coastal short cycle high potential mini-line breeding programme were germinated from true seed in the glasshouse at Mount Edgecombe research station in Durban, KZN. A week after germination, seedlings in trays were counted to determine the germination percentage. Seedlings were then transferred and grown outside the glasshouse. When the seedlings were five weeks old, they were transported to Empangeni research station where they were hardened for two weeks before transplanting into airbricks. The airbricks were filled with a mixture of sand, soil and baggase compost in the ratio of 1:1:2. The seedlings were left to

grow in airbricks for 10 months until they produced mini-stalks. The seedlings were irrigated three times a day to prevent moisture stress. Fertiliser (N:P:K = 5:1:5) was applied weekly at a rate of 10 kg per hectare to achieve optimum growth and replenish nutrients lost to leaching.

The vegetative stalks of each seedling were cut at the base and topped at the natural breaking point during harvesting. The vegetative material from each seedling was planted in the field in a tramline design. Tramline refers to a system where two adjacent rows are planted followed by an unplanted row. Trials were laid out in a RCBD with three replications per family. Genotypes from each family were counted and divided into three sets. Each set was randomly assigned to one of the three replications. The seedlings were raised in airbricks on concrete slabs for 10 months and the same design was carried over to field planting. The growing conditions in the airbricks were presumed to be uniform due to similar and uniformly prepared soil media and uniform irrigation, thus no carry over effects were expected.

4.3.4 Data collection

Stalk sampling to measure quality traits was done at 12 months harvest age. In each family plot, one stalk was randomly cut from each of the first 20 seedlings in the field. The stalks were bundled together and analysed in the sugarcane laboratory at Mount Edgecombe using standard methods (Schoonees-Muir et al., 2009). Samples were analysed for dry matter (DM) %cane, Fibre %cane, Brix %cane, Brix DM %cane, Pol %cane, purity %, and ERC %cane. The stalk samples were disintegrated using a blender and 2000 ml tap water was added to 1000 g of the disintegrated cane. The sugarcane water mixture was cold digested for 20 minutes before being squeezed through a mesh funnel to collect the extract. The extract was then poured into a 250 ml Schott bottle and placed in a cooling bath (RM6 LAUDA D6970) to bring the temperature to 20ºC. Once cooled, the extract was poured into filter paper containing 3 g of Celite and collected in a 250 ml beaker. The cooled juice was allowed to filter in the cooling bath. After 100 ml of filtered juice was collected it was analysed in the refractometer (RSM 500) and the Autopol (Rudolph research analytical 880) to obtain Brix %cane and Pol %cane readings. Brix %cane refers to the total soluble solids present in the sugarcane juice, expressed as a percentage. The Brix of sugarcane juice was obtained by measuring the refractive index of the juice. Pol %cane refers to the total sucrose present in the soluble solids in sugarcane juice expressed as a percentage. Pol %cane was measured using a saccharimeter. The saccharimeter measures the rotation of plane of the polarised light by sugarcane juice. Brix DM %cane is referred to as the ratio of Brix %cane to DM %cane. Purity % refers to the percentage ratio of sucrose (Pol) to

the total soluble solids (Brix) in sugarcane juice. Purity was calculated using the following formula:

$$\text{Purity \%} = \frac{pol}{Brix} X\ 100 \quad \text{.....Equation 4.1}$$

Estimable recoverable crystal (ERC) %cane refers to the total recoverable sucrose in sugarcane after loss from molasses and fibre, expressed as a percentage. ERC %cane was calculated using the following formula:

$$\text{ERC \%cane} = a \cdot S - b \cdot N - c \cdot F \quad \text{.....Equation 4.2}$$

Where, *S* is the sucrose %cane, *N* is the non-sucrose %cane (calculated as Brix %cane minus sucrose %cane), *F* is the fibre %cane and *a*, *b* and *c* are constant parameters related to the sucrose loss within the factory (Peacock and Schorn, 2002). The *a* in the ERC %cane formula refers to the sucrose loss in the filter cake, *b* refers to sucrose loss due to final molasses and *c* refers to sucrose loss due to bagasse per unit of fibre entering the mill in the cane supply. Fibre %cane refers to the total fibre present in sugarcane expressed as a percentage.

Dry matter %cane is the sum total of the soluble and non-soluble solids and cane was calculated using the formula:

$$\text{DM \%cane} = \text{Sucrose \%} + \text{Fibre \%} + \text{Non-sucrose \%} \quad \text{.....Equation 4.3}$$

4.3.5 Data analysis

Data were analysed using linear mixed models in the statistical analysis system (SAS) software (SAS, 2014) for variance components. The following statistical linear mixed model was used for family analysis:

$$Y_{ij} = \mu + R_i + F_j + RF_{ij} \quad \text{.....Equation 4.4}$$

Where Y_{ij} is the observation for family and replication, μ is the overall mean, R_i is the random effect of the i*th* replication, F_j is the random effect of the j*th* family and RF_{ij} is the random interaction effect of the i*th* replication by the j*th* family and is the residual error.

Family broad-sense heritability was calculated using the formula:

$$H = \sigma^2_F / (\sigma^2_F + \sigma^2_{RF/r}) \quad \text{Equation 4.5}$$

Where H is the family broad-sense heritability, r is the number of replications, σ^2_F is the family effects variance component, σ^2_{RF} is the variance component for the random interaction effects of family and replication also known as the residual effects variance component.

The estimates of standard errors for broad-sense heritability were calculated using Becker (1992):

$$SE = \sqrt{\frac{2(1-H)^2[1+H(q-1)]^2}{q(q-1)(n-1)}} \quad \text{Equation 4.6}$$

Where SE is the standard error, H is the broad-sense heritability, q is the number of observations per family and n is the number of families in the trial.

Predicted selection gains were calculated using:

$$Gs = H\sigma K \quad \text{Equation 4.7}$$

Where Gs is the predicted gains to selection, H is the broad-sense heritability, σ is the phenotypic standard deviation and K is the selection intensity. Percent predicted family selection gains was calculated as:

$$\%Gs = Gs/\mu \times 100 \quad \text{Equation 4.8}$$

Where $\%Gs$ is the predicted percentage gains to family selection, Gs is the predicted gains to selection and μ is the overall mean.

Pearson's correlation coefficient analysis was done in SAS using Proc CORR to determine the inter-relationships among quality traits for the coastal short cycle high potential mini-line breeding programme.

4.4 Results

4.4.1 Variance components and means

4.4.1.1 ERC %cane, Pol %cane, Brix %cane and Brix DM %cane

The family genetic variances (σ^2_F) for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane (Table 4.2), collectively known as sucrose content related traits, were highly significant (P<0.0001) for all four populations (TML10, TML11, TML12 and TML13). The family genetic variance for ERC %cane was highest in the TML12 population and lowest in the TML11 population. The order of magnitude for ERC %cane family variances across populations was TML12 > TML13 > TML10 > TML11. The family variance component for Pol %cane produced a similar trend to that of ERC %cane. The TML12 population produced the highest variance while TML11 produced the lowest. The order of magnitude for Pol %cane family variances across populations was TML12 > TML13 > TML10 > TML11. For both Brix %cane and Brix DM %cane, the TML12 population produced the highest family variance while TML11 produced the lowest. The order of magnitude of variances for both Brix %cane and Brix DM cane was TML12 > TML13 > TML10 > TML11. Sucrose content related traits produced similar trends in magnitudes of family variances. ERC %cane produced higher family variances than Pol %cane across the populations. The family genetic variances for ERC %cane and Pol %cane were higher than those for Brix %cane but lower than those of Brix DM %cane.

The family by replication interaction effect variance (σ^2_{RF} and also the residual variance) was highly significant (P<0.0001) for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane (Table 4.2) in all four populations (TML10, TML11, TML12 and TML13). The residual variance for both ERC %cane and Pol %cane was highest in the TML12 population and lowest in the TML13 population. The residual variance for ERC %cane and Pol %cane increased consistently from TML10 to TML12 populations and decreased in the TML13 population. The order of magnitude of residual variance for ERC %cane and Pol %cane across populations was TML12 > TML11 > TML10 > TML13. The residual variance for Brix %cane was highest in the TML12 population and lowest in the TML10 population. The family by replication variance for Brix DM %cane was highest in TML12 and lowest in TML13 population. The order of magnitude for family by replication variance of Brix %cane in populations was TML12 > TML13 > TML11 > TML10 while for Brix DM %cane it was TML12 > TML10 > TML11 > TML13.

Table 4.2. Variance components, broad-sense heritability (H), selection gains (Gs) and percentage selection gains (%Gs) for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane determined for the TML10, TML11, TML12 and TML13 populations

Statistic	TML10	TML11	TML12	TML13
		ERC %cane		
σ^2_F	0.35±0.06***	0.32±0.06***	0.68±0.12***	0.37±0.06***
σ^2_{RF}	0.51±0.04***	0.55±0.05***	0.62±0.07***	0.32±0.03***
H	0.67±0.02	0.64±0.02	0.77±0.02	0.77±0.02
Gs	0.57	0.55	0.70	0.51
%Gs	5.22	4.25	6.27	3.61
Mean±SD	10.83±0.73	12.85±0.74	11.19±0.79	14.19±0.57
R^2	0.66	0.66	0.80	0.74
CV %	6.70	5.73	7.05	4.02
		Pol %cane		
σ^2_F	0.31±0.05***	0.27±0.05***	0.63±0.11***	0.32±0.05***
σ^2_{RF}	0.42±0.03***	0.45±0.04***	0.54±0.06***	0.31±0.03***
H	0.69±0.02	0.64±0.02	0.78±0.02	0.76±0.02
Gs	0.53	0.50	0.66	0.49
%Gs	4.28	3.45	5.13	3.11
Mean±SD	12.35±0.66	14.35± 0.67	12.89±0.73	15.79±0.56
R^2	0.67	0.65	0.81	0.73
CV %	5.36	4.65	5.69	3.54
		Brix %cane		
σ^2_F	0.24±0.04***	0.18±0.03***	0.51±0.09***	0.27±0.05***
σ^2_{RF}	0.31±0.03***	0.34±0.03***	0.45±0.05***	0.34±0.03***
H	0.69±0.02	0.61±0.03	0.77±0.02	0.70±0.02
Gs	0.46	0.42	0.60	0.48
%Gs	3.21	2.58	3.99	2.74
Mean±SD	14.26±0.57	16.09±0.59	15.12±0.68	17.63±0.59
R^2	0.67	0.64	0.80	0.70
CV %	3.99	3.64	4.47	3.36
		Brix DM %cane		
σ^2_F	2.19±0.42***	1.75±0.37***	5.75±0.95***	2.64±0.44***
σ^2_{RF}	4.54±0.36***	3.84±0.32***	4.84±0.54***	3.49±0.31***
H	0.59±0.03	0.58±0.03	0.78±0.02	0.69±0.02
Gs	1.48	1.31	2.00	1.53
%Gs	2.83	2.52	3.74	2.81
Mean±SD	52.33±2.16	51.95±1.95	53.58±2.21	54.27±1.90
R^2	0.61	0.62	0.81	0.71
CV %	4.13	3.76	4.13	3.50

*** Significant at P<0.0001, σ^2_F – family variance, σ^2_{RF} – family by replication interaction variance, H – Broad-sense heritability, Gs – Gains to selection, %Gs – Percentage selection gains, SD – Standard deviation, R^2 – R-squared, CV – Coefficient of variation, Pol – Polarisation, ERC – Estimable recoverable crystal, TML – Coastal short cycle high potential mini-line populations planted from 2010 to 2013 at Empangeni research station

The mean values ranged from 10.8 to 14.2% for ERC %cane, 12.4 to 15.8% for Pol %cane, 14.3 to 17.6% for Brix %cane and 52.0 to 54.3 for Brix DM %cane across the four populations (Table 4.2). The TML13 population had the highest mean values for all four sucrose content related traits while the TML10 population had the lowest mean values for three of the traits. For Brix DM %cane, the TML11 population had the lowest mean. The R^2 value ranged from 0.66 to 0.80 for ERC %cane, 0.65 to 0.81 for Pol %cane, 0.64 to 0.80 for Brix %cane and 0.61 to 0.81 for Brix DM %cane. The coefficient of variation (CV %) was generally low (<10%) for all four sucrose content related traits in all populations. The CV % ranged from 4.0 to 7.1% for ERC %cane, from 3.5 to 5.7% for Pol %cane, from 3.4 to 4.5% for Brix %cane and from 3.5 to 4.1% for Brix DM %cane across the four populations. The TML12 population had the largest CV % values while the TML13 population had the smallest CV % values across all four sucrose contents related traits.

