

**GENOTYPE BY ENVIRONMENT INTERACTION AND RESOURCE
OPTIMIZATION IN SUGARCANE VARIETY EVALUATION IN
SWAZILAND**

BY

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DECLARATION

I hereby declare that the information contained in the following dissertation is the result of my own research efforts, unless otherwise stated. I further cede copyright of the thesis in favour of the University of the Free State.

Signed.....

Njabulo Dlamini

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CHAPTER 1

GENERAL INTRODUCTION

Sugarcane (*Saccharum* sp.) is one of the most important cash crops in Swaziland, and it is the main source of products such as sugar, ethanol and fertilizer. In addition, the bagasse residue from the mills has also gained importance in the co-generation of electricity (Da Silveira *et al.*, 2013). The sugar industry plays a key strategic role in the Swaziland economy, where it directly contributes 10% to national output, 35% to private sector wage employment and 11% to national wage employment (Crawford, 2014). The area under sugarcane cultivation during 2012/13 increased by 2 386 ha year-on-year to an all-time high of 57 262 ha, and industry leaders projected this figure to reach 65 139 ha in 2016 (Wade, 2014). The high demand of sugarcane products has provided significant impetus to the expansion of sugarcane cultivation in recent years (Da Silveira *et al.*, 2013). Year-on-year, cane yields increased from 5 774 344 tons in 2014 to 5 836 899 tons in 2015.

One of the most important components of a thriving sugar industry is the availability of high yielding, adaptable and stable sugarcane varieties. In South Africa, the development of improved varieties has been the major factor in sustaining a competitive sugar industry (Parfitt, 2005). According to Lyne and Clowes (2013), varieties are the main drivers of production (yield of sugar, fibre, ethanol etc.), risk and opportunity. The Swaziland sugar industry (SSI) does not have a sugarcane breeding programme owing to its relatively small size. According to Dlamini (2014), another contributing factor is the proximity of the SSI to South Africa as it allows the utilisation of facilities and expertise for sugarcane breeding established at the South African Sugarcane Research Institute (SASRI) using bilateral agreements. Since the late 1980s, a major function of the Technical Services of the Swaziland Sugar Association (SSA) has been to import newly released smut (*Ustilago scitaminea*) resistant irrigated sugarcane varieties (prefixed N-) from SASRI and evaluate their performance in the Swaziland Lowveld (Butler, 2001). In addition, to broaden and diversify the industry's variety base, SSA imported eight Mauritian varieties in year 2001 for testing in Swaziland. The importation of these varieties was administered under a bilateral agreement that exists between SSA and the Mauritian Sugar Industry Research Institute (MSIRI).

Due to the impact of genotype by environment interaction (GEI), all sugarcane varieties imported into Swaziland undergo a rigorous performance evaluation prior to release to growers.

In an ongoing evaluation programme, SSA imported varieties are planted in a series of replicated field trials, representing potential combinations of soil type and season of harvest as fully as possible (Butler, 2001). This is particularly necessary because conditions under which varieties are selected elsewhere do not match those in Swaziland (Clowes, 2001). The effects of specific soil types and climatic factors (harvesting season) on sugarcane growth and yields in the industry are extensive and well documented (Ramburan, 2012). According to Ong'injo and Olweny (2009) best-yielding sugarcane varieties are identified by cultivating them in different environments. The results from sugarcane evaluation trials are used to predict the likely performance of varieties under comparable commercial conditions (Ramburan, 2008). Sugarcane varieties imported into Swaziland are assessed on the following characteristics before they can be approved and released to industry stakeholders: i) sucrose yield must compare favourably with that of standard varieties; ii) yield performance must be sustainable for at least five crops (plant plus four ratoons); iii) resistance to sugarcane smut disease (*Ustilago scitaminea*) and other major pests and diseases; and iv) acceptable general agronomic and milling qualities.

GEI is said to occur when two or more genotypes are compared across different environments and their relative performance (responses to the environment) are found to differ. That is, one variety may have the highest performance in one environment but perform poorly in others (Acquaah, 2007). This complicates breeders' efforts in selecting, releasing and recommending a superior genotype across different environments. However, estimating the nature and effect of GEI assists in informing breeding programmes (Tarakanovas and Ruzgas, 2006), and this may be primarily achieved by conducting multi-environment trials (METs). METs play an important role in plant breeding and agronomic research, and data from these trials have three main objectives: a) to accurately estimate and predict commercial yield based on limited experimental data; b) to determine yield stability and the pattern of response of genotypes across environments; and c) to provide reliable guidance for selecting the best genotypes or agronomic treatments for planting in future years and at new sites (Crossa, 1990).

Several statistical methods have been proposed and are being used to analyse GEI and phenotypic stability in many plant breeding and variety evaluation programmes. MET data analysis techniques conventionally used in large-scale plant breeding studies, which have historically been empirical in nature, are becoming increasingly analytical due to the availability and adaptation of statistical techniques that allow for interpretation of GEI patterns

(Ramburan, 2011). At the beginning of this study, there was no record of any study that was undertaken to investigate the nature of GEI and stability analysis in the variety testing sites of the SSI, yet such studies are successfully carried out elsewhere using the contemporary GEI analytical techniques. This is in spite of the massive MET data that have been accumulated over the years. Crossa (1990) reported that although many countries conduct extensive trials, little attention has been devoted to the most effective analyses of the data generated. Little or no emphasis is placed on interaction of the varieties with the target environments, which is largely unpredictable (Rakshit *et al.*, 2012).

The test sites in Swaziland are located within commercial farms chosen based on expert knowledge of geographic, soil and climatic variability. These sites are meant to represent the general growing conditions of the respective areas at which they are located. Therefore there was a need to carry out a formalized study to validate such a decision. At the inception of this project, there was insufficient documentation to justify the quantities of resources such as test sites, trial replicates/blocks and crop cycles/ratoons used in the SSA variety evaluation programme (VEP). Yet in other industries, the GEI statistical techniques have been fully exploited to identify the optimal combinations of resources required to efficiently and effectively test the performance of sugarcane varieties. Conducting a similar exercise in the SSA VEP could ease the increasing budgetary constraints associated with such projects.

There has been a growing concern among Swaziland sugarcane growers that the VEP is lengthy as it takes not less than six years for a variety already released in South Africa to become available to them; the bone of contention being the delayed benefit from genetic gains already enjoyed by their South African counterparts. This has necessitated SSA to investigate possible practices that may bridge this gap, one of these being to investigate if there are similar testing sites between the two industries. If similar sites were to be identified, then SSA may utilize information from the SASRI test sites for variety recommendations, thus optimising resource use and maximize benefits obtainable from the research cooperation agreement that exists between the two industries. Redshaw *et al.* (2005) lamented that despite the geographical proximity, SSA and SASRI since the early 1980s independently evaluate sugarcane varieties that are common to both industries across a range of sites to provide recommendations to growers for different agro-climatic conditions and management practices.

The principal aim of this study was to assess the GEI in the sugarcane VEP of SSA in order to optimise future testing procedures. This involved an investigation of opportunities to optimise the use of available resources for more effective and efficient sugarcane variety evaluation.

The specific objectives of the study were:

1. To evaluate the adaptability and phenotypic stability of imported sugarcane genotypes in Swaziland.
2. To determine the optimum combination of locations, replications and crop-years necessary to provide an adequate level of discrimination among genotypes within the SSA VEP.
3. To undertake a combined data analysis of irrigated sugarcane variety trials in Swaziland and South Africa.

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CHAPTER 2

LITERATURE REVIEW

2.1 The sugarcane crop and its uses

Sugarcane is a tall growing, monocotyledonous crop plant, belonging to the grass family (*Poaceae*) that is cultivated in the tropical and subtropical regions of the world, primarily for its ability to store high concentrations of sucrose, or sugar, in the internodes of the stem. It is highly adapted to a wide range of tropical and subtropical climates, soils and cultural conditions and is propagated in over 100 countries situated between 37⁰ N in southern Spain, to 31⁰ S in KwaZulu-Natal in South Africa (Meyer and Clowes, 2013). Modern sugarcane varieties cultivated for sugar production are complex interspecific hybrids (*Saccharum* spp.) that are products of intensive selective breeding of species within the *Saccharum* genus primarily involving crosses between the species *Saccharum officinarum* L. (known as noble cane) and *S. spontaneum* L. (Cox *et al.*, 2000). Daniels and Roach (1987); Sreenivasan *et al.* (1987) and Irvine (1999) indicated possible contributions from *S. robustum*, *S. sinense*, *S. barberi*, and related grass genera such as *Miscanthus*, *Narenga*, and *Erianthus* to these modern varieties.

Sugarcane is vegetatively propagated, usually from setts (pieces of stalk which can be planted as single budded or two to four budded setts) or as a full stalk which is then cut into setts in the planting furrow. In Swaziland, the planting row spacing normally ranges from 1.5 to 1.9 m, the wider spacing being suited to infield mechanical trafficking. The buds on planted setts, or on the plant bases remaining after harvest, germinate after planting (or after harvest of the preceding crop) and germination is determined by climatic conditions and the variety involved. The regrowth after harvesting is termed ratooning, and the number of ratoons obtained from crop-years also depends on genotypic and environmental factors (Ramburan, 2012). Sugarcane farming is capital intensive and ratooning the crop for longer ensures greater profitability (Clowes and Breakwell, 1998).

Another important concept in sugarcane growing (that is often confused with ratooning) is ratoonability or ratooning ability. Ratooning ability is defined as the ability of a variety to sustain sucrose production with each successive crop (Chapman *et al.*, 1992; Ramburan, 2009). Milligan *et al.* (1995) defined ratooning ability in absolute and relative terms. In absolute terms, a good ratooning variety is one that produces high ratoon crop yields and/or several

economically rewarding ratoon crops. Relative to other varieties, a good ratooning variety is one whose ratoon crop yields are a higher, or similar, percentage of its plant cane or a younger crop's yields (Milligan *et al.*, 1995). Ratooning ability depends on the genotype, environment and crop management (Chapman *et al.*, 1992). The inherent potential of a variety to give better yields in plant and ratoon crops is of paramount importance for sustaining high productivity (Arain *et al.*, 2011).