4.4.1.2 Purity %

The family genetic variance for purity % was highly significant ($P<0.0001$) for all four populations (TML10, TML11, TML12 and TML13) (Table 4.3). The family genetic variance for purity % was highest in TML12 population and lowest in TML13 population. The order of magnitude of family genetic variance for purity % across populations was TML12 > TML11 > TML10 > TML13. The family by replication interaction effect was highly significant ($P<0.0001$ for all four populations. The family by replication interaction variance was 66% (TML10), 58% (TML11), 39% (TML12) and 27% (TML13) higher than the family genetic variance for the four populations. The TML10 population produced the highest family by replication variance while for the TML13 population it was the lowest. The order of magnitude of family by replication interaction variance for purity % across populations was TML10 > TML12 > TML11 > TML13.

The mean values fluctuated across populations with the TML13 population having the highest mean (89.6%) and TML12 population having the lowest mean (85.2%) (Table 4.3). The R^2 value ranged from 0.56 for TML10 to 0.75 for TML12. The R^2 value increased from TML10 to TML12, however, it decreased again in TML13. The CV % fluctuated across the four populations and ranged from 1.5 to 2.2%. The TML10 population had the highest CV % while TML13 had the lowest CV %.

Table 4.3. Variance components, broad-sense heritability (H), selection gains (Gs) and percentage selection gains (%Gs) for purity % determined for the TML10, TML11, TML12 and TML13 populations

Statistic	TML10	TML11	TML12	TML13
		Purity %		
σ^2_F	1.24±0.29***	1.30±0.31***	2.01±0.46***	1.23±0.22***
σ^2_{RF}	3.61±0.29***	3.10±0.27***	3.31±0.37***	1.69±.0.15***
H	0.51±0.03	0.56±0.03	0.65±0.02	0.69±0.02
Gs	1.13	1.11	1.36	1.04
%Gs	1.32	1.25	1.59	1.16
Mean±SD	86.53±1.92	89.15±1.72	85.17±1.81	89.55±1.30
R^2	0.56	0.64	0.75	0.70
CV %	2.22	1.93	2.13	1.45

*** Significant at P<0.0001, σ^2_F – family variance, σ^2_{RF} – family by replication interaction variance, H – Broad-sense heritability, Gs – Gains to selection, %Gs – Percentage selection gains, SD – Standard deviation, R^2 – R-squared, CV – Coefficient of variation, TML – Coastal short cycle high potential mini-line

4.4.1.3 Fibre %cane and DM %cane

The family genetic variance for fibre %cane and DM %cane (Table 4.4) were highly significant (P<0.0001) for all four populations (TML10, TML11, TML12 and TML13). The family genetic variance for fibre %cane and DM %cane fluctuated from TML10 to TML13. The magnitude of family genetic variance for fibre %cane increased with successive selection populations. The order of magnitude for fibre %cane family variance across populations was TML12 > TML13 > TML11 > TML10. The order of magnitude of family genetic variance was TML11 > TML10 > TML13 > TML12. Later populations (TML12 and TML13) had lower family genetic variance than earlier populations (TML10 and TML11). The family by replication variance was highly significant (P<0.0001) for fibre %cane and DM %cane in all four populations. The family by replication interaction variance was 54% (TML10), 35% (TML11), 7% (TML12) and 24% (TML13) higher than the family genetic variance for fibre %cane. The family by replication interaction variance for fibre %cane showed a decreasing trend from the TML10 to the TML13 population. Family by replication interaction variance for DM %cane fluctuated from TML10 to TML13. The order of magnitude of family by replication interaction variance across populations was TML10 > TML11 > TML12 > TML13. The order of magnitude of family by replication interaction variance for DM %cane across populations was TML10 > TML12 > TML11 > TML13.

Table 4.4. Variance components, broad-sense heritability (H), selection gains (Gs) and percentage selection gains (%Gs) for fibre %cane and DM %cane determined for the TML10, TML11, TML12 and TML13 populations

Statistic	TML10	TML11	TML12	TML13
		Fibre %cane		
σ^2_F	0.32±0.06***	0.42±0.08***	0.57±0.10***	0.46±0.08***
σ^2_{RF}	0.70±0.06***	0.65±0.06***	0.62±0.07***	0.60±0.05***
H	0.58±0.03	0.66±0.02	0.74±0.02	0.69±0.02
Gs	0.57	0.61	0.68	0.64
%Gs	4.35	4.11	5.18	4.27
Mean±SD	13.00±0.84	14.90±0.80	13.11±0.80	14.87±0.79
R^2	0.60	0.66	0.78	0.70
CV %	6.48	5.36	6.07	5.30
		DM %cane		
σ^2_F	0.48±0.01***	0.53±0.08***	0.36±0.08***	0.38±0.06***
σ^2_{RF}	0.68±0.06***	0.53±0.05***	0.58±0.06***	0.47±0.04***
H	0.68±0.02	0.75±0.02	0.65±0.02	0.71±0.02
Gs	0.66	0.63	0.58	0.57
%Gs	2.41	2.03	2.07	1.75
Mean±SD	27.26±0.83	30.99±0.72	28.22±0.77	32.50±0.69
R^2	0.66	0.72	0.73	0.69
CV %	3.05	2.33	2.74	2.13

*** Significant at P<0.0001, σ^2_F – family variance, σ^2_{RF} – family by replication interaction variance, H – Broad-sense heritability, Gs – Gains to selection, %Gs– Percentage selection gains, SD – Standard deviation, R^2 – R-squared, CV – Coefficient of variation, TML – Coastal short cycle high potential mini-line

The mean values fluctuated across the four populations and ranged from 13.0 to 14.9% for fibre %cane and from 27.3 to 32.5% for DM %cane (Table 4.4). The TML11 population had the highest mean value while the TML10 population had the lowest mean value for fibre %cane. The TML13 population produced the highest mean, while the TML10 population produced the lowest mean for DM %cane. The R^2 values for fibre %cane increased from TML10 to TML13 and ranged from 0.6 to 0.8 across the four populations. TML12 (0.78) and TML13 (0.70) had higher R^2 values than TML10 (0.60) and TML11 (0.66). The R^2 for DM %cane increased from TML10 to TML12 and ranged from 0.65 to 0.73. The CV % for fibre %cane fluctuated from TML10 to TML13 and ranged from 5.3 to 6.5%. The TML10 population had the highest CV % while the TML13 population had the lowest value. All populations had CV % less than 5% and ranged from 2.1% (TML13) to 3.1% for DM %cane.

4.4.2 Broad-sense heritability

4.4.2.1 ERC %cane, Pol %cane, Brix %cane and Brix DM %cane

Broad-sense heritability values ranged from 0.64 to 0.77 for ERC %cane, 0.64 to 0.78 for Pol %cane, 0.61 to 0.77 for Brix %cane and 0.58 to 0.78 for Brix DM %cane (Table 4.2). The order of magnitude of broad-sense heritability for ERC %cane was TML13 > TML12 > TML10 > TML11. Earlier populations (TML10 and TML11) had lower broad-sense heritability for ERC %cane than later populations (TML12 and TML13). Broad-sense heritability for Pol %cane, Brix %cane and Brix DM %cane fluctuated across the four locations (Figure 4.1). The order of magnitude of broad-sense heritability for Pol %cane, Brix %cane and Brix DM %cane across populations was TML12 > TML13 > TML10 > TML11.

4.4.2.2 Purity %

Broad-sense heritability ranged from 0.51 (TML10) to 0.69 (TML13) for purity % across populations (Table 4.3). The order of magnitude of broad-sense heritability was TML13 > TML12 > TML11 > TML10.

4.4.2.3 Fibre %cane and DM %cane

Broad-sense heritability ranged from 0.58 to 0.74 for fibre %cane and 0.65 to 0.75 for DM %cane (Table 4.4). For fibre %cane, broad-sense heritability increased from TML10 to TML12 and decreased from TML12 to TML13. The order of magnitude of broad-sense heritability was TML12 > TML13 > TML11 > TML10. Broad-sense heritability for DM %cane fluctuated across populations. The TML11 (0.75) population produced the highest broad-sense heritability while the TML12 (0.65) population produced the lowest broad-sense heritability. The order of magnitude of broad-sense heritability for DM %cane across populations was TML11 > TML13 > TML10 >TML12.

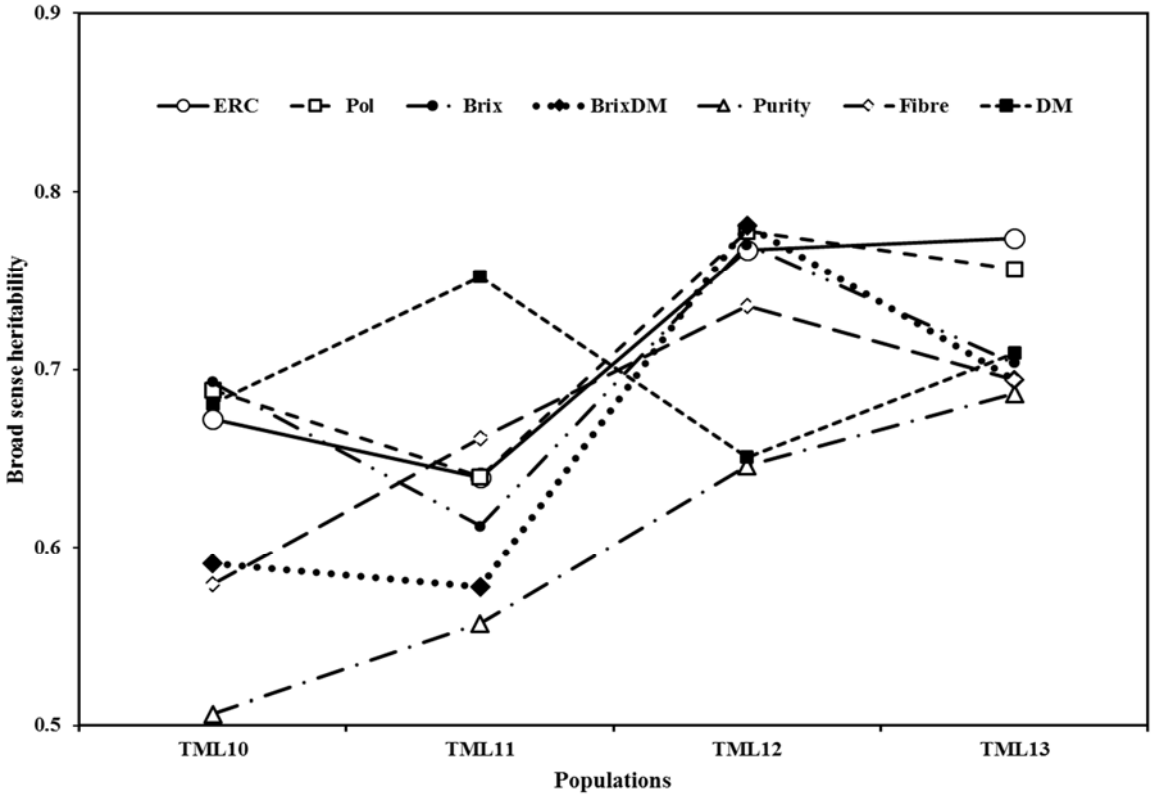

Figure 4.1. Broad-sense heritability for sugarcane quality traits plotted against the TML10, TML11, TML12 and TML13 populations. TML – Coastal short cycle high potential mini-line

4.4.3 Predicted selection gains

4.4.3.1 ERC %cane, Pol %cane, Brix %cane and Brix DM %cane

Predicted selection gains (Gs) ranged from 0.51 to 0.70 for ERC %cane, 0.49 to 0.66 for Pol %cane, 0.42 to 0.60 for Brix %cane and 1.31 to 2.00 for Brix DM %cane (Table 4.2). Percentage selection gains (%Gs) ranged from 3.61 to 6.27 % for ERC %cane, 3.11 to 5.13% for Pol %cane, 2.58 to 3.99% for Brix %cane and 2.52 to 3.74% for Brix DM %cane. The Gs comparison across traits is affected by the relative magnitude of traits' values, therefore, %Gs was interpreted for comparisons. The %Gs of all sucrose content related traits fluctuated across the four populations (Figure 4.2). The TML12 population had the highest %Gs for all traits while TML13 had the lowest for ERC %cane and Pol %cane and TML11 had the lowest values for Brix %cane and Brix DM %cane. The order of magnitude of %Gs for ERC %cane and Pol %cane was TML12 > TML10 > TML11 > TML13 while for both Brix %cane and Brix DM %cane it was TML12 > TML10 > TML13 > TML11.