Sugarcane is primarily grown as a source of sugar. According to Ming *et al.* (2006) and Malik (2010) about 75% of the world's sugar supply is from sugarcane, and the other 25% is from sugar beet (*Beta vulgaris* L., Chenopodiaceae). Besides the production of sugar from the sugarcane plant, there are several valuable by-products that are derived after the extraction of sucrose such as bagasse and molasses. Bagasse is the fibrous portion of sugarcane that remains after the juice has been removed. Swaziland sugar mills use the bagasse to generate power for the mill, estates and the excess is directed to the national electricity grid. The ash produced is mixed with other impurities (mud) left after the sugarcane juice is clarified and fine bagasse known as bagacillo to produce filter cake used as fertilizer on cane fields. In other countries, the bagasse is also used for papermaking as well as a livestock feed. With the advent of life cycle analysis and rising demand of energy, the cane residues known as trash (leaves and tops) have also proven to be highly valuable as they offer similar benefits as bagasse. Molasses is the thick syrupy residue left after the sucrose has been removed from the clarified sugar juice (syrup). It is utilized for the production of alcohol and/or fermented to produce fertilizer (vinasse or condensed molasses solubles) for cane fields.

2.2 Sugarcane breeding

The differential response of sugarcane varieties with respect to environment have compelled most sugar industries to establish their own breeding programmes to meet their specific industry requirements (Ramdoyal *et al.*, 2003). There are approximately 23 sugarcane breeding stations in the world (Rossi, 2002). Most maintain a large number of clones selected from local breeding programmes, clones imported from other stations, and clones of basic species imported from world germplasm collections (Ming *et al.*, 2006). For decades, sugarcane varieties have been produced through conventional means. The traditional sugarcane breeding methods consist of three steps: (i) parental selection from a source population, (ii) hybridization

using bi-parental crosses and poly-crosses, and (iii) progeny selection at several stages during clonal propagation.

Natural flowering in sugarcane only occurs under specific climatic conditions and is more widespread in most tropical areas (Ramburan, 2012). In subtropical and temperate areas, natural flowering is highly variable and pollen infertility in flowering varieties pose a great challenge for plant breeders. To overcome this challenge, the breeding stations situated in subtropical and temperate zones, such as SASRI, Sugar Research Australia (SRA - Australia), United States Department of Agriculture – Agricultural Research Service (USDA-ARS), Canal Point (Florida) and Houma (Louisiana) provide photoperiodic facilities to induce flowering in shy and non-flowering varieties (Nuss and Berding, 1999).

The breeding programme cycle starts with parent selection (Zhou, 2013). Selection of parents from a source population for crossing is based on the performance data of each parent and its progeny (Ming *et al.*, 2006). Most breeding stations evaluate progeny performance on the basis of a selection rate. If the progeny performs better than a standard, the progeny's parent will be identified as a proven parent (Ming *et al.*, 2006). The selection of sex in parents is based on the extent of anther dehiscence and pollen fertility (Malik, 2010). At SASRI, genotypes that produce large quantities of viable pollen (>30%) are classified as males, otherwise they are females (Zhou, 2013). Either bi-parental or poly-crosses can be used to generate segregating populations. The main advantage of bi-parental crosses is that the male and female parents are both known, whereas in poly-crosses, the exact male parent of the progeny is not readily known because several pollen sources are placed together to interbreed with only one female (Ramdoyal *et al.*, 2003; Scortecci *et al.*, 2012).

Hybridization (crossing) is the main procedure used in sugarcane to generate new genetic recombination events to further perform selection of superior genotypes, focusing on sugar, ethanol or biomass production (Scortecci *et al.*, 2012). Crossing procedures and techniques vary among breeding stations. Basic pollination procedures consist of harvesting tasseling stalks from field plots as flower anthesis begins, then moving the harvested stalks to a crossing shelter where they are placed in a weak acid solution for prolonging flower life to enable making either bi-parental crosses or poly-crosses (Ming *et al.*, 2006). At SASRI, during crossing at the glasshouse, the minimum temperature is kept at 20°C, humidity levels are maintained above 70% to ensure good pollen viability, pollen survival and seed set. Fourteen

days after crossing, when shedding of pollen ceases, the males are discarded and the female flower is left to ripen (Zhou, 2013).

Procedures for selecting varieties vary among breeding stations, depending primarily on the crop cycle length and number of ratoon cycles practiced by the local cane growers (Mamet and Domaingue, 1999). A selection cycle in sugarcane usually involves a sequence of about four to six stages (Skinner *et al.*, 1987) and typically takes about 12 to 15 years to complete. The first stage is the only stage, after hybridization, to be planted with true seed. Subsequent stages are planted using vegetative propagation, and progressively fewer clones are selected and evaluated in the more advanced stages. During this 12 to 15 year period, no opportunities exist for sexual recombination or the creation of new genetic variation that the breeder can exploit (Kimbeng and Cox, 2003). Selection priorities in the SASRI selection program include recoverable value (RV), yielding ability and pest and disease resistance (Ramburan, 2012).

2.3 The Swaziland sugar industry

The history of the Swaziland sugar industry dates back to the mid-1950's when commercial sugarcane growing resulted in the establishment of a small mill at Big Bend (in the south-eastern part of the country). This was replaced by a larger mill in 1960, the same year which also saw the establishment of a second mill at Mhlume in the northern lowveld. At that time, total sugar production was approximately 57 000 tons. In 1980, a third mill was established at Simunye (approximately 30 km south of Mhlume). The area under sugarcane cultivation increased substantially and the total sugar production grew to above 300 000 tons. By the end of the 2014/15 season, total sugar production stood at 695 410 tons. The mills at Big Bend, Mhlume and Simunye have developed sugarcane growing estates to ensure adequate throughput. By 2015, these mills owned 8 513 ha, 8 508 ha, 10 720 ha, respectively. In addition, growers individually or in association, have formed companies that produce and sell sugarcane to these millers, and payment is based on tons of sucrose delivered. Figure 2.1 shows the sugarcane growing areas of Swaziland.

In 1967, when the sugar industry act was passed into law, the Swaziland Sugar Association (SSA) was formed. SSA is responsible for providing the services necessary for the regulation and general development of the Swazi sugar industry as well as marketing of all the sugar and molasses produced in the country. SSA provides support services to the entire industry's value

chain which includes agricultural research and extension, cane testing, warehousing and distribution, marketing and policy advocacy. Concerning the research component, SSA through its Technical Services department import and evaluate the performance of sugarcane varieties at different locations (test sites), harvesting times and across multiple crop-years prior to release for commercial cultivation. These locations are within the three millers' sugarcane estates and represent the major sugarcane growing areas of the industry with their diverse soil types.

A majority of the sugarcane varieties grown within the industry are imported from SASRI, especially varieties tested and selected for the South African irrigated region. The sugarcane growing region of Swaziland is classified as semi-arid with hot summers and cool winters. Rain falls mostly during the late summer months of December to March and average annual rainfall varies between 675 mm in the southern areas of the country and 800 mm in the northern areas. The atmosphere typically extracts between 1500 and 1700 mm of water from the crop, thus irrigation is required to make up the shortfall between evapotranspiration and effective rainfall to grow an economically viable crop. Hence, sugarcane grown in the country is 100% irrigated.

The testing locations of the industry's VEP are classified into good draining, moderately draining and poor draining soils, represented by the Simunye, Big Bend and Mhlume sites, respectively. Harvesting times are classified into early (April to June), mid- (July to September) and late (October to December) seasons, and the harvesting period proceeds for nine months (April to December). Thus, sugarcane varieties in the industry are recommended according to their preferred soil type and harvesting season. Ideally, the cutting cycle is 12 months when the sugarcane crop is fully matured and sucrose accumulation within the stalks reaches a peak. The sugarcane production cycle (crop-years), depending on variety, soil type and biotic factors, typically lasts to at least five crops. But under ideal environmental conditions, management practices and variety choice, the cycle can be extended to over 30 crops, as is the case with some growers in the country (Meyer and Clowes, 2013).

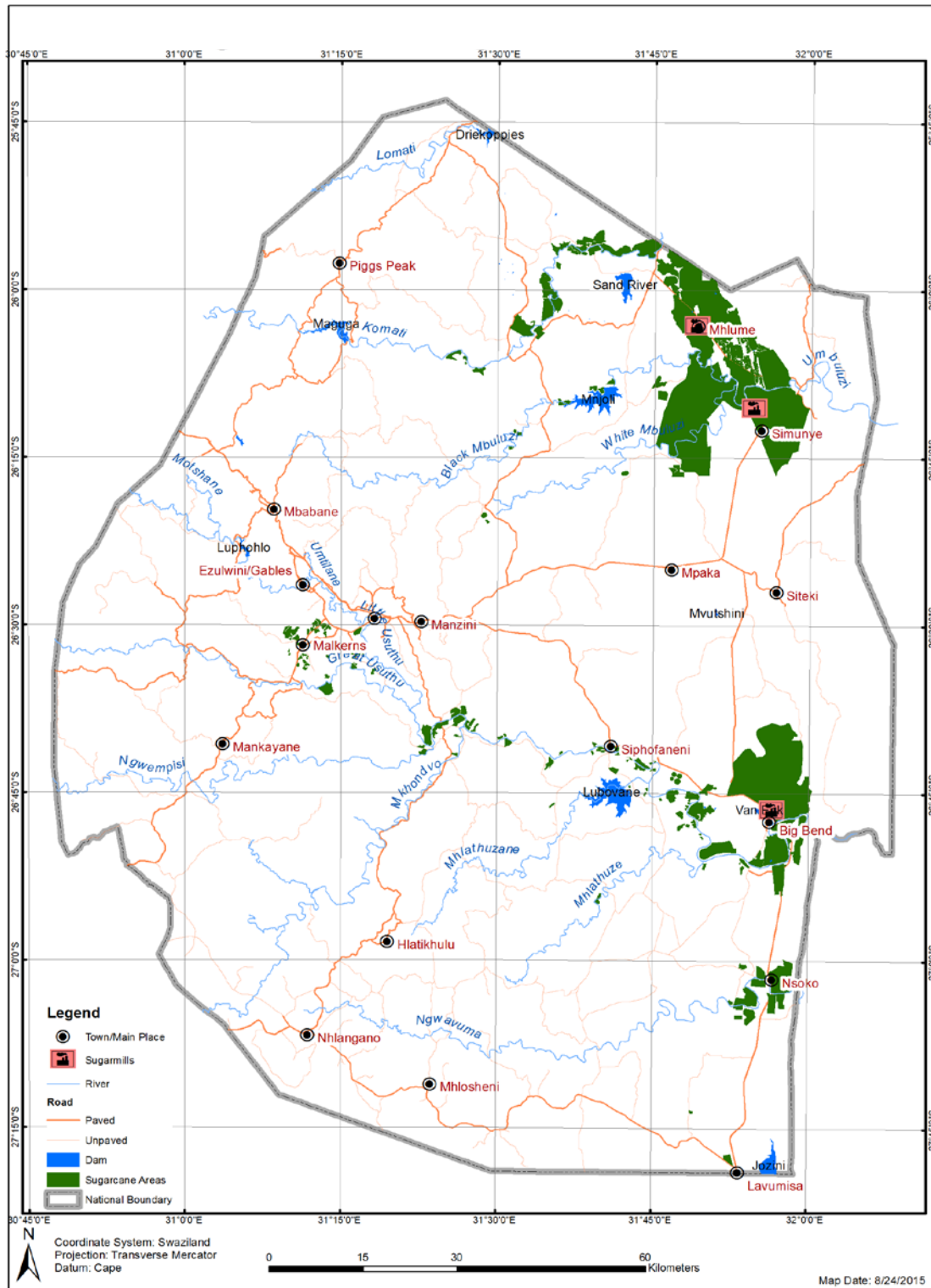


Figure 2.1: Sugarcane growing areas of Swaziland

2.4 Genotype by environment interaction

A genotype is defined as an individual's genetic makeup (Fan *et al.*, 2007). The environment, on the other hand, is defined as all non-genetic factors that influence the expression of the