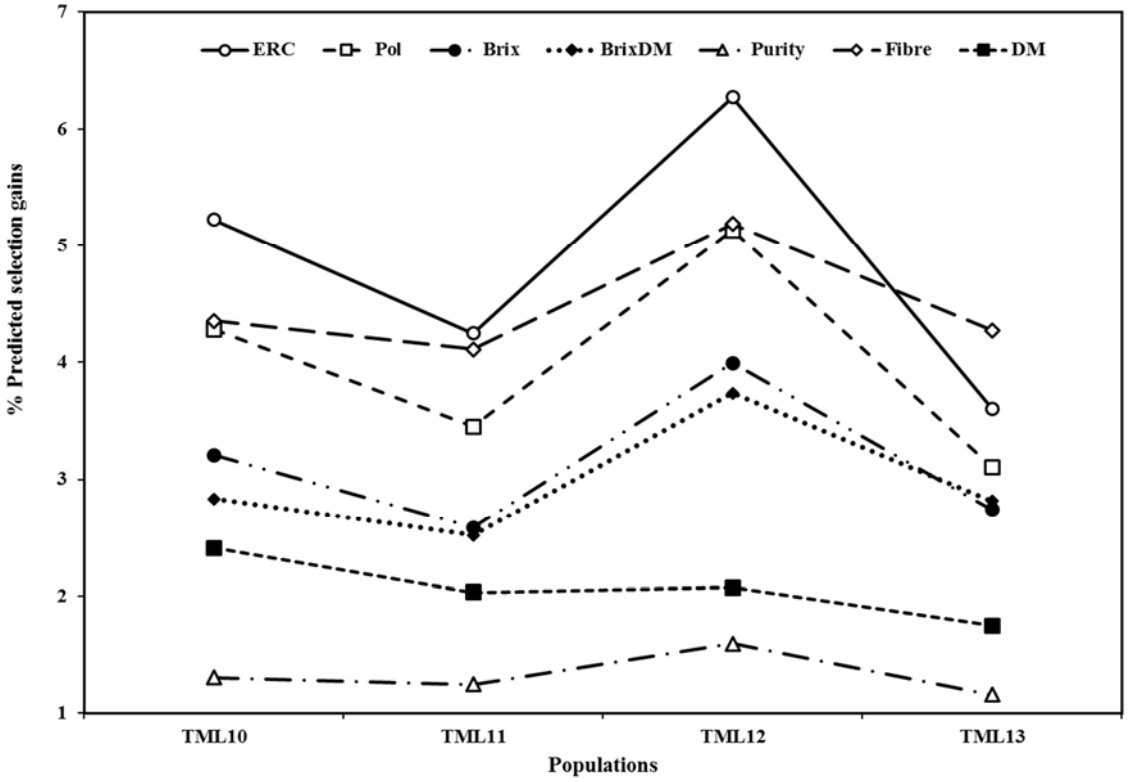

Figure 4.2. Percentage predicted selection gains of sugarcane quality traits plotted against the TML10, TML11, TML12 and TML13 populations. TML – Coastal short cycle high potential mini-line

4.4.3.2 Purity %

For purity %, the Gs ranged from 1.04 to 1.36 across populations while %Gs ranged from 1.16 to 1.59 % (Table 4.3). The %Gs fluctuated across populations (Figure 4.2) where TML12 (1.59%) had the highest value and TML13 (1.16%) had the lowest value. The order of magnitude of %Gs for purity % was TML12 > TML10 > TML11 > TML13.

4.4.3.3 Fibre %cane and DM %cane

The Gs for fibre %cane ranged from 0.57 to 0.68 while for DM %cane it ranged from 0.57 to 0.66 (Table 4.4). The Gs for fibre %cane increased from TML10 to TML12 while for DM %cane it decreased consistently from TML10 to TML13. TML10 (0.57) had the lowest Gs for fibre %cane while TML12 (0.68) had the highest values (Figure 4.2). The %Gs for fibre %cane ranged from 4.11 to 5.18% while for DM %cane it ranged from 1.75 to 2.41%. The %Gs for fibre %cane fluctuated across populations while for DM %cane it decreased from TML10 to TML13. For fibre %cane, the TML11 (4.11%) population had the lowest %Gs while TML12 (5.18%) had the highest value. The order of magnitude of %Gs for fibre %cane was TML12 >

TML10 > TML13 > TML11 while for DM %cane it was TML10 > TML12 > TML11 > TML13. Fibre %cane had higher %Gs than DM %cane across populations.

4.4.4 Phenotypic correlations among quality traits

Sucrose content related traits such as ERC %cane, Brix %cane and Pol %cane generally showed strong and highly significant (P<0.001) positive correlations (r-values ranging from 0.83 to 0.99) with each other (Table 4.5). Either one of these traits could be used to predict others (Figure 4.3). ERC %cane, Pol %cane and Brix %cane, respectively showed moderate to strong and highly significant positive correlations with Brix DM %cane in TML10 (r = 0.54 to 0.71), TML11 (r = 0.53 to 0.74), TML12 (r = 0.74 to 0.85) and TML13 (r = 0.47 to 0.81). Purity % showed weak to high positive correlations with the sucrose content related traits (r-values ranging from 0.05 to 0.88) and depending on the population. However, the correlation between purity % and Brix %cane was weak and not significant (r = 0.05) in TML13. DM %cane generally showed moderate but highly significant positive correlations with the sucrose related content traits across populations.

Fibre %cane generally showed weak to moderate but significant to highly significant negative correlations with the sucrose related content traits across populations. Brix DM %cane was highly significant and positively correlated with purity % for three of the four populations, namely TML10 (r = 0.18), TML12 (r = 0.43) and TML13 (r = 0.35). In TML11, Brix DM %cane showed a non-significant correlation with purity % (r = 0.06). Brix DM %cane showed strong and highly significant negative correlations with fibre %cane (r = -0.87 to -0.93) and weak but highly significant negative correlations with DM %cane (r = -0.18 to -0.37) or all four populations. Purity % showed weak to moderate but significant correlations with fibre %cane (r = 0.15 to 0.54) and DM %cane (r = 0.46 to 0.67). Fibre %cane and DM %cane had highly significant positive correlations (r = 0.55 to 0.74) across all four populations.

Table 4.5. Pearson's phenotypic correlations among sugarcane quality traits

	ERC %cane	Pol %cane	Brix %cane	Brix DM %cane	Purity %	Fibre %cane
			TML10			
Pol %cane	0.99***					
Brix %cane	0.92***	0.96***				
Brix DM %cane	0.54***	0.59***	0.71***			
Purity %	0.86***	0.79***	0.58***	0.18***		
Fibre %cane	-0.11*	-0.15***	-0.27***	-0.87***	0.15***	
DM %cane	0.54***	0.53***	0.44***	-0.32***	0.55***	0.74***
			TML11			
Pol %cane	0.99***					
Brix %cane	0.86***	0.92***				
Brix DM %cane	0.53***	0.60***	0.74***			
Purity %	0.80***	0.71***	0.39***	0.06		
Fibre %cane	-0.16***	-0.21***	-0.37***	-0.90***	0.18***	
DM %cane	0.46***	0.45***	0.34***	-0.37***	0.46***	0.74***
			TML12			
Pol %cane	0.99***					
Brix %cane	0.94***	0.97***				
Brix DM %cane	0.74***	0.78***	0.85***			
Purity %	0.88***	0.83***	0.68***	0.43***		
Fibre %cane	-0.44***	-0.48***	-0.58***	-0.92***	0.16**	
DM %cane	0.46***	0.44***	0.36***	-0.18***	0.52***	0.55***
			TML13			
Pol %cane	0.98***					
Brix %cane	0.83***	0.92***				
Brix DM %cane	0.47***	0.60***	0.81***			
Purity %	0.59***	0.43***	0.05	0.35***		
Fibre %cane	-0.15**	-0.28***	-0.55***	-0.93***	0.54***	
DM %cane	0.55***	0.47***	0.23***	-0.37***	0.67***	0.69***

* Significant at P<0.05, ** significant at P<0.01, *** significant at P<0.001. DM – Dry matter, Brix DM – Brix dry matter, Pol – Polarisation, ERC – Estimable recoverable crystal, TML – Coastal short cycle high potential mini-line

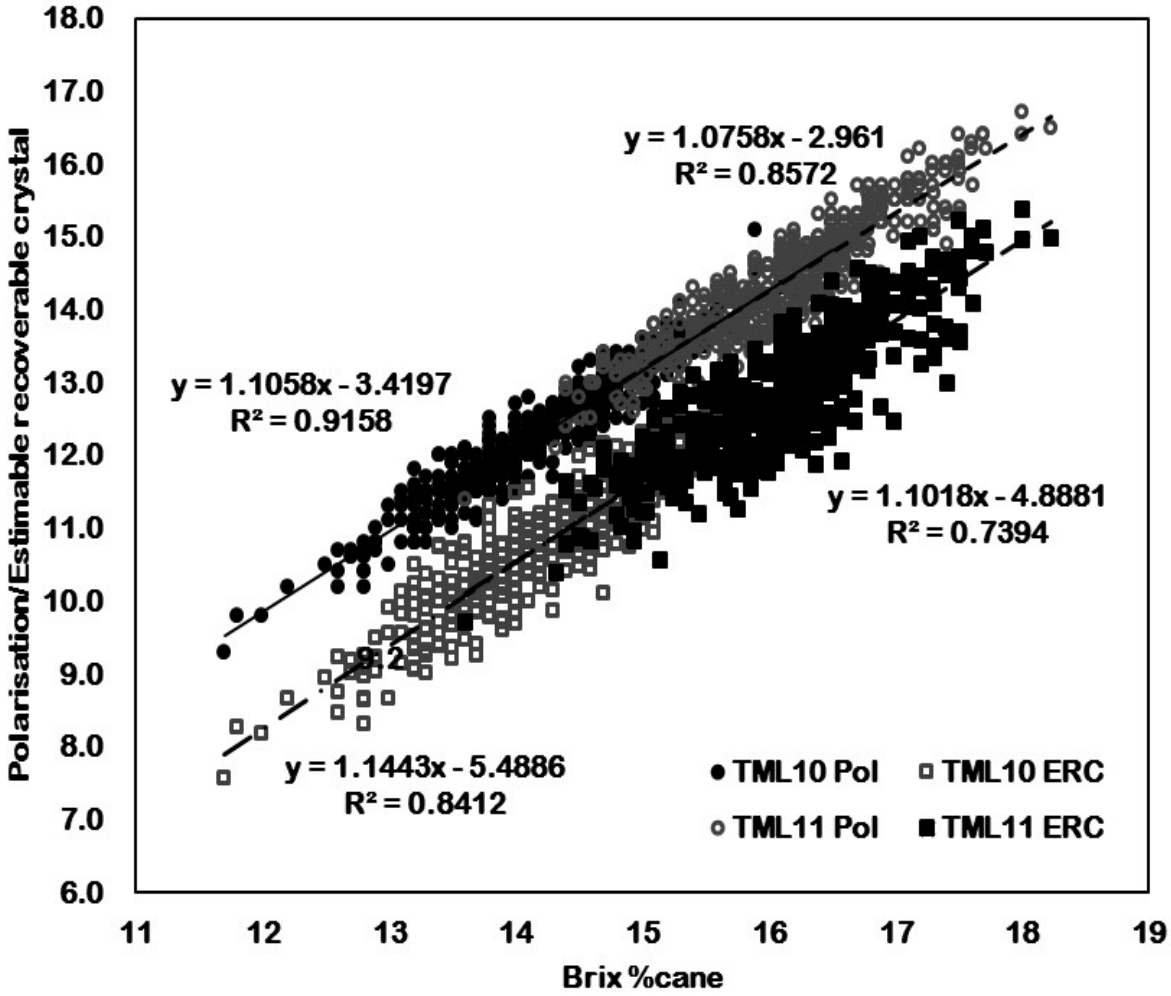

Figure 4.3. Prediction model for Pol %cane and ERC %cane using Brix %cane

4.5 Discussion

Family variances were significant for all traits, indicating the existence of genetic variability and potential for genetic improvement for quality traits through family selection. Genetic variance describes the magnitude of variation that is present for a particular trait. The existence of large variability among sugarcane families indicated the potential for identifying and selecting superior families within populations. By using family evaluation, poor performing families with a low probability of producing commercial varieties can be identified and discarded early. Several studies on family evaluation in sugarcane found family evaluation to be superior to individual genotype evaluation but Hogarth et al. (1990), Cox and Hogarth (1993) and Cox et al. (1996) all showed that selection of superior individuals within elite families was the most efficient method of family evaluation. Zhou and Lichakane (2012) and Zhou et al. (2013) studied family evaluation for quality traits in different breeding programmes and

reported significant family genetic variance for the coastal short cycle high potential mini-line breeding programme in South Africa.