genotype traits (Basford and Cooper, 1998). An environment is characterized by the combined effects of climatic, soil, management and biotic (pest and disease) factors (Ramburan *et al.*, 2010). A phenotype is the observable manifestation of a specific genotype (Acquaah, 2007; Schlegel, 2010). Phenotypic expression reflects the combined effects of the genetic and non-genetic factors on the development of the phenotype. It is widely accepted that the effects of genotype and environment on phenotypic expression are never independent (Comstock and Moll, 1963). This inconsistent genotypic responses to changes within or between environments is generally known as genotype by environment interaction (GEI). GEI involves changes in rank or order for phenotypes between environments and changes in the absolute and relative magnitude of the genetic, environmental and phenotypic variances between environments (Bowman, 1972). The importance of GEI in sugarcane selection is widely recognized (Milligan *et al.*, 1990).

The change in ranks across environments makes it difficult for breeders to determine the true genetic value of prospective genotypes, and to select among them (Kimbeng *et al.*, 2009; Setimela *et al.*, 2010). This retards genetic gains from selection and limits commercial production when varieties are incorrectly sited (Ramburan *et al.*, 2010). GEI lowers the correlation between phenotypic and genotypic values (Comstock and Moll, 1963; Kang and Gorman, 1989), thereby reducing progress from testing and selection (Rashidi *et al.*, 2013). This implies that, where GEI is large, the performance of a genotype in one environment cannot be used to predict its performance elsewhere. GEI is a problem for any kind of breeding programme, be it during selection or in the recommendations of varieties (Ngeve and Bouwkamp, 1993; Guerra *et al.*, 2009; Hassanpanah, 2009). As a result, the major task of a breeder in selecting consistently high performing varieties across a range of environments is often inefficient unless the effects of GEI are considered.

The magnitude and nature of GEI determines the features of a selection and testing programme (Rashidi *et al.*, 2013). Where conditions between targeted environments differ significantly, the magnitude of GEI is likely to be high. A larger GEI does not only make the prediction of genotype performance across environments difficult, but also reduces the heritability and the precision of the selection across the environments (Rodríguez *et al.*, 2010; Kamutando *et al.*, 2013; Khan *et al.*, 2013). However, environments that interact similarly with genotypes induce corresponding responses in plants, and lead to strong genetic correlations (Malosetti *et al.*, 2013). The challenge for breeders then is to accurately group the target environments

according to their similarities to effectively exploit GEI and derive maximum benefit from genetic gains.

To manage, most plant breeders feel that they should exploit rather than ignore the potential for yield increases that reside in GEI (Parfitt, 2000). GEIs are important sources of variation in any crop and the term stability is used to characterize a genotype which shows a relatively constant yield, independent of changing environmental conditions (Tiawari *et al.*, 2011). Where GEI is significantly large, breeders face a choice between selecting genotypes with high yield across all trial environments (broadly adapted) versus those that perform well in a subset of trials but perhaps poorly in others (specifically adapted) (Parfitt and Thomas, 2001). These choices have proven to be the most effective strategies that breeders have adopted in managing GEI. The terms stability and adaptability are discussed later on in this chapter.

To evaluate the performance of potential commercial varieties under a range of different conditions breeders conduct multi-environment trials (METs) (Ramburan and Zhou, 2011). Plant variety trials are routinely conducted to compare multiple genotypes in multiple environments (years and location) for multiple traits (Yan and Tinker, 2006). METs facilitate quantification of the genotype, environment and GEI effects (Farshadfar *et al.*, 2013), and they play a significant role in plant breeding and agronomic research (Ma'ali, 2008). Ferreira *et al.* (2006) recommended that in the last phase of plant breeding programmes, candidate varieties with market potential should be evaluated under a range of conditions similar to the real conditions that they will experience when released for commercial propagation. The results from METs are used to predict the likely performance of varieties under comparable commercial conditions (Ramburan, 2008).

In perennial crops like sugarcane, the different conditions as defined by locations, seasons (harvesting times) and crop-year/cycles are considered individually or in combination as environmental conditions. In such cases, the components of GEI such as genotype x location (GxL), genotype x season (GxS), genotype x crop-year (GxC), genotype x location x season (GxLxS), genotype x location x crop-year (GxLxC) and genotype x season x crop-year (GxSxC) are used to evaluate genotype performance. GxL refers to the interaction of genotype with locations and evaluates the performance of genotypes across locations; GxS refers to the interaction of genotype with seasons and evaluates the performance of genotypes across

seasons; GxC refers to the interaction of genotype with crop-years and evaluates the genotypes' performance across ratooning cycles; GxLxS evaluates combined effect of location and season on genotype performance; GxLxC estimates ratooning ability of genotypes as influenced by locations; and GxSxC evaluates ratooning ability of genotypes as influenced by seasons (Zhou *et al.*, 2012).

Some scenarios that can occur when comparing the performance of pairs of genotypes across environments are presented in Figure 2.2. Figure 2.2A shows the case where there is no GEI, the genotype and the environment behave additively and the reaction norms are parallel. The other graphs show different situations in which GEI occurs: divergence (Figure 2.2B), convergence (Figure 2.2C), and the most critical one, crossover interaction (Figure 2.2D).

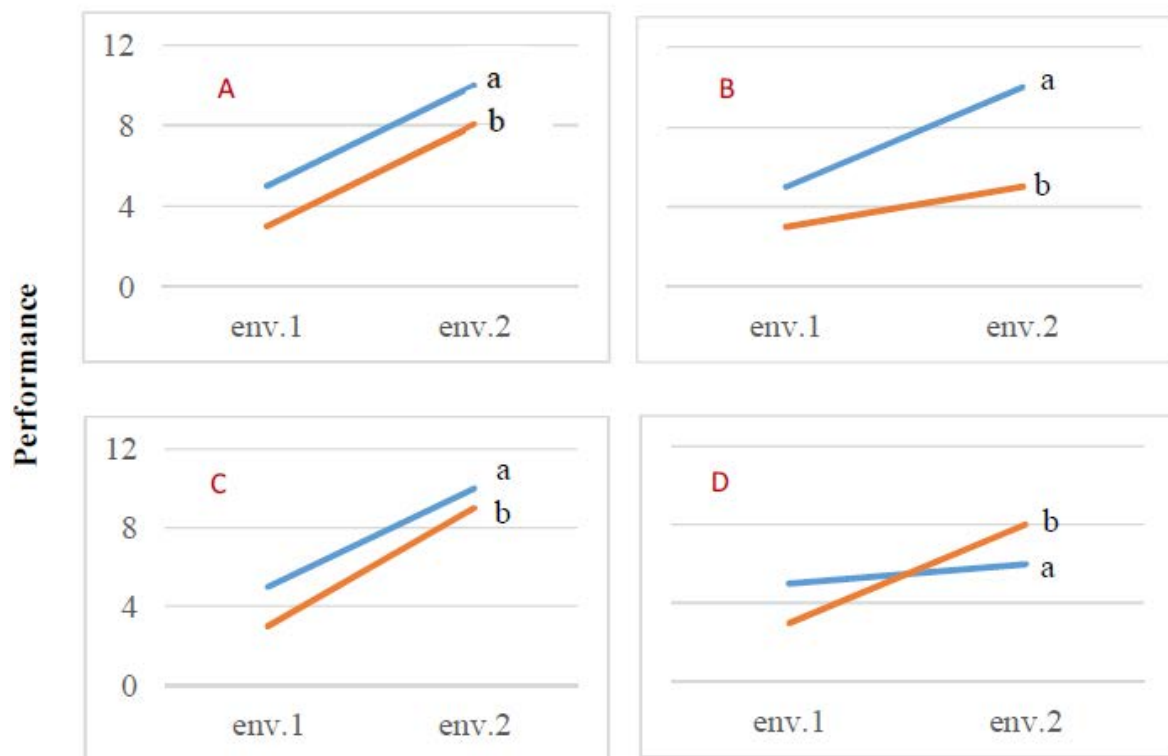


Figure 2.2: Genotype by environment interaction in terms of changing mean performances across environments: A) additive, B) divergence, C) convergence, and D) crossover interaction

Understanding the causes of non-crossover and crossover GEI can help develop an understanding of the genotypic characteristics that contribute to a superior variety, and environmental factors that can be manipulated to facilitate selection for such varieties. The

change in genotype ranks across environments implies the existence of crossover GEI. Crossover interactions are the most important for breeders as they imply that the choice of the best genotype is determined by the environment (Kamutando *et al.*, 2013; Malosetti *et al.*, 2013). It is the large real crossover-type GEI that invalidates recommending to farmers the variety giving the highest average yield across test environments (Farshadfar *et al.*, 2013).

2.5 Adaptability and phenotypic stability

In most developing countries, where research funds are limited and farmers scarcely use farming inputs such as fertilizers on a regular basis to improve environmental conditions, a good stable variety once developed, should be able to serve many growers in the country (Ngeve and Bouwkamp, 1993). Farmers and scientists aim at identifying a genotype which is superior over a wide range of environmental conditions and also over a number of years (Muungani *et al.*, 2007; Rodrigues *et al.*, 2008). The terms ‘stability’ and ‘adaptability’ refer to consistent high performance of genotypes across diverse sets of environments (Romagosa and Fox, 1993). Yield stability is a measure of the ability of a genotype to produce high and consistent yields over a wide range of environments, seasons and times of planting (Petersen, 1994; Ferreira *et al.*, 2006). It gives a measure of the response of a variety to favourable and unfavourable growing conditions (Zhou *et al.*, 2011).

In general, stable genotypes should perform more or less the same across a wide range of environments (Kamutando *et al.*, 2013). Ideal varieties should be both high yielding and highly stable (Yan and Tinker, 2006; Kumar *et al.*, 2011; Mostafavi *et al.*, 2012). On the other hand, adaptability refers to the variety’s capability to take advantage of environmental variations in a positive way (Scortecci *et al.*, 2012). According to Dabholkar (1999), adaptation is the property of a genotype which permits its survival under selection. In short, an adapted genotype is simply one which survives the selection procedure of a breeder, that is, one which performs comparatively better than the standard.

Productivity of a genotype in favourable environments does not indicate its adaptability and stability, whereas performance of a genotype in diverse environments truly evaluates its inherent potential for adaptation (Pandey *et al.*, 1981). This implies that a stable genotype is less sensitive to temporal environmental changes. The analysis of adaptability and stability for yield and quality traits is therefore very important and necessary for the identification and

recommendation of superior genotypes in different environments (Khan *et al.*, 2004; Tiawari *et al.*, 2011). An appropriate stable variety is capable of utilizing resources that are available in high yield environments, while maintaining above average productivity in all other environments (Finlay and Wilkinson, 1963). Productivity stability is shown by some varieties in both predictable and unpredictable environments (Khan, 1981). According to this author, in a predictable environment (that is climatic, soil type, day length and controllable variables such as fertilization, sowing dates and harvesting methods), a high level of GEI is desirable, so as to ensure a maximum yield or financial return. In an unpredictable environment (inter and intra-season fluctuation, fluctuation in quantity and distribution of rainfall and prevailing temperature), a low level of interaction is desirable so as to ensure maximum uniformity of performance over a number of locations or seasons.

It is important for breeders to screen and identify specific genotypes adapted to and/or stable at different environments prior to their release as varieties (Hagos and Abay, 2013; Kulsum *et al.*, 2013). The development of varieties that are adapted to a wide range of diversified environments is actually a major goal of plant breeders in an improvement programme (Dehghani *et al.*, 2006). As indicated earlier, in order to identify the most stable and high yielding genotypes, it is important to conduct METs (Farshadfar *et al.*, 2013). Stability measurement, and testing varieties at multi-locations is very important to ensure that the selected varieties have acceptable performance in variable environments (Mostafavi *et al.*, 2012; Delacy *et al.*, 1996; Yan *et al.*, 2000; Yan and Rajcan, 2002). According to Eberhart and Russell (1966), selecting and recommending genotypes with better stability across a wide range of environments mitigate the GEI effect.

While there can be genotypes that do well across a wide range of conditions (widely adapted genotypes), there are also genotypes that do relatively better than others exclusively under a restricted set of conditions (specifically adapted genotypes) (Kang *et al.*, 2004; Malosetti *et al.*, 2013). This implies that, if a range of varieties is to be tested in contrasting environments, varieties showing wide or specific adaptations should be identified (Chimonyo *et al.*, 2014). This varied performance of varieties in different environments indicates their adaptability to specific regions or over wide areas (Khan *et al.*, 2004). Significant GEI warrants the release of varieties for specific environments where they have a greater adaptation (Campbell and Jones, 2005). In sugarcane, the identification of these specific positive interactions becomes especially important because the renewal of the sugarcane fields usually happens after a long period of

six or seven harvests (years). Thus, when a variety is erroneously recommended, the economic damage may be extended for many years (Da Silveira, 2013). Therefore, knowledge of the magnitude of GEI is important to assess the stability and adaptability of genotypes where they are intended to be introduced (Contreras and Krarup, 2000; Muungani *et al.*, 2007). Consequently, GEI must be either exploited by selecting superior genotypes for each specific target environment or avoided by selecting widely adapted and stable genotypes across wide range of environments (Ceccaralli, 1989).

Irrespective of how a stability parameter is measured, one of the most critical questions is whether it is genetic? If the characteristic measured by the parameter is non-genetic, it is not heritable and thus selection for such parameter is fruitless (Lin and Binns, 1988; Jalata *et al.*, 2011). For a sugarcane breeding programme to be successful it is important to know which traits give the highest estimates of heritability and which are the most repeatable over a number of seasons (O'Reilly *et al.*, 1995). If stability is heritable, the next step in the genetic analysis is identification of the chromosomal location of the genes controlling the character (Farshadfar *et al.*, 2012).

Lin *et al.* (1988); Becker and Léon (1988) and Acquaaah (2007) distinguished between two concepts of phenotypic stability: static and dynamic. Static phenotypic stability (also called biological stability) exists when a genotype maintains its performance independently of variations in the environmental conditions. Static stability is analogous to the biological concept of homeostasis, that is, a stable genotype tends to maintain a constant yield across environments (Acquaaah, 2007). Dynamic stability is when a stable genotype has a yield response in each environment such that it is always parallel to the mean response of the genotypes evaluated in the trial (Acquaaah, 2007). This kind of stability is called agronomic stability (Ferreira *et al.*, 2006). Becker and Léon (1988) clarified that all stability procedures based on quantifying GEI effects belong to the dynamic stability concept.

2.6 Mega-environments

There are two major tasks for researchers working on stability analysis. The first is to determine whether the target region is homogenous or should it be divided into different mega-environments (MGE); and, the second is to select superior varieties for a given MGE (Mostafavi *et al.*, 2012). A MGE may be defined as a portion of a crop species' growing region

with a homogenous environment that causes some genotypes to perform similarly (Gauch and Zobel, 1997). International Maize and Wheat Improvement Center (CIMMYT) defines a MGE as a broad, not necessarily contiguous area, occurring in more than one country and frequently transcontinental, defined by similar biotic and abiotic stresses, cropping system requirements, consumer issues, and for convenience by volume of production (Braun *et al.*, 1996). Investigations of MGEs are a prerequisite for any meaningful variety evaluation and recommendation procedures (Yan and Hunt, 2001), and they are primarily identified through the analyses of MET data (Ramburan and Zhou, 2011).

The primary goal of METs is to allow breeders to select the best-performing genotype(s) for their target regions by assessing the relative performance of genotypes under a variety of locations and environmental conditions (Xu, 2010). A secondary, but important, goal is to develop an understanding of the target region and, in particular, to determine if the target region can be subdivided into different MGEs (Yan *et al.*, 2000; Ramburan and Zhou, 2011). In addition to enabling thorough selections, METs also provide data for estimating broad sense heritability (repeatability) and for studying the extent and pattern of GEI that can provide information on how genotypes respond to different environments (Cooper *et al.*, 1996). Multi-year data are required to confirm if the pattern is repeatable (Yan and Tinker, 2006). If crossover GEI is repeatable across years, then target environments can be divided into MEs and genotypes can be recommended based on METs (Voltas *et al.*, 2002; Yan and Tinker, 2006).

Mostafavi *et al.* (2012) suggested two criteria that are required to detect the existence of different MGEs. First, there should be different winning genotypes in different test locations. Second, the between-group variation should be significantly greater than the within-group variation, a common criterion for clustering. These clusters or subdivisions represent different MGEs. Therefore to optimize growers' yields, dividing the target environment into different MGEs and deploying different genotypes in different MGEs is the best way to utilize GEI in plant breeding (Parfitt, 2000; Yan and Tinker, 2006; Mostafavi *et al.* 2012). Such subdivisions are necessary for the implementation of regional specific breeding strategies, which can ensure the greatest gains from selection, as environmental variance between selection sites is minimized (Ramburan and Zhou, 2011). Although subdivision of a crop production area into different environments implies more resources, it also implies faster progress for plant breeders and higher yields for growers (Gauch and Zobel, 1997). Ramburan and Zhou (2011)

emphasized that the subdivision of the target environments is important for the development of variety recommendation domains.

If the breeding goal is wide adaptation, the best strategy would be to identify several different environments within the region and place a test location in each to select for adaptability (Gauch and Zobel, 1997). The use of a single selection site to identify elite clones or families may limit the gains from selection compared to clones or families evaluated over multiple locations (Parfitt, 2000). In the South African sugar industry, the commissioning of new farms more representative of the major ecological areas placed the plant breeding department in a better position to develop varieties more specifically bred and selected for the different regions (MGEs) (Parfitt and Thomas, 2001). In Australia, separate sugarcane selection programmes are conducted within six different MGEs that are separated by latitude. The regional programmes are used as a basis for allocating resources, to rationalise germplasm exchange, to increase heritability in genetic populations being tested and to improve efficiency of selection (Jackson and McRae, 1998). Where only one MGE exists, it is not essential to have separate breeding programmes for the various environments. The existence of one MGE also shows that crossover interactions could be occurring within a few varieties and thus, selection of stable genotypes is needed (Kamutando *et al.*, 2013).

2.7 Discriminating ability and representativeness of testing sites

Within a single MGE, the objectives of data analysis are twofold: genotype evaluation to identify genotypes with both high performance and high stability, and test environment evaluation to identify test environments that are both informative (discriminating) and representative (Yan and Tinker, 2006). Discriminating ability refers to the ability to detect significant differences between test genotypes and the control (Zhou and Kimbeng, 2010), and it is an important measure of a test environment. The most discriminating environments are good as testing environments for both early generation testing and advanced testing (Kamutando *et al.*, 2013). If the environments are consistently non-discriminating (non-informative) they may be discarded as testing environments as they provide little information (Yan and Tinker, 2006; Muungani *et al.*, 2007).

Another equally important measure of a test environment is its representativeness of the target environment. The success of the plant breeding programme is highly dependent on the

representativeness of the test sites (Ramburan, 2012). A test environment that is representative should be able to provide the same information about genotypes as the target environment. The presence of GEI dictates that breeders sample the appropriate environmental conditions likely to be encountered by the target environments under which the prospective genotypes will eventually be grown (Kimbeng *et al.*, 2009). Thus, an ideal test environment should be more discriminating of the genotypes in terms of the genotypic main effect and more representative of the overall environment (Dehghani *et al.*, 2006; Mostafavi *et al.* 2012). Yan and Tinker (2006) stress that test environments that are both discriminating and representative are good test environments for selecting generally adapted genotypes.

2.8 Measuring genotype by environment interaction

The importance and scope of multi-environment testing of sugarcane varieties is a reflection of a successful breeding programme which, when coupled with a suitable statistical model for GEI analysis, can be extremely helpful in the identification of stable varieties adapted to wider cultivable areas (Kumar *et al.*, 2011). Genotypes adaptable to target environments are selected under an optimum strategy, this strategy is determined by measuring GEI (Annicchiarico, 1997; Khan *et al.*, 2013). This phenomenon of GEI is of primary interest in plant breeding, hence it has resulted in a large body of literature on models and strategies for its analysis (Malosetti *et al.*, 2013).