The magnitudes of family genetic variance were larger for TML12 and TML13 compared to TML10 and TML11 for ERC %cane, Pol %cane, Brix %cane and fibre %cane, respectively, indicating larger genetic variability in later breeding populations (TML12 and TML13). The larger family genetic variability in later populations highlighted the effectiveness of family evaluation for improving sugarcane quality traits for the coastal short cycle high potential mini-line breeding programme with advancing populations. ERC %cane had larger family genetic variance estimates compared to Pol %cane and Brix %cane, indicating larger variability associated with families for ERC %cane. Family genetic variance fluctuated across populations for ERC %cane, Pol %cane and Brix %cane indicating unstable family genetic variance trends over time. Results revealed an increasing family genetic variance trend for purity % and fibre %cane from TML10 to TML12, indicating increased genetic variability over time. Results thus highlighted the possibility of genetic improvement for purity % and fibre %cane with advancing populations. Family genetic variance fluctuated over time for DM %cane indicating unstable family genetic variance across populations. Family genetic variance was larger for TML10 and TML11 compared to TML12 and TML13 for DM %cane, indicating reduced family genetic variability for later populations compared to earlier populations.

Broad-sense heritability is described as the proportion of total variance that is attributable to the genetic variance in the population (Zhou and Joshi, 2012). According to Piepho and Möhring (2007) heritability determines the response to selection. Broad-sense heritability values closer to one indicate that most of the variability in the trial was attributed to genetic variance. High broad-sense heritability values also indicate the ability to obtain selection gains. Broad-sense heritability can be used for quantifying precision of selection. Selection in vegetatively propagated crops such as sugarcane takes advantage of broad-sense heritability, since recombination events are limited to segregation that occurs only at crossing (Zhou and Joshi, 2012). Studying trends in broad-sense heritability over time can be useful for evaluating changes in breeding populations and also to evaluate the accuracy of selection. Broad-sense heritability ranged from 0.51 to 0.78 across traits and populations indicating that more than 50% of the phenotypic variation was attributable to genetic variance. Traits such as ERC %cane, Brix %cane, Pol %cane and DM %cane had broad-sense heritability values above 0.60 across years, highlighting improved accuracy of selection. Results indicated that a large proportion of

the total variance was heritable and family selection for these traits would be effective. High broad-sense heritability values also indicated that superior families can be easily identified and selected for using family evaluation.

Broad-sense heritability values fluctuated over time for ERC %cane, Pol %cane, Brix %cane, Brix DM %cane and DM %cane, indicating unstable trends over time and difficulty in predicting future performance of families using family evaluation. However, the sucrose related content traits (ERC %cane, Pol %cane and Brix %cane) had higher broad-sense heritability values in TML12 and TML13 (H>0.70) compared to TML10 and TML11 (H<0.70). This highlighted the potential to increase broad-sense heritability through family selection over time and the precision of family selection. Brix DM %cane had lower broad-sense heritability values for populations TML10 (H=0.59) and TML11 (H=0.58), indicating difficulty in identifying superior families for selection. However, TML12 (H=0.78) and TML13 (H=0.69) had higher broad-sense heritability values, indicating higher precision of family selection in later populations than earlier populations. Purity % had the lowest broad-sense heritability values among the cane quality traits, indicating lower accuracy of identifying and selecting superior families for this trait. Zhou et al. (2013) also found purity % to have the lowest broad-sense heritability compared to other cane quality traits. However broad-sense heritability showed an increasing trend for purity % and fibre %cane over time, indicating the potential for increase with advancing populations for these traits.

Knowledge on predicted selection gains (%Gs) is essential for evaluating the effectiveness of family evaluation (Zhou and Lichakane, 2012). For effective selection of superior genotypes, there must be adequate discriminating ability among families and genotypes. Predicted selection gains generally showed fluctuating trends for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane, indicating instability over time and the difficulty in predicting future selection gains. ERC %cane (3.61 to 6.27%) had the highest percentage predicted selection gains compared to other sugarcane quality traits. This indicated that family evaluation would be effective for ERC %cane. However, it is expensive and time consuming to measure ERC %cane and the possibility of using another correlated trait needs to be investigated. To measure ERC %cane, 20 stalks are collected from family plots in the field and sent to the sugar laboratory for analysis. Pol %cane (3.11 to 5.13%) had similar magnitudes and trends in percentage predicted selection gains and broad-sense heritability as ERC %cane.

Brix %cane (ranging from 2.58 to 3.99%) had a lower predicted selection gains compared to ERC %cane (ranging from 3.61 to 6.27%) and Pol %cane (ranging from 3.11 to 5.13%) but it had similar magnitudes in broad-sense heritability. Brix %cane can be used for family evaluation to indirectly estimate sucrose content (Kimbeng and Cox, 2003) and it is measured using a hand held refractometer in the field. A hand held refractometer is not expensive and provides quick measurements. Measuring Brix %cane with a hand held refractometer in the field would save data collection time and costs that can be used to plant and evaluate more families. Zhou and Lichakane (2012) have reported an increased percentage predicted selection gains over time for ERC %cane, Pol %cane and Brix %cane suggesting that these traits would benefit from family selection.

Purity % had the lowest predicted selection gains estimates compared to other cane quality traits. This indicated that purity % would not be an ideal trait for family evaluation. Purity % is further known to change depending on time of harvest, thus making it ineffective for family evaluation. Zhou et al. (2013) also reported lower predicted selection gains for fibre %cane compared to other cane quality traits. Fibre %cane had higher predicted selection gains estimates than Pol %cane, Brix %cane, Brix DM %cane, purity % and DM %cane. However, fibre %cane is considered undesirable for sugarcane quality breeding. On the other hand, high fibre is often associated with high Eldana borer (*E. saccharina*) resistance (Nuss and Atkinson, 1993; Nuss, 1991; Zhou and Lichakane, 2012), thus making families that have a high fibre content and sufficient levels of sucrose content suitable for family evaluation in areas where Eldana borer is prevalent. Eldana borer is prevalent at the Empangeni research station, thus, fibre %cane would be suitable for family evaluation for the coastal short cycle high potential mini-line breeding programme in South Africa.

Family by replication variance (residual variance) was highly significant for all quality traits across all populations. With a few exceptions, the residual variance was larger than the family genetic variance for most quality traits, suggesting that larger variability was not accounted for by the experimental design. Results implied difficulty in increasing the efficiency of comparing families. Residual variance increased from TML10 to TML12 for ERC %cane, Pol %cane and Brix %cane, indicating an increase in residual variability with advancing populations. Residual variance was smaller than family genetic variance in TML12 and TML13 populations for both ERC %cane and Pol %cane indicating larger family genetic variability than residual variability in later populations for these traits. This further indicated the effectiveness of family evaluation

for sucrose content related traits. The residual variance fluctuated across populations for Brix DM %cane, purity % and DM %cane, highlighting unstable trends in residual variability over time. For fibre %cane, residual variance decreased from TML10 to TML13, suggesting a decrease in residual variability over time. Results suggested the need to optimise trial designs and sample size. The efficiency of the experimental design could be improved by implementing an optimum sample size (number of genotypes) and number of replications to adequately determine family values. A study by Zhou (2014) suggested that four replications would be adequate to estimate sugarcane breeding parameters. Previous studies also reported larger residual variability over genetic variability for cane yield (Zhou, 2014), *E. saccharina* (Zhou and Mokwele, 2015) and quality traits (Zhou and Lichakane, 2012; Zhou et al., 2013).

The R^2 value, also known as the coefficient of determination, is the ratio of explained variation by a linear model to the total variation in the trial (Hamilton et al., 2015). An R^2 value of 1.0 indicates that all variability in the trial can be explained by the linear model. An R^2 value closer to zero indicates that most of the variability in the trial cannot be explained by the statistical model used for data analysis. The TML12 population generally had the highest R^2 ($R^2 > 0.80$) values for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane compared to the other populations. ERC %cane, Pol cane, Brix %cane, Brix DM %cane had R^2 values ranging from 0.60 to 0.82 indicating that more than 60% of the variation in the trial was explained by the statistical model. Purity % (ranging from 0.56 to 0.75), fibre %cane (ranging from 0.59 to 0.78) and DM %cane (ranging from 0.65 to 0.73) had lower R^2 values compared to the sucrose content related traits.

Significant positive correlations were observed among Brix %cane, ERC %cane and Pol %cane indicating that when selection is applied for one of these traits, an indirect improvement could be observed in the other traits. Among the sucrose content related traits, Brix %cane can be measured in the field using a hand held refractometer. Using a hand held refractometer to measure Brix %cane is not expensive and provides quick measurements. By measuring Brix %cane of all individual genotypes in the trial with a hand held refractometer, it would be more effective to quantify within family variability. Results suggested that expenses involved in collecting, transporting and analysing samples for Pol %cane and ERC %cane will be reduced by measuring Brix %cane using hand held refractometer. Sucrose content related traits (ERC %cane, Pol %cane, Brix %cane and Brix DM %cane) were negatively correlated with fibre %cane, suggesting the need to determine optimum trait values between these traits. In sugarcane

breeding, a higher fibre content has been associated with lower Eldana damage (Nuss, 1991; Zhou and Lichakane, 2012), which further emphasises the need to determine optimum trait values to achieve high sucrose with low Eldana damage. The low to moderate correlation between Brix %cane and purity % (ranging from 0.05 to 0.68) suggested poor prediction of crop maturity using hand held refractometer measurements, particularly in highly variable breeding populations. The significant and positive correlation between purity % and fibre %cane might indicate an indirect association of these traits caused by their increasing values with advancing crop age (Wagih et al., 2004).

4.6 Conclusions

Families had highly significant genetic variances for all sugarcane quality traits, indicating the presence of variability that can be exploited during selection. The presence of genetic variability implied that superior families can be selected within the populations. Large genetic variability was associated with large broad-sense heritability in later populations for ERC %cane, Pol %cane, Brix %cane, Brix DM %cane and fibre %cane indicating the effectiveness of family evaluation for these traits. Family evaluation produced fluctuating predicted selection gains over time, indicating difficulty in predicting future selection gains from family selection. ERC %cane, Pol %cane and fibre %cane had large broad-sense heritability and predicted selection gains values, suggesting these traits were suitable for family evaluation. However, these traits are expensive to measure. The study revealed that Brix %cane, measured using a hand held refractometer, would be suitable for family evaluation, providing quick and less expensive measurements. Hand held refractometer Brix %cane would also provide more data to quantify within family variability. Phenotypic correlations showed high correlations among Brix %cane, Pol %cane and ERC %cane, which further emphasize that Brix %cane (used to indirectly measure sucrose content) would be suitable for family evaluation. The study revealed negative correlations between sucrose content related traits and fibre %cane, highlighting the difficulty of breeding for high sucrose content and indirectly for low Eldana damage. High fibre content is associated with low Eldana damage, therefore, families that have high fibre and acceptable levels of sucrose content would be considered suitable for family evaluation in areas where Eldana borer is abundant. Purity % had lower broad-sense heritability and predicted selection gains values, indicating that family selection for this trait would not be effective. The study revealed that the selection strategy based on Brix %cane, could potentially result in the improvement of sucrose content for the coastal short cycle high potential mini-line breeding programme at Empangeni research station in South Africa.

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Chapter 5

Using best linear unbiased prediction (BLUP) to evaluate parent and family performance for quality traits in the coastal short cycle breeding programme

5.1 Abstract

Parental selection is a critical stage in sugarcane breeding. Family evaluation determines the performance of progenies from a cross. Family evaluation data can be useful for evaluating the performance of parental genotypes. The aim of this study was to use best linear unbiased predictors (BLUP) to identify families and parents with high BLUP values for quality traits in sugarcane breeding. Data for quality traits were collected from 20 stalks that were randomly chosen from the first 20 sub-plots of sugarcane families planted in four trials. The trials were laid out as randomized complete block designs with three replications at Empangeni research station. The data were analysed on statistical analysis software (SAS) to estimate BLUP values for family and parents using mixed models. Results showed highly significant ($P<0.001$) family, female and male variances highlighting the existence of genetic differences among families. Results showed lower proportions of families (< 10 %) and parents (< 10 %) with significantly higher BLUP values compared to the population mean. Results suggested the need to use breeding values to guide parent selection in the coastal short cycle breeding programme. Similar trends were observed for ERC %cane, Pol %cane and Brix %cane for family and parent evaluation, suggesting that Brix %cane, measured with a hand held refractometer, can be used to reduce costs.

Key words: Parent evaluation, Family evaluation, BLUP

5.2 Introduction

In plant breeding, parent selection identifies genotypes used for crossing to produce progeny populations. Parents are generally selected based on their genetic values, which is their performance in plant breeding trait evaluation trials. After initial crossing, parental genotypes can be further evaluated using progeny data. Genotypes that produce progenies with higher BLUP values are more desirable for use in future crossing as parents.

Family evaluation is used to determine genetic values of populations (progenies) derived from a cross (Falconer and MacKay, 1996). In sugarcane breeding, families that produce progenies

with high trait values are selected for further testing and commercial selection in larger plots. This is done to identify individual genotypes within families with desirable combinations of commercial BLUP values. Progenies from families producing higher BLUP values are planted in larger numbers where individual genotype selection is focused. Family evaluation in sugarcane breeding was first described by Hogarth (1971). However, statistical methods for determining families with high trait values are still limited to analysis of variance and selection based on family ranking.

Prior to family and parent evaluation, the proven cross and parent system was used (Bond, 1977; Heinz and Tew, 1987) to select populations (Skinner, 1971). The proven cross system assumed that crosses and parents, with larger number of progenies advanced through the breeding stages, are more valuable (Skinner, 1971; Skinner et al., 1987; Ming et al., 2006). The proven cross and parent system created a potential bias against new crosses because they had fewer progenies tested and advanced. This approach was time consuming to obtain data because of long breeding cycles in sugarcane (Milligan and Legendre, 1990; Kimbeng and Cox, 2003). Since the proven cross system used limited statistical analysis (Zhou, 2009) there is need to explore statistical methods to further optimise parent and family evaluation.

Best linear unbiased prediction (BLUP) was proposed by Henderson (1974) and has been widely used in animal and forestry breeding. BLUP has been used to predict yield of crosses in maize (*Zea mays* L.) single crosses (Viana et al., 2010) and sugarcane (Chang and Milligan, 1992; Zhou, 2014; Zhou and Mokwele, 2015). According to Stringer et al. (2011), results from family evaluation trials can be used to estimate breeding values of parent clones that were used to produce those families. BLUP has the potential to improve accuracy of family estimated potential and to predict future performance of families and parents. BLUP is expected to focus selection efforts on elite parents and families, thereby improving populations. The aim of this study was to use BLUP to identify families and parents with high BLUP values for quality traits in sugarcane breeding.

5.3 Materials and methods

5.3.1 Experimental materials

Parent varieties were selected and planted in the glasshouse and photoperiod house to initiate and produce viable flowers for crossing. Crosses used for this study were made in the glasshouse at Mount Edgecombe research station (29.7ºS, 31.03ºE, 96 m above sea level) Durban, KZN.

Three mating designs, namely bi-parental cross (BP), males only (MO) and melting pot (MP) were used. A male pollinates a female to produce BP populations. With MO, at least two males inter-cross and seed is collected from all parents. MP involves several males pollinating a single female. The trials, cross and parent details are shown in Table 5.1.

Table 5.1. Trial, number of families, parents (female and male), planting and harvesting dates and type of cross for the coastal short cycle high potential mini-line breeding programme (Empangeni research station)

Trial	Families	Female	Male	Planted	Harvested	MO/MP	BP
TML10	199	66	47	2010	2011	93	106
TML11	198	65	43	2011	2012	108	90
TML12	199	69	46	2012	2013	126	73
TML13	193	67	34	2013	2014	134	59

TML - Short cycle high potential mini-line

5.3.2 Description of research site

The coastal short cycle high potential mini-line breeding programme, based at Empangeni (28º43'S, 31º53'E, 102 m above sea level), was established in 1997 to develop sugarcane cultivars with high cane yield, high sucrose content and maturity at 12 months harvest age. The coastal short cycle high potential region is characterised by deep and rich soils with a high rainfall and temperatures. The conditions are conducive to fast sugarcane growth and high cane yields. To obtain a high sucrose content at 12 months, early maturity and accelerated sucrose accumulation in the sugarcane stalks are required.

5.3.3 Experimental design

Trials were laid in a RCBD with three replications per family. After germination, the number of seedlings (genotypes) from each family were counted and divided into three sets. Each set was randomized to one of the three replications. The seedlings were raised in airbricks on concrete slabs for 10 months and the same design was carried over to field planting. The growing conditions in the airbricks are presumed to be even due to similar and uniformly prepared soil media and uniform irrigation, thus no carry over effects were expected.

5.3.4 Trial establishment and management

Seedlings for coastal short cycle high potential breeding programme were germinated from true seed in the glasshouse at Mount Edgecombe research station in Durban, KZN. A week after germination, the seedlings in trays were counted to determine the germination percentage and then transferred to be grown outside the glasshouse. When the seedlings were five weeks old, they were transported to Empangeni research station where the seedlings were acclimatized for two weeks before transplanting into airbricks. The airbricks were filled with a mixture of sand, soil and baggase compost in the ratio of 1:1:2. The seedlings were left to grow in airbricks for 10 months until they produced mini-stalks that were 1 m long. Seedlings were irrigated three times a day to prevent moisture stress. Fertiliser (N:P:K = 5:1:5) was applied weekly at a rate of 10 kg per hectare to achieve optimum growth and replenish nutrients lost to leaching. The vegetative stalks of each seedling were cut at the base and topped at the natural breaking point during harvesting. The vegetative material from each seedling was planted in the field in a tramline design.

5.3.5 Data collection

Stalk sampling for quality traits measurements was done at 12 months harvest age. In each family plot, one stalk was picked randomly from each of the first 20 seedlings in the field. The stalks were bundled together and analysed in the sugarcane laboratory using the standard methods (Shoonees-Muir et al., 2009). The samples were analysed for dry matter (DM) %cane, Fibre %cane, Brix %cane, Brix DM %cane, polarisation (Pol) %cane, purity % and estimable recoverable crystal (ERC) %cane. Brix %cane refers to the total soluble solids present in the sugarcane juice expressed as a percentage. The Brix %cane of sugarcane juice was obtained by measuring the refractive index of the juice. Pol %cane refers to the total sucrose present in the soluble solids in sugarcane juice expressed as a percentage and was measured using a saccharimeter. The saccharimeter measures the rotation of plane of the polarised light by sugarcane juice. Purity refers to the percentage ratio of sucrose (pol) to the total soluble solids (Brix) in sugarcane juice and is an index of crop maturity. Purity % was calculated using the formula:

$$\text{Purity} = \frac{pol}{Brix} X\ 100 \qquad \text{Equation 4.1}$$

ERC %cane refers to the total recoverable sucrose in sugarcane after loss from molasses and fibre and is expressed as a percentage. ERC %cane was calculated using the formula:

$$\text{ERC \%cane} = a \cdot S - b \cdot N - c \cdot F \quad \text{Equation 4.2}$$

Where, *S* is the sucrose %cane, *N* is the non-sucrose %cane (calculated as Brix %cane minus sucrose %cane), *F* is the fibre %cane and *a*, *b* and *c* are constant parameters that are related to the sucrose loss within the factory (Peacock and Schorn, 2002). The *a* in the ERC %cane formula refers to the sucrose loss in the filter cake, *b* refers to sucrose loss due to final molasses and *c* refers to sucrose loss due to fibre and is also known as bagasse. Fibre %cane refers to the total fibre present in sugarcane and is expressed as a percentage. DM %cane is the sum total of the soluble and non-soluble solids and was calculated using the formula:

$$\text{DM} = \text{Sucrose\%} + \text{Fibre\%} + \text{Non-sucrose\%} \quad \text{Equation 4.3}$$

5.3.6 Data analysis

Data were subjected to analysis using linear mixed models of SAS statistical software (SAS, 2014). BLUP analysis for family data was done using the linear mixed model:

$$Y_{ij} = \mu + R_i + F_j + RF_{ij} \quad \text{Equation 4.4}$$

Where Y_{ij} is the observation for family and replication, μ is the grand mean, R_i is the random effect of the ith replication, F_j is the fixed effect of the jth family and RF_{ij} is the random interaction effect of the ith replication by the jth family and is the residual error. BLUP analysis for parental effects was done using the linear mixed model:

$$Y_{ijk} = \mu + R_i + Fe_j + M_k + RFeM_{ijk} \quad \text{Equation 4.5}$$

Where Y_{ijk} is the observation for replication, females and males, μ is the grand mean, R_i is the random effect of the ith replication, Fe_j is the random effect of the jth female parent, M_k is the random effect of the kth male parent and RFM_{ijk} is the random interaction effect of the ith replication by the jth female parent by the kth male parent and was the residual error.

5.4 Results

5.4.1 Variance components

In general, both family variances and residual variances were highly significant (P<0.0001) for all traits across all trials (Table 5.2). The R^2 values, ranged from 0.64 to 0.81 for ERC %cane, Pol %cane and Brix %cane while for fibre %cane it ranged from 0.56 to 0.75, for purity % it ranged from 0.56 to 0.75 and for DM %cane it ranged from 0.66 to 0.73. Purity % (1.45 to 2.22%) and DM %cane (2.13 to 3.05%) had the lowest CV % values compared to other traits which ranged from 3.36 to 7.05%.

5.4.2 Best linear unbiased prediction (BLUP) analysis

BLUPs are estimates of random effects of individual families relative to the grand mean of the population (Zhou, 2014; Zhou and Mokwele, 2015). The BLUP values can either be positive (when the family produced higher values than the grand mean) or negative (when the family produced lower values than the grand mean). BLUP values were used to identify families with significantly (P<0.05) higher BLUP values than the population mean. A sample of BLUP estimates is shown in Table 5.3 for ERC %cane from the TML10 trial. Family SS0023 produced significantly (P<0.05) higher ERC %cane than the population mean while families PP0128 and SS0183 produced significantly lower values (Table 5.3).

Across the three sugar content related traits (ERC %cane, Pol %cane and Brix %cane), families UU0065, UU0221, UU0259, UU0400, UU0431, UU0440, UU0636, UU703, UU0704, UU0737 and UU0823 produced significantly (P<0.05) higher BLUP values for the TML10 population (Table 5.4). For the TML11 population, families UU0065, VV0140, VV0371 and VV0457 produced significantly higher BLUP values across the three sugar content related traits. Similarly, WW0185, WW0260, WW0712, WW0937, WW0941 and WW0953 produced significantly higher BLUP values for the TML12 population and WW0214 and WW0711 for the TML13 population. Families UU0065, UU400 and UU0431 (for TML10), VV0371 and VV0457 (for TML11), and WW0712 and WW0941 (for TML12) produced significantly higher BLUP values for Brix DM %cane. For purity %, families SS0023, UU0065, UU0221, UU0440 and UU0520 (for TML10), UU0440, VV0303, VV0457 and VV0966 (for TML11), and WW0212, WW0466, WW0467, WW0525, WW0581, WW0582, WW0636, WW0639, WW0711, WW0937, WW0953 and XX0272 (for TML13) had significantly higher BLUP values. BLUP values

Table 5.2. Family covariance parameter estimates, their standard error (SE) and probability for a larger Z-value (P>Z), coefficient of variation, R-squared, mean and standard deviation for sugarcane quality traits

Statistic	TML10	TML11	TML12	TML13
		ERC %cane		
σ^2_F	0.35±0.06***	0.33±0.06***	0.71±0.13***	0.37±0.06***
σ^2_{RF}	0.51±0.04***	0.55±0.05***	0.64±0.07***	0.32±0.03***
R^2	0.66	0.66	0.80	0.74
CV %	6.70	5.73	7.05	4.02
Mean ± SD	10.83 ± 0.73	12.85 ± 0.74	11.19 ± 0.79	14.19 ± 0.57
		Pol %cane		
σ^2_F	0.32±0.05***	0.27±0.05***	0.64±0.11***	0.33±0.05***
σ^2_{RF}	0.42±0.03***	0.45±0.04***	0.55±0.06***	0.31±0.03***
R^2	0.67	0.65	0.81	0.73
CV %	5.36	4.65	5.69	3.54
Mean ± SD	12.35 ± 0.66	14.35 ± 0.67	12.89 ± 0.73	15.79 ± 0.56
		Brix %cane		
σ^2_F	0.24±0.04***	0.18±0.03***	0.50±0.09***	0.29±0.05***
σ^2_{RF}	0.31±0.03***	0.34±0.03***	0.47±0.05***	0.35±0.03***
R^2	0.67	0.64	0.80	0.70
CV %	3.99	3.64	4.47	3.36
Mean ± SD	14.26 ± 0.57	16.09 ± 0.59	15.12 ± 0.68	17.63 ± 0.59
		Brix DM %cane		
σ^2_F	2.19±0.42***	1.75±0.37***	5.73±0.97***	2.90±0.49***
σ^2_{RF}	4.54±0.36***	3.84±0.32***	4.95±0.57***	3.58±0.32***
R^2	0.61	0.62	0.81	0.71
CV %	4.13	3.76	4.13	3.50
Mean ± SD	52.33 ± 2.16	51.95 ± 1.95	53.58 ± 2.21	54.27 ± 1.90
		Purity %		
σ^2_F	1.26±0.30***	1.32±0.31***	2.20±0.51***	1.24±0.22***
σ^2_{RF}	3.63±0.29***	3.09±0.27***	3.42±0.04***	1.69±.0.15***
R^2	0.56	0.64	0.75	0.70
CV %	2.22	1.93	2.13	1.45
Mean ± SD	86.53 ± 1.92	89.15 ± 1.72	85.17 ± 1.81	89.55 ± 1.30
		Fibre %cane		
σ^2_F	0.32±0.06***	0.42±0.08***	0.57±0.10***	0.50±0.08***
σ^2_{RF}	0.70±0.06***	0.65±0.06***	0.63±0.07***	0.62±0.05***
R^2	0.60	0.66	0.78	0.70
CV %	6.48	5.36	6.07	5.30
Mean ± SD	13.00 ± 0.84	14.90 ± 0.80	13.11 ± 0.80	14.87 ± 0.79
		DM %cane		
σ^2_F	0.48±0.08***	0.53±0.08***	0.36±0.08***	0.38±0.06***
σ^2_{RF}	0.68±0.06***	0.53±0.05***	0.58±0.06***	0.47±0.04***
R^2	0.66	0.72	0.73	0.69
CV %	3.05	2.33	2.74	2.13
Mean ± SD	27.26 ± 0.83	30.99 ±0.72	28.22 ± 0.77	32.50 ± 0.69