The GEI has been studied by different researchers, and several methods have been proposed to analyse it, and these include univariate methods such as the Francis and Kanneberg (1978) coefficient of variability, Plaisted and Peterson's (1959) mean variance component for pairwise GEIs, Wricke's (1962) ecovalence, Shukla's (1972) stability variance, Finlay and Wilkinson's (1963) regression coefficient, Perkins and Jinks's (1968) regression coefficient, Eberhart and Russell's (1966) joint regression analysis. Recently, two multivariate analytical techniques (biplot analysis), the AMMI biplot [the statistical model of additive main effect and multiplicative interaction (Gauch, 1988; Zobel *et al.*, 1988)] and the GGE biplot [genotype main effect plus genotype by environment interaction (Yan *et al.*, 2000)] have been introduced and are extensively used to visualize genotype by environment two-way data (Dehghani *et al.*, 2006). This section explains some of these analytical techniques.

2.8.1 Conventional analysis of variance

In GEI studies, first of all, it is necessary to establish if there is an interaction effect present in the MET data; then one has to consider this effect and importance as a subsequent work (Rodrigues *et al.*, 2008). If one considers a trial in which the yield of G genotypes is measured in E environments, each with R replicates, then the classical model for analysing the total yield variation contained in the GER observations is the analysis of variance (ANOVA) (Fisher, 1925). After removing the replicate effects, the GE observations are partitioned into the additive main effects for genotypes and environments, and the non-additive effects due to the GEI (Crossa, 1990). The ANOVA model of the combined data is then expressed as:

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + e_{ij} \quad (1)$$

Where, Y_{ij} is the expected yield of the i^{th} genotype in the j^{th} environment; μ is the grand mean; G_i , E_j , and GE_{ij} represent the effects of the genotype, environment, and the genotype by environment interaction respectively; and e_{ij} is the error term. In this model, the non-additivity interaction implies that the expected value of the i^{th} genotype in the j^{th} environment depends not only on the levels of G and E separately, but also on the way in which G interacts with E (Crossa, 1990).

A combined ANOVA can be used to quantify GEI and describe the main effects (Chimonyo *et al.*, 2014). However, even though ANOVA is effective in partitioning the total sum of squares (SS) into genotype main effect, environment main effect and the GEI, it does not provide insight into the GEI structure (Kumar *et al.*, 2011). ANOVA does not fully explain the interaction between the genotypes and environments (Admassu *et al.*, 2008), thus failing to distinguish varieties that exhibit specific or wide adaptation (Chimonyo *et al.*, 2014). Nonetheless, several researchers have successfully utilised ANOVA to quantify GEI and describe the main effects. According to Gauch and Zobel (1996), in routine METs, the environment (E) accounts for 80% of the total yield variation, while genotype (G) and GEI each account for about 10%. Gauch (2006) and Verma *et al.* (2006) concluded that for yield trials, the most common outcome is that environmental main effects are largest, followed by the interaction effects and then the genotype main effect.

Partitioning of the SS of 15 maize (*Zea mays* L.) genotypes' grain yield planted over four locations across north-western Ethiopia showed contribution of locations to be 68.30% of the

total variation, and 5.15 and 10.65% were contributed due to genotype and GEI, respectively (Anley *et al.*, 2013). A grain yield ANOVA of 13 winter wheat (*Triticum aestivum* L.) genotypes tested in eight environments in Lithuania showed that 77.1% of the total SS was attributable to environmental effects; only 7.1% to genotypic effects and 15.8% to GEI (Tarakanovas and Ruzgas, 2006). While an ANOVA of maize grain yield by Muungani *et al.* (2007) indicated that the environment explained 88.6%, the GEI and genotype effects explained 7.48% and 3.94%, respectively.

Akbarpour *et al.* (2014) working on 20 promising barley (*Hordeum vulgare* L.) varieties grown in 14 environments over two growing seasons found that 79% of the total SS was attributable to environmental effects; only 1% to genotype effects, and 20% to GEI effects. Badu-Apraku *et al.* (2003) studying GEI on 10 maize genotypes learnt that the test environments explained 85% of the total variation in yield while the genotypes and GEI sources of variation explained only 4 and 11% of the total variation, respectively. The results of combined ANOVA for grain yield of 16 field pea (*Pisum sativum* L.) genotypes tested across 12 environments showed that 79.68% of the total SS were attributed to environmental effects, whereas genotypic and GEI effects explained 4.53 and 5.70%, respectively (Fikere *et al.*, 2014). Rashidi *et al.* (2013) working on 20 chickpea (*Cicer arietinum* L.) genotypes tested in eight environments found that 81.62% of the total SS was explained by environmental fluctuations. Only a small portion (6.31%) was attributed to genotypic effects and GEI explained 12.57% of the treatment variation in grain yield. A combined ANOVA on bread wheat showed that grain yields were significantly affected by environment, which explained 81% of the total variation, while genotype and GEI accounted for 7.3% and 11.7%, respectively (Kaya *et al.*, 2006).

The results of ANOVA for cane yield data showed that locations (test sites) were the most important source of variation, accounting for 65.2%, while the GEI and genotypes accounted for 25.8% and 9.0% of the total SS, respectively (Rodríguez *et al.*, 2010). Results of a similar trial conducted by Klomsa-ard *et al.* (2013) indicated that on cane yield, the total SS were 55.97%, 36.03% and 8.00% attributable to environment, genotype and GEI, respectively. Bissessar *et al.* (2001) reported that ANOVA showed significant differences among genotypes and environments for three characters; cane yield, tons sucrose and sucrose content, but a non-significant GEI was realised. While a number of the above results seem to agree with Gauch and Zobel's (1996) assertion that under routine METs, the environment accounts for 80% of the variation, there are exceptions. Yan and Kang (2003) working on wheat-barley disomic

addition lines found that for yield data the environment effect accounted for 21.7% of total SS, while the GEI and genotype accounted for 55.3% and 15.6%. Kumar *et al.* (2011), working on nine sugarcane genotypes in 14 environments learnt that 61.11% of the total SS was attributable to GEI effects, and environments and genotypes accounted for 22.34% and 16.05%, respectively. The large GEI, relative to genotype effect, suggest the possible existence of different MEs with different top-yielding genotypes.

2.8.2 Joint linear regression

The joint linear regression (JLR) is an important model for analysing and interpreting the non-additive structure (interaction) of two-way classification data. This approach has been extensively used in genetics, plant breeding, and agronomy for determining yield stability (Crossa, 1990). When applied on two-way tables obtained from multi-environment trials, JLR aims to determine the stability of the genotypes or agronomic treatments over a wide range of environmental conditions and to interpret the interaction (non-additivity) (Rodrigues *et al.*, 2008). JLR analysis was developed by Yates and Cochran (1938) but slightly different models have since been proposed and popularised by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). The model partitions the GEI into two components i) a component due to linear regression (b_i) and ii) a component due to deviations from regression (d_{ij}) so that equation (1) becomes:

$$Y_{ij} = \mu + G_i + E_j + (b_i E_j + d_{ij}) + e_{ij} \quad (2)$$

The model uses the marginal means of the environments as independent variables in the regression analysis and restricts the interaction to a multiplicative form (Crossa, 1990). In the JLR model, varieties are grouped according to the size of their regression coefficients (b_i), less than, equal to, or greater than one and according to the size of variance of the regression deviations (d_{ij}) (equal to or different from zero). Those varieties with regression coefficients greater than one would be more adapted to favourable growth conditions, those with regression coefficients less than one would be adapted to unfavourable environmental conditions, and those with regression coefficients equal to one would have an average adaptation to all environments. Genotypes with variances in regression deviations equal to zero would have highly predictable behaviour, whereas with a regression deviation greater than zero, they would have low predictability because of the environmental stimulus (Scapim *et al.*, 2000).

Despite being one of the more popular techniques used in GEI analysis, the linear regression method has limitations (Ramburan, 2012). Crossa (1990) classified the limitations of the regression model into two: statistical and biological. The first statistical limitation of regression analysis is that the genotype mean is not independent of the marginal means of the environments. Regressing one set of variables on another that is not independent violates one of the assumptions of regression analysis. The second statistical limitation is that errors associated with the slopes of genotypes are not statistically independent because the SS for deviation cannot be divided orthogonally among the genotypes. The third statistical problem is that the regression model assumes a linear relationship between interaction and environmental means. When this assumption is violated, the effectiveness of the analysis is reduced, and the results are misleading (Mungomery *et al.*, 1974).

A major biological problem with regressing genotype means on environmental means arises when only a few very low or very high yielding sites are included in the analysis. Another biological limitation of the regression method is that the relative stability of any two genotypes depends not only on the particular set of locations included in the analysis but also on the other genotypes that are included in the regression calculation. Nevertheless, the method is still a valuable technique that can be used to describe the structures and patterns of GEI as it provides an easily interpretable measure of yield stability (that is, the slope of the regression line) (Ramburan, 2012).

Khan *et al.* (2004; 2013) utilised the stability parameters (regression coefficient and deviation from regression coefficient) to estimate the stability and adaptability of new promising sugarcane clones under different agro-climatic conditions of Sindh, Pakistan. Tiawari *et al.* (2011) using the JLR analysis identified two out of 16 sugarcane genotypes established in three areas as stable genotypes with high mean values of cane yield and high sucrose content which may be commercially cultivated over a wide range of environments. Ramburan *et al.* (2010) characterised sugarcane varieties according to their stability and adaptability using the regression model popularised by Finlay and Wilkinson (1963), which was eventually reviewed by DeLacy *et al.* (1996).

Kamutando *et al.* (2013) successfully used the Finlay and Wilkinson (1963) regression model to identify stable and high yielding maize hybrid genotypes. Kulsum *et al.* (2013) used the JLR

analysis to conduct stability analysis for protein content, amylose content and yield of 13 promising rice (*Oryza sativa* L.) hybrids in five environments. Tekalign (2007) employed the JLR analysis to estimate the responsiveness and stability of elite sweet potato (*Ipomea batatas* L.) genotypes. Arain *et al.* (2001) used the JLR analysis to establish the adaptability and stability of 12 wheat (*Triticum aestivum* L.) genotypes evaluated over 11 locations in Sindh province (Pakistan).