*** Significant at P<0.0001, σ^2_F – family variance, σ^2_{RF} – family by replication interaction variance, R^2 – R-squared, CV – Coefficient of variation, Pol – Polarisation, ERC – Estimable recoverable crystal, DM – Dry matter, TML – Coastal short cycle high potential mini-line

Table 5.3. Sample output family best linear unbiased prediction (BLUP), standard error of BLUP (SE), Satterthwaite estimated degrees of freedom (DF), t-statistic and probability of a larger t-statistic (Pr>|t|) for the TML10 population for ERC %cane

Family	BLUP	SE	DF	t-statistic	Pr > |t|
PP0128	-0.8091	0.3419	283	-2.37	0.0186
QQ0427	-0.3190	0.3419	283	-0.93	0.3516
QQ0620	0.2766	0.3874	283	0.71	0.4758
QQ0745	0.1159	0.4588	283	0.25	0.8008
QQ0977	0.2670	0.4588	283	0.58	0.5610
QQ0996	-0.2558	0.4588	283	-0.56	0.5775
QQ1273	0.5121	0.4588	283	1.12	0.2653
RR0448	-0.2943	0.3419	283	-0.86	0.3902
RR0487	-0.4584	0.3419	283	-1.34	0.1811
SS0023	0.8366	0.3419	283	2.45	0.0150
SS0183	-0.8653	0.3419	283	-2.53	0.0119
SS0767	-0.1077	0.3419	283	-0.31	0.7531
SS0836	0.4229	0.3419	283	1.24	0.2171
SS1219	0.6130	0.3874	283	1.58	0.1146
TT0098	-0.02897	0.3419	283	-0.08	0.9325
TT0309	-0.07169	0.3419	283	-0.21	0.8341
TT0474	-0.5191	0.3419	283	-1.52	0.1301
TT0583	-0.4368	0.3874	283	-1.13	0.2604
TT0586	-0.6153	0.4588	283	-1.34	0.1809
TT0590	0.3015	0.3419	283	0.88	0.3786
TT0605	0.3982	0.3419	283	1.16	0.2452
TT0624	-0.2935	0.3107	283	-0.94	0.3456
TT0827	0.1722	0.3874	283	0.44	0.6570
TT0839	0.4251	0.3419	283	1.24	0.2147
TT0866	0.4361	0.3874	283	1.13	0.2612

Table 5.4. Summary of families that produced significantly (P<0.05) positive BLUP values for sugarcane quality traits for the coastal short cycle high potential mini-line trials

Trait	TML10	TML11	TML12	TML13
ERC %cane	SS0023, UU0065, UU0221, UU0259, UU0400, UU0431, UU0440, UU0520, UU0636, UU0703, UU0704, UU0737, UU0823	TT0141, UU0065, UU0440, UU0726, VV0140, VV0323, VV0371, VV0457, VV0575, VV0966	WW0185, WW0260, WW0526, WW0676, WW0711, WW0712, WW0937, WW0941, WW0953, WW0981	WW0214, WW0256, WW0260, WW0581, WW0636, WW0637, WW0638, WW0639, WW0711, XX0383, XX1022, XX1228
Pol %cane	SS0023, UU0065, UU0221, UU0259, UU0400, UU0431, UU0440, UU0520, UU0636, UU0703, UU0704, UU0737, UU0823	TT0141, UU0065, UU0123, UU0726, VV0140, VV0160, VV0216, VV0323, VV0371, VV0455, VV0457, VV0575	WW0185, WW0260, WW0526, WW0638, WW0676, WW0679, WW0712, WW0937, WW0941, WW0953, WW0981	WW0214, WW0256, WW0260, WW0581, WW0636, WW0638, WW0639, WW0711, XX0383, XX1228
Brix %cane	UU0065, UU0074, UU0110, UU0219, UU0221, UU0259, UU0293, UU0400, UU0431, UU0440, UU0636, UU0703, UU0704, UU0737, UU0823	UU0065, UU0123, VV0140, VV0160, VV0161, VV0216, VV0371, VV0457	WW0132, WW0185, WW0260, WW0531, WW0638, WW0712, WW0937, WW0941, WW0953	WW0213, WW0214, WW0711, XX0275, XX0276
Brix DM %came	UU0065, UU0149, UU0397, UU0400, UU0431, UU0635	VV0457, UU0123, UU0635, VV0163, VV0371	VV0065, VV0140, VV0163, WW0132, WW0531, WW0585, WW0712, WW0841, WW0941, WW0956	WW0213, XX0731, XX0737, XX0749, XX1257, XX1264
Purity %	SS0023, UU0065, UU0221, UU0440, UU0520	UU0440, VV0303, VV0457, VV0966		WW0212, WW0466, WW0467, WW0525, WW0581, WW0582, WW0636, WW0639, WW0711, WW0937, WW0953, XX0272
Fibre %cane	TT0474, UU0103, UU0190, UU0435, UU0600, UU0634, UU0730, UU0731	TT0166, UU0322, UU0730, VV0084, VV0188, VV0302, VV0456, VV0457, VV0458, VV0574, VV0735, VV1016	VV0041, VV0105, VV0167, VV0265, WW0053, WW0054, WW0093, WW0184, WW0258, WW0336, WW0337, WW0374, WW0579, WW0846	WW0466, WW0467, WW0563, WW0582, WW0639, XX0131, XX0184, XX0195, XX0198, XX0609, XX0715, XX0754, XX0830, XX0970
DM %cane	SS0836, SS0023, TT0474, TT0839, UU0103, UU0221, UU0322, UU0431, UU0435, UU0440, UU0520, UU0600, UU0634, UU0703, UU0730, UU0731, UU0737, UU0823	TT0166, UU0322, UU0730, VV0039, VV0062, VV0216, VV0223, VV0302, VV0323, VV0446, VV0456, VV0457, VV0458, VV0508, VV0574, VV0575, VV0967	WW0053, WW0184, WW0185, WW0260, WW0579, WW0643	WW0212, WW0214, WW0259, WW0466, WW0581, WW0582, WW0636, WW0637, WW0638, WW0639, WW0676, WW0711, XX0383, XX0715, XX0830, XX1105, XX1228

Pol – Polarisation, ERC – Estimable recoverable crystal, DM – Dry matter, TML – Coastal short cycle high potential mini-line

For both fibre %cane and DM %cane (Table 5.4), families TT0474, UU0103, UU0435, UU0600, UU0634, UU0730, UU0731 (for TML10), TT0166, UU0322, UU0730, VV0302, VV0456, VV0457, VV0458 and VV0574 (for TML11), WW0053, WW0184 and WW0579 (for TML12), and WW0466, WW0582, WW0639, XX0715 and XX0830 (for TML13) produced significantly higher BLUP values. Across sucrose, purity and fibre traits, families SS0023, UU0065, UU0221, UU0440 and UU0520 (for TML10), WW0457 (for TML11), WWW0256, WW0214, WW0639, WW0711 (for TML13) generally produced significantly higher BLUP values than the population mean.

5.4.3. Proportion of families with high BLUP values

The number of families that produced significantly higher BLUP values than the population mean ranged from 10 to 13 (for ERC %cane and Pol %cane), five to 15 (for Brix %cane) and five to 10 (for Brix DM %cane) from TML10 to TML13 (Table 5.5). For ERC %cane, the proportion of elite families was highest in TML10 (6.53%) and lowest in TML12 (5.03%). For Pol %cane, the proportion of families was highest in TML10 (6.53%) and lowest in TML13 (5.18%). For Brix %cane, the proportion of families was highest in TML10 (7.54%) and lowest in TML11 (4.04%) Brix %cane. For Brix DM %cane, the proportion of families was highest in TML12 (5.03%) and lowest in TML11 (2.53%).

Table 5.5. Proportion of families with high BLUP values for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane for the coastal short cycle mini-line breeding programme

Trial	Total	ERC %cane		Pol %cane		Brix %cane		Brix DM %cane	
		HTV	%	HTV	%	HTV	%	HTV	%
TML10	199	13	6.53	13	6.53	15	7.54	6	3.02
TML11	198	10	5.05	12	6.06	8	4.04	5	2.53
TML12	199	10	5.03	11	5.53	9	4.52	10	5.03
TML13	193	12	6.22	10	5.18	5	5.29	6	3.11
Total	789	45	22.83	46	23.3	37	21.39	27	13.69

TML – Coastal short cycle high potential mini-line, ERC – Estimable recoverable crystal, Pol – Polarisation, HTV – number of families that produced significant ($P<0.05$) BLUP values

The number of elite families that produced significantly higher BLUP values than the population mean ranged from zero to 12 (for purity %), eight to 14 (for fibre %cane) and six to eight (for DM %cane) from TML10 to TML13 (Table 5.6). For purity%, the proportion of

families was highest in TML13 (6.22%) and lowest in TML12 (0%). For fibre %cane the proportion of families was highest in TML13 (7.25%) and lowest in TML10 (4.02%). For DM %cane, TML10 (9.05%) had the highest proportion of families while TML12 (3.02%) had the lowest.

Table 5.6. Proportion of families with high BLUP values for purity %, fibre %cane and DM %cane for the coastal short cycle high potential mini-line breeding programme

Trial	Total	Purity %		Fibre %cane		DM %cane	
		HTV	%	HTV	%	HTV	%
TML10	199	5	2.51	8	4.02	18	9.05
TML11	198	4	2.02	12	6.06	17	8.59
TML12	199	0	0	14	7.04	6	3.02
TML13	193	12	6.22	14	7.25	17	8.81
Total	789	21	10.75	48	24.37	58	29.47

TML – Coastal short cycle high potential mini-line, ERC – Estimable recoverable crystal, Pol – Polarisation, DM – Dry matter, HTV – number of families that produced significant (P<0.05) BLUP values

5.4.4. Parents covariance estimates

The female effects variance for ERC %cane, Pol %cane, Brix DM %cane, purity %, fibre %cane and DM %cane were all significant (P<0.05) across the four populations (TML10, TML11, TML12 and TML13) (Table 5.7). TML12 had the largest female effects family variance, respectively for ERC %cane (0.80), Pol %cane (0.73), Brix %cane (0.55), Brix DM %cane (6.41), purity % (2.00) and fibre %cane (0.50). With the exception of TML11, the largest female variance effects were generally observed for Brix DM %cane (ranging from 0.34 to 6.41). With the exception of TML12, the lowest female variance effects were generally observed for Brix %cane (ranging from 0.04 to 0.55). The residual variance was highly significant (P<0.01) across all populations and quality traits and were larger than both the female and male effects variance for all traits and across all populations.

5.4.5. Best linear unbiased prediction (BLUP) estimates for parents

Parental BLUP analysis is shown in Table 5.8. The interpretation is similar to that for family BLUP. For example, genotypes 94W1043, 96F0701, 97E0474 and 03T3487 have significantly (P<0.05) higher BLUP values and, therefore, produced progenies with higher ERC %cane.

Table 5.7. Covariance estimates for female and male effects, their standard error (SE) and probability for a larger Z-value (P>Z)

Effect	TML10	TML11	TML12	TML13
		ERC %cane		
Female	0.15± 0.05**	0.13 ± 0.05**	0.80 ± 0.21***	0.19 ± 0.06***
Male	0.13 ± 0.05**	0.12 ± 0.05**	0.12 ± 0.07*	0.19 ± 0.06***
Residual	0.59 ± 0.04***	0.62 ± 0.05***	0.66 ± 0.07***	0.39 ± 0.03***
		Pol %cane		
Female	0.13 ± 0.05**	0.09 ± 0.01**	0.73 ± 0.19***	0.16 ± 0.05***
Male	0.12 ± 0.04**	0.11 ± 0.04**	0.10 ± 0.06^{ns}	0.18 ± 0.06***
Residual	0.50 ± 0.04***	0.51 ± 0.04***	0.58 ± 0.06***	0.38 ± 0.03***
		Brix %cane		
Female	0.09± 0.03**	0.04 ± 0.02*	0.55 ± 0.15***	0.13 ± 0.04**
Male	0.09 ± 0.03**	0.10 ± 0.03***	0.06 ± 0.05^{ns}	0.16 ± 0.05**
Residual	0.38 ± 0.03***	0.38 ± 0.02***	0.50 ± 0.05***	0.42 ± 0.04***
		Brix DM %cane		
Female	0.79 ± 0.34**	0.34 ± 0.20*	6.41 ± 1.70***	1.54 ± 0.49***
Male	1.02 ± 0.39**	1.00 ± 0.31***	0.75 ± 0.53	1.22 ± 0.46**
Residual	5.28 ± 0.39***	4.24 ± 0.31***	5.52 ± 0.54***	4.27 ± 0.35***
		Purity %		
Female	0.54 ± 0.24*	0.78 ± 0.29**	2.00 ± 0.78**	0.75 ± 0.23***
Male	0.42 ± 0.22*	0.29 ± 0.20^{ns}	0.82 ± 0.45*	0.40 ± 0.18*
Residual	3.92 ± 0.28***	3.29 ± 0.25***	3.30 ± 0.33***	1.74 ± 0.14***
		Fibre %cane		
Female	0.17 ± 0.06**	0.17 ± 0.06**	0.50 ± 0.16***	0.32 ± 0.09***
Male	0.11 ± 0.05*	0.16 ± 0.05**	0.15 ± 0.07**	0.16 ± 0.07**
Residual	0.75 ± 0.06***	0.72 ± 0.054**	0.69 ± 0.07***	0.68 ± 0.06***
		DM %cane		
Female	0.31 ± 0.09***	0.31 ± 0.08***	0.22 ± 0.08**	0.27 ± 0.08***
Male	0.10 ± 0.05*	0.15 ± 0.05**	0.16 ± 0.06**	0.12 ± 0.05**
Residual	0.71 ± 0.05***	0.57 ± 0.04***	0.60 ± 0.06***	0.47 ± 0.04***

* Significant at P<0.05, ** significant at P<0.01, *** significant at P<0.001, Pol – Polarisation, ERC – Estimable recoverable crystal, DM – Dry matter

Table 5.8. Sample output female and male best linear unbiased prediction (BLUP), standard error of BLUP (SE), Satterthwaite estimated degrees of freedom (DF), t-statistic, and probability of a larger t-statistic (Pr>|t|) for the TML10 population for estimable recoverable crystal (ERC) %cane

Role	Parent	BLUP	SE	DF	t statistic	Pr > |t|
Female	**94W0947**	0.08999	0.282	350	0.32	0.7498
Female	**94W1043**	0.7415	0.2851	350	2.60	0.0097
Female	**95H0039**	-0.1351	0.3161	350	-0.43	0.6694
Female	**95W1786**	-0.1755	0.1616	350	-1.09	0.2781
Female	**96F0269**	-0.1192	0.163	350	-0.73	0.4651
Female	**96F0701**	0.4959	0.2402	350	2.06	0.0397
Female	**96M0058**	0.2991	0.3111	350	0.96	0.3371
Female	**96M0646**	-0.194	0.3161	350	-0.61	0.5399
Female	**96W0246**	-0.01991	0.3294	350	-0.06	0.9518
Female	**97E0410**	-0.06978	0.3242	350	-0.22	0.8297
Female	**97E0474**	0.6761	0.1452	350	4.66	<.0001
Female	**97E1414**	0.2811	0.3363	350	0.84	0.4039
Female	**97E1455**	0.2061	0.2511	350	0.82	0.4122
Female	**97M0653**	-0.2236	0.3525	350	-0.63	0.5263
Female	**97W0101**	0.00836	0.2587	350	0.03	0.9742
Male	**02U0198**	-0.1528	0.3117	350	-0.49	0.6242
Male	**03T2530**	-0.00089	0.2262	350	0.00	0.9969
Male	**03T3487**	0.4912	0.2099	350	2.34	0.0198
Male	**03T3936**	-0.03475	0.3335	350	-0.10	0.9171
Male	**04X0016**	-0.1433	0.2302	350	-0.62	0.534
Male	**04X0022**	-0.9298	0.2735	350	-3.40	0.0008
Male	**04X0027**	-0.2081	0.3116	350	-0.67	0.5047
Male	**04X0054**	-0.2263	0.3366	350	-0.67	0.5018
Male	**66N2008**	-0.189	0.3041	350	-0.62	0.5347
Male	**76W1050**	0.08238	0.3338	350	0.25	0.8052
Male	**86F3326**	0.05463	0.3063	350	0.18	0.8586
Male	**87F2019**	0.3236	0.2876	350	1.13	0.2612
Male	**87L0573**	-0.1754	0.2999	350	-0.58	0.5591
Male	**89W1916**	-0.04045	0.2342	350	-0.17	0.863
Male	**90E0667**	-0.1667	0.3116	350	-0.53	0.593

Genotype 94W0947 (Table 5.9) produced progenies with significantly higher BLUP values, respectively for populations TML11 (for Brix %cane and DM %cane), TML12 (for ERC %cane, Pol %cane, Brix %cane, Brix DM %cane, purity % and DM %cane) and TML13 (for ERC %cane, Pol %cane and Brix %cane). Genotype 99F2694 produced progenies with significantly higher BLUP values, respectively in populations TML10 and TML11 for ERC %cane, Pol %cane and Brix DM %cane. Genotypes 97E0474 and 95W1786 produced progenies with significantly higher BLUP values for fibre %cane and DM %cane, respectively in populations TML10 and TML11. For TML12 and TML13, genotypes 92E0901 (for purity %), 92W0511 (for ERC %cane) and 00U1422 (for purity % and DM %cane) produced progenies with significantly higher BLUP values compared to the population mean. For TML10, genotypes 97E0474 and 99F2694 had progenies with significantly higher BLUP values, respectively for ERC %cane, Pol %cane, Brix %cane, purity % and DM %cane. In addition, for TML10, genotypes 94W1043, 96F0701, 03T3487 and 96W0246 had significantly higher BLUP values for ERC %cane, Pol %cane and Brix %cane, respectively while 89E0822 and 95W1786 had progenies with significantly higher BLUP values, respectively for fibre %cane and DM %cane.

For trial TML11 (Table 5.9), genotype 91W1462 produced progenies with significantly higher BLUP values, respectively for ERC %cane, Pol %cane, purity %, fibre %cane and DM %cane, 99F2694 (respectively for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane), 94W0947 (respectively for Brix %cane and DM %cane) and 95W1786, 97E0474 and 98G0115 (respectively for fibre %cane and DM %cane). For TML12, genotypes 94W0947 (respectively for ERC %cane, Pol %cane, Brix %cane, Brix DM %cane, purity % and DM %cane), 02T0005, 03T2791, 03U1293 and 05T2497 (respectively for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane), and 05T2658 and 92E0901 (respectively for ERC %cane, Pol %cane and Brix %cane) produced significantly higher BLUP values. For TML13, genotypes 00U1422 (respectively for ERC %cane, Pol %cane, purity %, fibre %cane and DM %cane), 94W0947 and 01S1672 (respectively for ERC %cane, Pol %cane and Brix %cane), and 06T3120, 04T2686, O6T3608, 91W1482 and 92W0461 (respectively for at least two of the quality traits) produced significantly higher BLUP values.

Table 5.9. Summary of parents (female and male) that produced significantly positive best linear unbiased prediction (BLUP) values for sugarcane quality traits for the coastal short cycle high potential trials

Trait	TML10	TML11	TML12	TML13
ERC %cane	94W1043, 96F0701, 97E0474, 03T3487, 96W0246, 99F2694	91W1462, 99F2694	02T0005, 03T2791, 03U1293, 05T0740, 05T2497, 05T2658, 92E0901, 92W0511, 94W0947, N40	00U1422, 06T3120, 92W0511, 94W0947, 00U1422, 01S1672
Pol %cane	94W1043, 96F0701, 97E0474, 03T3487, 96W0246, 99F2694	91W1462, 04X0033, 94M0017, 99F2694	02T0005, 03T2791, 03U1293, 05T0340, 05T2497, 05T2658, 92E0901, 92W0511, 94W0947	00U1422, 06T3120, 94W0947, 01S1672
Brix %cane	03T3487, 94W1043, 96F0701, 97E0474, 03T3487, 96W0246, 99F2694	94W0947, 99F2694	02T0005, 03T2791, 03U1293, 05T2497, 05T2658, 92E0901, 92W0511, 94W0947	04T2686, 94W0947, 01S1672
Brix DM %cane	96W0246, 99F2694	99F2694	01T2730, 02T0005, 03T2791, 03U1293, 04X0016, 05T0740, 05T2497, 92W0511, 94W0947, SP901368	04T0330, 04T2686, 06T1932, 03T2530
Purity %	97E0474, 99F2694	91W1462, 95W1786, 97E1414	92E0901, 92W0511, 94W0947, N40, 00U1422	00U1422, 02T2631, 06T3608, 91W1482, 92E0901, 92W0461, 00U1422, 05U0645
Fibre %cane	89E0822, 95W1786, 95W1786, 97E0474	75E0247, 91W1462, 95W1786, 97E0474, 91W1462, 95W1786, 98G0115	05U0114, 91W1482, EGYPT, SELFPGN8, SELFSES2	05T0375, 05T2178, 06T2836, 06T3608, 92W0461, N35, 00U1422
DM %cane	89E0822, 94W1043, 95W1786, 97E0474, 99F0198, N40, 95W1786, 97E0474, 99F2694,	91W1462, 94M0997, 94W0947, 95W1786, 97E0474, 91W1462, 98G0115	00U1422, 91W1482, 94W0947, 00U1422, 94W0947	00U1422, 06T3600, 06T3608, 91W1482, 92W0461, 92W0511, 00U1422

Pol – Polarisation, ERC – Estimable recoverable crystal, TML – Coastal short cycle high potential mini-line

5.4.6. Proportion of parents that produced progenies with high BLUP values

In total, more elite parents were observed for ERC %cane and Pol %cane than for Brix %cane and Brix DM %cane (Table 5.10). The proportion of high BLUP value parents for ERC %cane ranged from 1.85% (TML11) to 8.70% (TML12), for Pol %cane from 3.70% (TML11) to 7.83% (TML12), for Brix %cane from 1.85% (TML11) to 6.96% (TML12) and for Brix DM %cane from 0.93% (TML11) to 8.70% (TML12). The highest proportion of high BLUP value parents was consistently observed for TML12 across all four quality traits, while the lowest proportion was observed for TML11 across the same quality traits.

Table 5.10. Proportion of parents that produced progenies with high BLUP values for ERC %cane, Pol %cane, Brix %cane and Brix DM %cane for the coastal short cycle high potential mini-line breeding programme

Trial	Total	ERC %cane		Pol %cane		Brix %cane		Brix DM %cane	
		HVP	%	HVP	%	HVP	%	HVP	%
TML10	113	6	5.31	6	5.31	7	6.19	2	1.77
TML11	108	2	1.85	4	3.70	2	1.85	1	0.93
TML12	115	10	8.70	9	7.83	8	6.96	10	8.70
TML13	101	6	5.74	5	4.95	3	2.97	3	3.96
Total	437	24	21.6	24	21.79	20	17.97	17	15.43

TML – Coastal short cycle high potential mini-line, ERC – Estimable recoverable crystal, Pol – Polarisation, DM – Dry matter, HVP – Number of parents that produced significant ($P<0.05$) best linear unbiased prediction (BLUP) values

For purity %, fibre %cane and DM %cane, respectively the number of high BLUP value parents ranged from two (TML10) to eight (TML13), four (TML10) to eight (TML13) and five (TML12) to nine (TML10) (Table 5.11). TML13 had the highest proportion of high BLUP value parents for both purity % (7.92%) and fibre %cane (6.93%), while TML10 had the lowest for purity % (1.77%) and fibre %cane (3.54%). TML12 and TML13, respectively had a higher proportion of high BLUP value parents than TML10 and TML11 for purity %. For DM %cane, TML10 (7.96%) had the highest proportion of high BLUP value parents while TML12 (4.35%) trial had the lowest.