2.8.3 Multivariate analysis

2.8.3.1 Clustering

Cluster analysis (CA) is one of the most important methods in analysing and structuring GEI (Aastveit and Mejza, 1992), and it is the most widely used technique for classifying environments or genotypes into homogeneous groups (Huhn and Truberg, 2002). Two types of classification can be distinguished: non-hierarchical and hierarchical (Crossa, 1990). Non-hierarchical classification assigns each item to a class, and the relationships among the classes are not characterized. Hierarchical classification groups individuals into clusters and arranges these into a hierarchy for the purpose of studying relationships in the data. CA requires a measure of similarity between the individuals to be classified, and it imposes a discontinuity in the data. CA has been used to study genotype adaptation by simplifying the pattern of responses and to subdivide genotypes and environments into more homogeneous groups (Crossa, 1990).

Crossa (1990) identified the following disadvantages associated with the use of CA: (i) numerous hierarchical groupings exist, and each of them may produce different cluster groups; (ii) the truncation level of the classificatory hierarchies may be decided arbitrarily; (iii) many different similarity measures can be used yielding different results; and (iv) CA may produce misleading results by showing structures and patterns in the data when they do not exist.

Klomsa-ard *et al.* (2013) used the CA method to group test sites and identified efficient test sites for multi-environment evaluation of sugarcane genotypes in Thailand. Sahin *et al.* (2012) using the same method grouped 17 orchard grass (*Dactylis glomerata* L.) ecotypes into four distinct groups based on mean yield and estimated stability parameters in Erzurum, Turkey. Using the CA, Badu-Apraku *et al.* (2003) classified 10 maize genotypes and 30 testing environments in West and Central Africa into three groups each. Dehghani *et al.* (2006) subjecting a two-way grain yield data of 11 lentil (*Lens culinaris* Medik.) to the clustering

procedure, characterised the genotypes into two major groups. Rodríguez *et al.* (2010) using the CA to study GEI and phenotypic stability on sugarcane genotypes detected that one test site out of four was the major contributing location to GEI due to a different soil type.

2.8.3.2 Principal component analysis

Principal component analysis (PCA) is the most used technique for reducing the data set while preserving significant features (Rodrigues *et al.*, 2008). Its aim is to transform the data from one set of coordinate axes to another, which preserves as much as possible, the original configuration of the set of points, and concentrates most of the data structure in the first component axis (Crossa, 1990). This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data possible), and each succeeding component in turn has the highest variance possible under the constraint that it be orthogonal to (that is, uncorrelated with) the preceding components (Rodrigues *et al.*, 2008). This analysis can effectively reduce the structure of a two-way GEI data matrix of genotype points in environment dimensions in a subspace of fewer dimensions (Crossa, 1990). The PCA model may be written as:

$$Y_{ij} = \mu + \sum \lambda_k \alpha_{ik} \delta_{jk} \quad (3)$$

Where, Y_{ij} is the value of the i^{th} genotype in the j^{th} environment; μ is the grand mean; λ_k is the singular value for PC axis k ; α_{ik} and δ_{jk} are the PC scores for axis k of the i^{th} genotype and j^{th} environment, respectively.

Another goal of PCA is the visualization of variables underlying the original structure (the principal components, PC) which have physical meaning and, therefore, help to see the initial structure from another point of view (Rodrigues *et al.*, 2008). This is achieved by superimposing the objects and the variables on the same biplot so that their relationships may be studied. Although PCA is more efficient than the linear regression method in describing genotypic performance (Crossa, 1990), the interpretation of resulting principal components is difficult (Aastveit and Mejza, 1992). More weak points of PCA found in the literature are: (i) the reduction of dimensionality of multivariate data may lead to distortions on interpretations (Crossa, 1990); (ii) if the proportion of variance explained by the first PC is small, the individuals that are quite different may be represented by points that are close together (Gower,

1967); (iii) as a multiplicative model it has the problem of not describing the additive main effects (Zobel *et al.*, 1998) and confuses the additive (main effects of genotypes and environments) structure of the data with non-additivity effects (GEI) (Crossa, 1990); (iv) and non-linear association in the data prevents PCA from efficiently describing the real relationships between entities (Williams, 1976).

The PCA showed important effects of locations and time of harvest on cane and sugar yields, and confirmed the dependence of cane yield on its components - stalk population, length and diameter (Milanés-Ramos *et al.*, 2010). Dehghani *et al.* (2006) utilised the PCA technique to understand the relationship between 19 univariate methods used to study GEI. Similarly, Tadege *et al.* (2014) used the PCA model to establish the relationship among 13 measures of stability. These workers learnt that 11 genotypes formed three distinct groups (namely static stability, dynamic stability and yield performance measures) while the other two were not affiliated to any of the groups. Using the same technique, Mahasi *et al.* (2009) classified 30 safflower (*Carthamus tinctorious* L.) genotypes originating from Asia, North America and Australia in Kenya. Laurentin and Montilla (1999) successfully used the PCA method to determine GEI and identify the most stable among eight sesame (*Sesamum indicum* L.) genotypes.

2.8.3.3 Factor analysis

Factor Analysis (FA) is an ordination procedure related to PCA, the “factors” of the former being similar to the PC of the latter (Crossa, 1990). As in PCA, in FA a large number of correlated variables is reduced to a small number of main (uncorrelated) factors (Cattell, 1965), and assumed that each variable can be expressed as a linear compound of k (lower than the number of individuals) hypothetical variables, the common factors, plus an additional term depending only on the particular variable and known as the specific factor (Gower, 1967). Another difference between PCA and FA is that in FA the rotation of the obtained factors is possible. A rotation which requires the factors to remain uncorrelated is an *orthogonal* rotation, while others are *oblique* rotations. Oblique rotations often achieve a simpler structure, though at the cost that one must also consider the matrix of factor inter-correlations when interpreting results.

2.8.3.4 REML

The restricted maximum likelihood (REML) algorithm is designed to analyse linear mixed models also known as multilevel models. The word ‘mixed’ indicates that the model contains fixed terms like treatments, as well as random terms, like rows and columns of a field experiment (Payne *et al.*, 2012). In general, METs must be analysed using a mixed model because they contain a mixture of fixed and random effects (Vargas *et al.*, 2013). The three stability analysis models, that is, joint linear regression (Finlay and Wilkinson, 1963); Eberhart and Russell (1966) and stability variance (Shukla, 1972) may be embedded in the mixed model framework where genotypes are the fixed effect and environments are a random effect. While the ANOVA and regression analysis methods assume independence of errors, a procedure which in more complex situations is imprecise, the REML method may not take this assumption into consideration and can be more flexible in its application (Gonçalves *et al.*, 2014). The mixed linear model may be written in this form:

$$Y = X\beta + Zu + e \quad (4)$$

Where, Y is the vector of observations, β and u are vectors of fixed and random effects, respectively, X and Z are the associated design matrices, and e is a random residual vector.

The REML method allows the consideration of different structures of variance and covariance for the GEI effects, which makes the model more realistic (Ferraudo and Perecin, 2014). The major advantage of mixed model approach is its applicability to analyse unbalanced data for the stability measures. The data may be unbalanced owing to missing data on some plots, varying number of replications among the trials and some GEI combinations that may not be tested (Kassa *et al.*, 2006). Unbalanced data and mixed effects preclude the estimation of variances using the standard fixed effects model, instead, variances are estimated by REML (Holland, 2006).

In GEI studies, REML has a great ability to explain GEI, to inform about specific positive or negative interactions with environments and to decompose the interaction on terms of “pattern” or “noise” (Ferraudo and Perecin, 2014). Ramburan *et al.* (2009) used REML to assess the effects of variety, harvest age and Eldana (*Eldana saccharina*) damage on coastal sugarcane production. REML was used by Redshaw *et al.* (2002) to predict yields for sugarcane varieties at specific locations over a number of years. The author indicated that REML predicted the

same ranking of varieties as that of the actual means, except for those environments where there were missing values. Gonçalves *et al.* (2014), selecting the most productive clones of sugarcane for a region in the southeast of Brazil, concluded that the mixed model methodology proved adequate for evaluating adaptability and stability. Redshaw *et al.* (2005) employed REML to identify and group similar Swaziland and South African sugarcane testing sites using unbalanced MET data. Ramburan *et al.* (2010) developed a variety selection decision support system for the South African sugar industry by conducting REML analyses within the sugarcane growing regions to determine varietal adaptability to different harvest age and seasons. Moiana *et al.* (2014) used the REML methodology to determine the genotypic stability and adaptability of nine cotton (*Gossypium hirsutum L.*) varieties with unbalanced data and heterogeneity of variances of errors in Mozambique.

Holland (2006) comparing multivariate analysis of variance (MANOVA) and REML, found that both methods provided similar results when data were balanced or only 5% of data were missing. However, when 15 or 25% data were missing, the REML method generally performed better, resulting in higher power detection of correlations and more accurate 95% confidence intervals. Samples of at least 75 genotypes and two environments were required to obtain accurate confidence intervals using REML.

2.8.3.5 AMMI

The ANOVA can only describe the main effects effectively as it is an additive model (Snedecor and Cochran, 1980), and the PCA being multiplicative, does not describe the additive main effects (Zobel *et al.*, 1988). While the linear regression models (by combining both additive and multiplicative components) explain both main effects and the interaction, the interaction gets confounded with the main effects compromising the power of general significance test (Wright, 1971). Zobel *et al.* (1988) proposed the additive main effects and multiplicative interaction (AMMI) model by integrating additive and multiplicative components into an integrated, powerful least squares analysis, which can explain GEI more effectively. The AMMI uses ANOVA to study the main effects of genotypes and environments, and PCA for the residual multiplicative interaction among genotypes and environments (Rodrigues *et al.*, 2008; Da Silveira, 2013). It is this dual function that makes researchers describe it as a hybrid statistical model (Kumar *et al.*, 2011; Farshadfar *et al.*, 2013). The AMMI model equation is:

$$Y_{ij} = \mu + G_i + E_j + \sum \lambda_k \alpha_{ik} \delta_{jk} + R_{ij} + \varepsilon \quad (5)$$

Where, Y_{ij} is the value of the i^{th} genotype in the j^{th} environment; μ is the grand mean; G_i is the deviation of the i^{th} genotype from the grand mean; E_j is the deviation of the j^{th} environment from the grand mean; λ_k is the singular value for principal component (PC) axis k ; α_{ik} and δ_{jk} are the PC scores for axis k of the i^{th} genotype and j^{th} environment, respectively; R_{ij} is the residual and ε is the error term (Gauch, 1992).