Table 5.11. Proportion of parents that produced families with high BLUP values for purity %, fibre %cane and DM %cane for the coastal short cycle high potential mini-line breeding programme

Trial	Total	Purity %		Fibre %cane		DM %cane	
		HVP	%	HVP	%	HVP	%
TML10	113	2	1.77	4	3.54	9	7.96
TML11	108	3	2.78	7	6.48	7	6.48
TML12	115	5	4.35	5	4.35	5	4.35
TML13	101	8	7.92	8	6.93	7	6.93
Total	437	18	16.82	23	21.21	28	25.72

TML – Coastal short cycle high potential mini-line, ERC – Estimable recoverable crystal, Pol – Polarisation, DM – Dry matter, HVP – Number of parents that produced significant ($P<0.05$) best linear unbiased prediction (BLUP) values

5.5 Discussion

Results showed highly significant family, female and male variances suggesting that significant genetic differences were obtained among the populations derived from different female and male parent cross combinations. This also indicated presence of genetic variability among families planted for commercial selection, suggesting the potential for achieving genetic gains by selecting families that are made up of progenies with high BLUP values for quality traits. The significant female and male variances was an indication of the effectiveness of parent selection for quality traits. By selecting parental genotypes that produce high BLUP values, progenies with higher BLUP values will be produced from the resulting families after crossing. Further, the significant female and male variances also indicate presence of additive genetic effects for quality traits. Additive genetic control of traits can be used in plant breeding by selection of parents, and crossing among those parents with higher BLUP values. The presence of additive genetic effects indicates that breeding values of male and female parents can be quantified and eventually used in parent selection during breeding for quality traits.

Results have shown that there were families and parents that produced significantly higher BLUP values across the quality traits. Such families and parents would be ideal for enhancing breeding for multiple quality traits, as is practiced in sugarcane breeding. Families with significantly higher BLUP values across several traits suggested that there is a potential to improve several quality traits. Results also indicated that during crossing, several traits can

potentially be combined in the progeny. This suggested that it is possible to create populations combining several desirable traits in sugarcane breeding. The presence of parents that produce progenies with combination of high BLUP values suggest the presence of high additive genetic effects for several traits among some of the parental genotypes. Results further indicated that genotypes with high breeding values for several traits can be identified within the breeding populations.

The proportion of families producing high BLUP values was very low across different breeding cycles and largely less than 10%. This highlighted the inefficiency of mass selection, also known as individual genotype selection, compared to family evaluation and selection in sugarcane breeding for quality traits. Further, results indicated the potential value to be derived from adopting family evaluation for quality traits in sugarcane breeding. Previous studies by Zhou and Lichakane (2012) also showed significant family differences for quality traits as well as some gains in certain quality traits over cycles of breeding. With family evaluation, crosses producing lower progeny values as well as lower proportion of progenies with high BLUP values will be discarded early. Families with higher BLUP values will be planted more frequently and at time of crossing such families are repeated to generate more progenies for selection, a situation that is not done when family evaluation is not practiced. As a result, the number of families producing high BLUP values will increase. Results from family evaluation studies in Australia (Kimbeng and Cox, 2003) shows that approximately 40% of families within their breeding programmes produce high BLUP values. This high proportion of high trait value families is a result of over 40 years of continuous family evaluation.

The proportion of parents producing progenies with high BLUP values was very low and less than 9%. The low proportion of parents could be a result of the many years of using genetic values to guide parent selection for the breeding programmes. A recent study (Zhou and Mokwele, 2015) has shown that genetic values of a genotype were not always associated with its ability to produce progenies with high BLUP values. Therefore, the consistent selection of parents using genetic values may result in very little improvement in parent populations and in most cases a dilution effect where a large number of low value parents are used. This scenario could also be associated with the low proportion of families producing progenies with high BLUP values observed in this study. Parents that produce progenies with high BLUP values are categorised as high breeding value parents. Continuous analysis and testing for high breeding values is expected to increase the identification of parents producing high BLUP

values while those producing low BLUP values can be discarded. Over time, the proportion of high breeding value parents will increase which will further increase the proportion of families producing high BLUP values as has been realised in Australia (Kimbeng and Cox, 2003).

BLUP analysis is a statistical method suitable for analysing highly unbalanced data. Family evaluation data, which are also used for parent evaluation in sugarcane breeding, are made up of highly unbalanced data. The unbalanced data emanates from variable seed set at crossing, variable germination when sowing seed as well as variable seedling survival after germination. Sugarcane germinates as tiny seedlings. As a result, in most early stage plant breeding trials, a variable number of genotypes are planted from a cross, resulting in a variable number of observations for data collected from plots. A robust method, such as BLUP analysis, provides efficient analysis of the data. BLUP analysis can improve estimation of family values compared to analysis of variance (Chang and Milligan, 1992).

ERC %cane, Pol %cane and Brix %cane (or sucrose related traits) produced similar trends for family and parent evaluation, indicating similarity of the traits with regards to the families and parents that produced significant positive BLUP values. Sampling for one trait, such as Brix %cane that can be measured easily in field using a hand held refractometer, may cut the cost of evaluating for sucrose related traits among families and parents in early stages of sugarcane breeding. Brix %cane, measured using a hand held refractometer, can be done for each individual genotype providing more data to estimate within family variability.

Parents 94W1043, 96F0701, 97E0474, 03T3487, 96W0246, 99F2694, 02T0005, 03T2791, 03U1293, 05T2497, 05T2658, 92E0901, 92W0511, 94W0947 and 01S1672 produced progenies with significantly higher BLUP values for sucrose related traits (ERC %cane, Pol %cane and Brix %cane). Parents 89E0822, 95W1786, 97E0474, 91W1462, 97E0474, 98G0115, 91W1482, 06T3608 and 00U1422 produced progenies with significantly higher BLUP values for fibre %cane and DM %cane. These parents could be used to improve fibre content that is associated with *E. saccharina* resistance since previous studies by Nuss (1991) and Zhou and Lichakane (2012) have indicated that a high fibre content is associated with lower levels of damage by *E. saccharina*. However, by crossing parents that have high contents of sucrose traits with parents that have high fibre content and biomass could be explored to create progenies with both high sucrose and optimum resistance to Eldana borer.

5.6 Conclusions

The highly significant family variances indicated the potential to identify and select families that produced significantly higher trait values compared to the trial mean. The significant parental effects (female and male) indicated the effectiveness of estimating parent breeding values using family evaluation data. The highly significant parent (female and male) variances also highlighted the potential to select for parents with high BLUP values for crossing. Results further indicated the potential to identify and discard families and parents of low breeding value, thereby improving the efficiency of family selection. Families producing significantly higher BLUP values have been identified that could be used more frequently to build superior populations. The low proportion of families and parents that produced high BLUP values highlighted the need to use breeding values to improve the identification of parents producing progenies with higher trait values.

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Chapter 6

General discussion, conclusions and recommendations

6.1 General discussion

Family evaluation and selection have been found to produce larger selection gains compared to individual genotype selection. Early research (Kimbeng et al., 2000; Kimbeng and Cox, 2003) has showed that families can be replicated, providing more accurate estimates of genetic values compared to individual genotypes. After family evaluation, superior families are identified and planted to larger numbers. Individual genotype selection is focused on families that produce higher BLUP values. Previous studies have indicated the potential to obtain larger broad-sense heritability and in turn selection gains for cane yield, *E. saccharina* and quality traits (Zhou and Lichakane, 2012; Zhou et al., 2013; Zhou, 2014; Zhou and Mokwele, 2015). Zhou and Lichakane (2012) showed the benefits of family evaluation for sucrose content. However, efforts are still needed to increase selection efficiency and gains from selection for quality traits. The aim of this study was to explore family evaluation at early stages of the breeding programme. The specific objectives were: 1) optimizing family evaluation for sucrose content in sugarcane breeding (Chapter 3), determining family breeding parameters and phenotypic correlations among quality traits in sugarcane breeding populations (Chapter 4) and, 3) using BLUP to evaluate parent and family performance for quality traits using data from the coastal short cycle mini-line breeding programme (Chapter 5).

The study demonstrated significant genetic variance for both families and individual genotypes. Families had larger genetic variances compared to individual genotypes across all populations, indicating higher genetic variability associated with families that could be exploited during selection. Families produced higher broad-sense heritability and predicted selection gains. Results indicated that Brix %cane would benefit from family evaluation in early stages of the breeding programme.

At SASRI, a sample size of 20 genotypes and three replications is used to estimate breeding parameters. However, results from this study showed that a sample size of 10 genotypes and four replications would provide adequate discriminating ability to estimate breeding parameters, produce higher broad-sense heritability and reduce costs of sampling compared to 20 genotypes and three replications. Using a hand held refractometer to determine Brix %cane,

instead of full quality analysis, would reduce costs associated with transport and mill room analysis and aid in quantifying within family genetic variability.

The significant family variances for all quality traits highlighted the existence of genetic variability and potential for identifying and selecting superior families. Increased genetic variability observed in later populations highlighted the potential for genetic improvement with advancing populations. The study revealed that traits such as ERC %cane, Pol %cane, Brix %cane and DM %cane had high broad-sense heritability, highlighting the effectiveness of family evaluation for these traits. Sucrose related traits had high broad-sense heritability in later populations, indicating improved selection precision for these traits.

The study indicated significant positive correlations among sucrose related traits (ERC %cane, Pol %cane and Brix %cane). Brix %cane, measured using a hand held refractometer which provide quick measurements and save costs associated with transport and mill room analysis of quality traits, can be used to predict ERC %cane and Pol %cane. Brix %cane could also be used to provide insight into within family variability. However, sucrose related traits were significantly and negatively correlated with fibre %cane, indicating the difficulty of breeding for high sucrose content with low Eldana damage. Fibre %cane has been associated with lower Eldana damage thus there is a need to determine optimum BLUP values to achieve high sucrose content with low Eldana damage.

The significant family genetic variances indicated the existence of genetic differences among populations which highlighted the potential for improving genetic gains by selecting families with progenies that had high BLUP values for quality traits. The study revealed families that produced higher BLUP values across traits would be ideal for enhancing breeding for multiple traits. However, a low proportion of families producing high BLUP values (< 10 %) was observed, which indicated the need to improve selection precision of family evaluation. With family evaluation, families producing lower BLUP values would de identified and discarded early. Breeding efforts can be focused on families with high BLUP values which can be planted more frequently, thus the number of families producing high BLUP values would increase.

Results showed highly significant female and male variances, indicating genetic differences among populations derived from different female and male parents cross combinations. The significant parental variances also highlighted the effectiveness of parent selection for quality

traits. Results also suggested the existence of additive effects, which indicate that breeding values of parents can be quantified for use in parent selection during crossing for quality traits. There were parents that had progenies with high BLUP values for quality traits, suggesting these parents can be selected to potentially produce families with high BLUP values after crossing. The low proportion of parents producing progenies with high BLUP values could be due to the use of genetic values to guide parent selection. The low proportion of parents with high BLUP values could also be associated with the low proportion of families with high BLUP values. Results suggested that frequent testing of breeding values would increase the identification and selection of parents which produce progenies with high BLUP values. Parents and families producing low BLUP values can be identified and discarded early in the breeding programme, thus increasing the number of parents producing high BLUP values.

6.2 Conclusions

Family evaluation proved to be superior to individual genotype evaluation, producing significantly larger family genetic variances, higher broad-sense heritability and predicted selection gains. Selecting individual genotypes from selected superior families increases the probability of selecting elite genotypes in later stages, thus enhancing the efficiency of family evaluation and selection. A sample size of 10 genotypes and four replications could potentially save time and costs that are associated with data collection and transport. Further, change in the sample size could improve the efficiency of allocating available resources for sugarcane breeding programmes. The significant variance, high broad-sense heritability and predicted selection gains suggested that ERC %cane, Pol %cane and Brix %cane are suitable for family evaluation. The significantly positive high correlation coefficients highlighted that either one trait can be used to predict the others. The study revealed that a selection strategy based on Brix %cane, measured with a hand held refractometer, could result in the improvement of sucrose content. Using a hand held refractometer to predict sucrose content would also provide data to quantify within family genetic variation. The negative correlation among sucrose related traits and fibre %cane highlighted the need to determine optimum BLUP values for sucrose content and low Eldana damage. The significant family and parent genetic variances indicated the potential to identify and discard progenies with low BLUP values at early stages. The efficiency of family selection could be improved by identifying and planting families with high BLUP values more frequently. The study suggested that the use of breeding values would improve the identification of parents producing higher BLUP values. Parents producing progenies with high BLUP values could be used to build families with progenies with high BLUP values.

6.3 Recommendations

1. The study showed the effectiveness of family evaluation over individual genotype evaluation for the irrigated, sandy soils and coastal short cycle average potential mini-line breeding programmes. Further studies should focus on investigating the magnitudes of benefits of family evaluation across all SASRI breeding programmes.

2. This study revealed that a hand held refractometer Brix %cane could potentially be used to estimate sucrose content. Further studies should quantify within family genetic variability to evaluate potential genetic gains that could be achieved from family selection.

3. Phenotypic correlations showed a negative association among sucrose related traits and fibre %cane. Further studies should determine optimum BLUP values between the two traits to achieve high sucrose content with low Eldana damage.

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