The AMMI model has demonstrated to be effective in understanding GEI, increasing the precision of making variety recommendations to different target sites, evaluating test environments (Chimonyo *et al.*, 2014) and obtaining better yield estimates (Bissessur *et al.*, 2001). This model provides the GEI SS with a minimum number of degrees of freedom (Da Silveira *et al.*, 2013). It is essentially effective where the assumption of linearity of responses of genotypes to a change in environment is not fulfilled (Zobel *et al.*, 1988; Farshadfar and Sutka, 2003) a requirement in stability analysis techniques (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966).

Crossa (1990) pointed out three main purposes of AMMI models. The first is model diagnosis. The AMMI model is more appropriate in the initial statistical analysis of yield trials because it provides an analytical tool for diagnosing other models as subclasses when these are better for a particular data set (Gauch, 1988). Secondly, AMMI clarifies GEI. The AMMI models summarize patterns and relations of genotypes and environments (Zobel *et al.*, 1988; Crossa, 1990). The third is that AMMI improves the accuracy of yield estimates. Gains have been obtained in the accuracy of yield estimates that are equivalent to increasing the number of replicates by a factor of two to five (Zobel *et al.*, 1988; Crossa, 1990). This therefore implies that costs may be saved by reducing the number of replications, or by including more treatments in the experiment thus improving efficiency in selecting the best genotypes.

Differences in genotype stability and adaptability to environment can be qualitatively assessed using the biplot graphical representation that scatters the genotypes according to their PC values (De Vita *et al.*, 2010). Biplot analysis is possibly the most powerful interpretive tool for AMMI models (Kulsum *et al.*, 2013). A biplot, by definition, is a scatter plot that graphically summarizes two factors in such a way that relationships among the factors and underlying

interactions between them can be visualized simultaneously (Rakshit *et al.*, 2012). This technique can provide useful information on grouping similar genotypes and/or environments, and can also provide some useful information about the GEI to identify genotypes which are well-adapted to a particular environment (Zobel *et al.*, 1988; Crossa, 1990; Anley *et al.*, 2013) or MEs in which to conduct tests (Gauch and Zobel, 1996; Ferreira *et al.*, 2006).

There are two basic AMMI biplots - the AMMI 1 biplot, where the main effect and IPCA-1 scores for both genotype and environment are plotted against each other, and AMMI 2 biplot where scores of IPCA-1 and IPCA-2 are plotted simultaneously (Kumar *et al.*, 2011). Graphically, the GEI and adaptation of genotypes to the respective environments are explained by the AMMI model 1 biplot where IPCA 1 scores are plotted against the yield (Ma'ali, 2008). The IPCA scores of a genotype in the AMMI analysis are an indication of the stability of a genotype over environments. The greater the IPCA scores, either negative or positive as it is a relative value, the more specifically adapted a genotype is to certain environments. The closer IPCA scores approach zero, the more stable the genotype is over all environments. To further explain the GEI and adaptation, an additional biplot (AMMI 2 model) between the IPCA 1 and IPCA 2 scores is created. Yield stability is determined by the AMMI stability value (ASV) described by Purchase (1997). The combination of the AMMI 2 mapping and environmental characteristics may then be used to identify suitable locations and reduce duplication of homogenous locations (Redshaw *et al.*, 2002).

Kumar *et al.* (2011) demonstrated that the AMMI model was very effective for studying the pattern of GEI and interpreting sugarcane yield data from multi-environment trials. Quemé *et al.* (2001) engaged the AMMI model to identify stable sugarcane varieties in Guatemala. Guerra *et al.* (2009) using the AMMI biplot analysis identified sugarcane genotypes that had high phenotypic stability and wide adaptability in plant and ratoon crops for northern Paraná in Brazil. Ramburan (2011) used the AMMI analysis model to characterise sugarcane varieties according to their adaptability to time of harvest, and identified environmental factors influencing such adaptability. Ma'ali (2008) showed the usefulness of this model in identifying stable varieties tested across eastern and western maize growing regions in South Africa. Yan *et al.* (2007) demonstrated the usefulness of biplots generated by the AMMI model in evaluation and identification of MEs and test environments. Rashidi *et al.* (2013) used the AMMI biplot to predict the stability of 20 chickpea genotypes and to explain the interaction of each genotype and environment.

Purchase *et al.* (2000); Alberts (2004); Oosthuizen (2005); Mohammadi *et al.* (2007); Ma'ali (2008) and Asfaw *et al.* (2009) found that the AMMI model was useful to rank genotypes according to their stability and for recommendation of specific genotypes for specific environments. Purchase *et al.* (2000) comparing the stability analysis results of Wricke (1962) ecovalence; Finlay and Wilkinson (1963) regression analysis; Shukla (1972) stability of variance; Eberhart and Russel (1966) joint regression analysis; Lin and Binns (1988) variety performance measure; and AMMI model (Gauch, 1988) on winter wheat data concluded that if a single method of describing the stability of a genotype had to be selected, the AMMI model would be the most appropriate. Abera *et al.* (2004) when assessing stability analysis of maize genotypes in Ethiopia using Wricke's (1962) ecovalence, Shukla (1972) stability of variance and AMMI model (Gauch, 1988) found close similarity and effectiveness in detecting stable genotypes over a range of environments. Comparing the GEI analysis of three methods - joint regression analysis, REML and AMMI, Ferraudo and Perecin (2014) concluded that each method detects the GEI effect in a different way. They suggested that the methods can be used in a complimentary way to a better understanding of the complex phenomenon (GEI), provided that they are carried out in accordance with the limitations inherent in each of the methods and that the assumptions are verified during the practical application of each method.

2.8.3.6 GGE

The GGE biplot method developed by Yan *et al.* (2000) has recently gained popularity as a tool to investigate various aspects of GEI (Ramburan and Zhou, 2011). Yan *et al.* (2000) combined G and GE, denoted as G + GE or GGE, repartitioned this into non-crossover GEI and crossover GEI. For variety evaluation, both G and GE must be considered simultaneously (Yan and Hunt, 2001). The difference between the AMMI and GGE is that the AMMI applies singular value decomposition (SVD) to the data minus the genotype and environment means, while GGE biplot applies SVD to the data minus the environment means only (Gauch, 2006). As a result, conventional AMMI biplots describe only genotype by environment effects, while GGE biplots describe genotype and genotype by environment effects. This is based on the concept that effects of environments are usually large, however, these effects are not relevant to variety evaluation and focus should therefore be on the genotype and genotype by environment effects only (Ramburan, 2012). The GGE biplot model is:

$$Y_{ij} - E_j = \sum \lambda_k \alpha_{ik} \delta_{jk} + R_{ij} + \epsilon \quad (6)$$

The GGE biplot is an effective method based on PCA to fully explore MET data (Farshadfar *et al.*, 2012). It has been recognized as an innovative methodology in biplot graphic analysis to be applied in plant breeding (Sandhu *et al.*, 2012) as it graphically displays GEI in a two-way table (Yan *et al.*, 2000) and in a way that facilitates visual variety evaluation and MGE identification (Dehghani *et al.*, 2006). It allows visual examination of the relationships among the test environments, genotypes and the GEI. The GGE biplot is an effective tool for: (i) MGE analysis (for example, “which-won-where” pattern), whereby specific genotypes can be recommended to specific MGEs (Yan and Kang, 2003; Yan and Tinker, 2006), (ii) genotype evaluation (the mean performance and stability), and (iii) environmental evaluation (the power to discriminate among genotypes in target environments) (Ding *et al.*, 2007).

The GGE biplot is constructed by plotting the first two principal components (PC1 and PC2, primary and secondary effects, respectively) derived from SVD of environment-centered data (Yan *et al.*, 2001). In the GGE biplots, a polygon is then drawn by connecting genotypes that are located furthest away from the biplot origin. A perpendicular line is drawn from each side of the polygon through the biplot origin such that the biplot is divided into sectors (MGEs). Environments within the same sector share the same winning genotype, and environments in different sectors have different winning genotypes. Thus, the polygon view indicates the presence or absence of crossover GEI, and is suggestive of the existence or absence of different MGEs among the test sites (Yan and Rajcan, 2002). Ideally, different sites should form separate MGEs (Ramburan, 2012). Visualization of the “which-won-where” pattern of MET data is necessary for studying the possible existence of different MGEs in the target environment (Gauch and Zobel, 1997; Yan *et al.*, 2000).

GGE biplots can aid in the visualisation of GEI not explained in general ANOVA, thereby allowing their exploitation by breeders (Muungani *et al.*, 2007). A common misconception is that biplot analysis is equivalent to PCA. While both biplot analysis and PCA use SVD as a key mathematical technique, biplot analysis is a fuller use of SVD to allow two interacting factors to be visualized simultaneously (Yan and Tinker, 2006). Compared to AMMI analysis, the GGE biplot explains a higher proportion of the SS of the GEI and is more informative with regards to environments and variety performance than the AMMI analysis (Sandhu *et al.*, 2012). The AMMI model is misleading in identifying “which-won-where” (Yan *et al.*, 2007). Thus, the GGE biplot is more logical and biological as compared to AMMI in explaining PC1 score, which represents genotypic effect rather than additive main effect (Yan and Rajcan,

2002). Another major advantage of the GGE biplot model is that information on external environmental or genotypic variables can be used directly with the model (Badu-Apraku *et al.*, 2003) a function that is not available on the AMMI model.

Rodríguez *et al.* (2010) indicated that the AMMI and the GGE biplot models were similar in showing genotype performance across environments with differences in the contribution explained in terms of GEI variance (GGE > AMMI 1). Yan and Hunt (2001) submitted that although strategies may differ in overall appropriateness, different methods usually lead to the same or similar conclusions for a given dataset. These authors highlighted that it is the quality of the data rather than the method of analysis that is more limiting to the understanding of GEI.

Luo *et al.* (2014) demonstrated that the GGE biplot analysis is a good tool for identifying sugarcane varieties suited for propagation under different ecological zones in China. Sandhu *et al.* (2012) utilised the GGE biplot technique to visualize mean performance and stability of 17 sugarcane genotypes across eight environments in India. Ramburan (2012) used the GGE biplot analysis to characterise sugarcane testing sites for the Midlands region in South Africa, and positively identified a redundant site which needed to be relocated. Klomsa-ard *et al.* (2013) found three sites that were sufficiently differentiating among the sugarcane genotypes and at the same time being representative of the nine testing sites in Thailand. Using the GGE biplot analysis, Ramburan and Zhou (2011) identified sugarcane genotypes that were ideal for cultivation under rainfed conditions, and further suggested that these genotypes be used as controls in selection trials to improve genetic gains. Ramburan and Sewpersad (2009) cherished the GGE biplot analysis as a valuable tool for interpreting variety x cutting cycle interactions for coastal sugarcane production in South Africa.

The GGE biplot analysis is successfully used in other crops besides sugarcane to characterise genotypes and environments by interpreting GEI. Yan and Tinker (2006) in a study of yield of 18 winter wheat genotypes tested at nine Ontario locations demonstrated that the GGE biplot model is an effective tool for quantifying and interpreting the GEI. Kamutando *et al.* (2013) used the GGE biplot to identify better performing maize hybrids in specific environments and effectively grouped five test sites into one ME. Farshadfar *et al.* (2012) used the polygon view of the GGE biplot analysis to positively visualize the “which-won-where” pattern of MET wheat-barley disomic addition lines. Setimela *et al.* (2010) found that the GGE biplot was useful in displaying maize hybrids that were high yielding and stable across the tested

environments, as well as in indicating hybrids with specific adaptation to a particular environment. Muungani *et al.* (2007) utilised the GGE biplots to identify MEs, high yielding and stable maize varieties, discriminating and representative test environments.

Dehghani *et al.* (2006) showed the possibility of improving progress from selections in diverse conditions by applying the GGE biplot procedure on barley yield in Iran. Using the same analytical technique (GGE biplot), Asfaw *et al.* (2012), predicted the stability and adaptability of seven mung bean (*Vigna radiate* L.) genotypes across six test locations in Ethiopia. Kaya *et al.* (2006) used the GGE biplot analysis to interpret the magnitude and causes of genotype, environment and GEI on yield performance of 25 bread wheat genotypes across different environments in Turkey. Rakshit *et al.* (2012) using a GGE biplot approach demonstrated the utility of biplot graphical approach in analysing the complex GEI in MET data of 10 rainy season grain sorghum (*Sorghum bicolor* L.) hybrids across 12 locations for two years.

2.9 Resource optimization

Plant breeders test large numbers of family lines, inbreds, hybrids and/or clones before discarding most and releasing a few as varieties. Genotypes that perform well across a wide range of environmental conditions are most useful to growers and seed companies, because such genotypes have greater probability of performing well in future years and in diverse production areas. Therefore, breeding trials are usually conducted over years, seasons (or planting dates), and locations to provide a number of test environments, and with replications (Swallow and Wehner, 1989). Conducting METs is laborious, time consuming and costly (Klomsa-ard *et al.*, 2013), so the question naturally arises 'how can resources be optimally allocated over years, seasons, locations, and replications to provide as much information as possible, and as cost-effectively as possible (Swallow and Wehner, 1989)?'

In general, the efficiency of yield trials for selecting superior genotypes is affected strongly by experimental designs, including the choice of number of replications, locations and years (Issa, 2009). Increasing the number of locations or replications may be expensive, and adding years can seriously delay a breeding programme. Therefore, knowing the best application of resources in the yield trial is important to ensure an efficient allocation of meagre resources of research and to get precise information from a breeding programme. The number of materials evaluated and the number of test environments required in METs affects the cost of plant

breeding (Kamutando *et al.*, 2013). A number of researchers working with a variety of crops have conducted studies to determine the optimal number and/or combinations of resources required in METs to achieve breeding objectives.

Swallow and Wehner (1989) working on cucumber (*Cucumis sativus*) found that efficient allocation of resources, when both yield and quality are of interest, using more seasons with fewer locations and /or replications is recommended. For Agriseeds to reduce cost of research, Kamutando *et al.* (2013) recommended the use of more replications per site and reduce the number of sites (which are in the same ME) in order to detect the same difference in maize yield. On the other hand, Kamutando *et al.* (2013) found that an increase in the number of locations can reduce the number of testing years.

Klomsa-ard *et al.* (2013) found that five test sites were equally effective in performance evaluation of sugarcane genotypes as the original nine test sites in Thailand. Statistical grouping of sugarcane variety trial sites suggested that there are similarities between some trial sites in Swaziland and South Africa (Redshaw *et al.*, 2005), as such it may be possible to make more effective use of resources, and variety evaluation and recommendations be made on a regional basis. Ramburan and Zhou (2011) using the GGE biplot were able to identify post-release evaluation sites that needed consideration for removal to optimise resources and improve the efficiencies of both selection and evaluation. Parfitt (2000) found that one breeding and selection site was sufficient for sugarcane variety recommendations for the irrigated region of South Africa instead of two. If the relative performance of genotypes is similar across sites, some sites may be redundant and should therefore be removed from the network in favour of more diverse sites (Ramburan, 2012).

2.9.1 Variance components

To determine the relative magnitude of GEI components namely, genotype x location (GxL), genotype x crop-years (GxC), and genotype x location x crop-years (GxLxC), herewith called variance components, sucrose yield and its components (cane yield and sucrose content) from the different test sites can be subjected to an analyses of variance (ANOVA) using the following model (Ramburan and Zhou, 2011):

$$Y_{ijkl} = \mu + L_l + R(L)_{kl} + G_i + GL_{il} + GR(L)_{ikl} + C_j + LC_{jl} + CR(L)_{jkl} + GC_{ij} + GLC_{ijl} + E_{ijkl} \quad (7)$$

Where, Y_{ijkl} is observation for genotype i , in crop-year j , in rep k nested within location l ; μ is the overall mean; L_l is the effect of the l^{th} location; $R(L)_{kl}$ is the effect of the k^{th} rep nested within the l^{th} location (Error 1); G_i is the effect of the i^{th} genotype; GL_{il} is the interaction effect between the i^{th} genotype and l^{th} location; $GR(L)_{ikl}$ is the interaction effect between the i^{th} genotype and the k^{th} rep nested within the l^{th} location (Error 2); C_j is the effect of the j^{th} crop-year; LC_{jl} is the interaction effect between the l^{th} location with the j^{th} crop-year; $CR(L)_{jkl}$ is interaction effect between the j^{th} crop-year and the k^{th} rep nested within the l^{th} location (Error 3); GC_{ij} is the interaction effect between the i^{th} genotype and j^{th} crop-year; GLC_{ijl} is the interaction effect between the i^{th} genotype, l^{th} location and j^{th} crop-year; and E_{ijkl} is the residual term (Error 4).

The model is defined to split all variance components so that they are separated as much as possible, thereby allowing for an evaluation of the variance components of interest (Ramburan and Zhou, 2011). Variance component analyses have been used widely to evaluate the relative contributions of relevant terms to variability and to assess the precision of trial networks to optimise resources (Ceretta and van Eeuwijk, 2008).

Jackson and Hogarth (1992) indicated that genotype x location (GxL) interactions are of greater importance than other interactions such as genotype x years (GxY) and genotype x location x years (GxLxY). Milligan *et al.* (1990) in a study, in which crop and year effects were studied independently, concluded that the potential gain strongly suggested selection across locations instead of crop year. Mirzawan *et al.* (1994) emphasized the need to concentrate more on testing across locations than on ratooning ability within a location. Ramburan and Zhou (2011) found that testing across locations was more important than testing across years in the rainfed region of South Africa. The work of these authors supported testing on several locations, while testing multiple crops on same location was found to be the reason for minimal gain.

On the other hand, Rattey and Kimbeng (2001) highlighted that when sugarcane was grown under irrigation, then GxY was of larger magnitude than GxL which was negligible in its effect on response to selection in the Burdekin region of Queensland. Similar results were reported by Kimbeng *et al.* (2009) where GxY was identified as the more important of the two first GEI components (GxL and GxY) at Rio Grande Valley Region of Texas where cane is grown under irrigation. These findings highlighted the importance of testing for ratooning ability, suggesting that substantial gains from selection could be achieved by increasing the number of crop-years

over which genotypes are tested for irrigated cane. These discoveries suggest that the results from GEI studies in sugarcane are not universal as such, the implications and potential selection strategies that develop from them may differ among sugarcane improvement programmes (Kimbeng *et al.*, 2009; Khan *et al.*, 2013). For example, SASRI breeding trials in rainfed areas are harvested in the plant and two ratoon crops, whereas the irrigated trials are harvested in the plant and three ratoon crops (Zhou *et al.*, 2012).

2.9.2 Resource allocation: the variance of a genotype mean and broad sense heritability

To declare one genotype as superior to another, one must be able to adequately discriminate between their means (Kimbeng *et al.*, 2009) and this discrimination must be repeatable or consistent. Therefore, broad sense heritability in this case is used to measure repeatability or the consistency with which one can distinguish between genotypic means, based on the proportion of the variation among genotype means that is due to genotypic effects. This assists in determining the number of the three testing resources (locations, crop-years and replications) required to detect repeatable differences among genotypes.

Variance components with their approximate standard errors (Anderson and Bancroft, 1952) are estimated from this analysis. The variance components are used to estimate the variance of a genotype mean (V_k) for different combinations of locations, replications and crop-years within a location (Fehr, 1987):

$$V_k = \sigma^2_E/ljc + \sigma^2_{GLC}/lc + \sigma^2_{GL}/l + \sigma^2_{GC}/c \quad (8)$$

Where, σ^2_E is the variance component for the residual term in the model; σ^2_{GLC} is the variance component for the interaction between genotype, location and crop-year; σ^2_{GL} is the variance component for the interaction between genotype and location and σ^2_{GC} is the variance component for the interaction between genotype and crop-year.

From the above, it is possible to calculate broad sense heritability or genetic repeatability (h^2) on a plot mean basis as:

$$h^2 = \sigma^2_G/(\sigma^2_G + V_k) \quad (9)$$

Where, σ^2_G is the variance component for the main effect of genotype.

Repeatability is an indispensable parameter in the genetic improvement of perennial crops. This is because repeatability enables the breeder to consider the characters that are repeated more than once during the period of cultivation and to estimate a minimum measurement number for real prediction of individuals (Rosa *et al.*, 2010). By altering the number of replications, locations and crop-years one can determine what resources would be required to detect repeatable differences among the genotypes (Kimbeng *et al.*, 2009).

Zhou *et al.* (2012) found that the number of replications in variety trials at Dwangwa sugar estate in Malawi may be reduced from eight to four per trial and testing years may be reduced from five to four crops. Kimbeng *et al.* (2009) reported that increasing the number of replications beyond two, locations beyond four, and crop-years beyond three did little to increase the heritability values at Texas. At the Burdekin region in Queensland (Australia), Rattey and Kimbeng (2001) found that increasing the number of replicates beyond two, locations beyond four and crop-years beyond two did not increase heritability markedly. Brown and Glaz (2001) were able to reduce replications by half from eight to four, and three crop-years were found appropriate for testing ratooning ability in Florida. Milligan (1994) realised marginal gains in broad sense heritability beyond two replications at Louisiana Sugarcane Variety Development Program. The implication of reducing the number of testing resources is that resources saved could be reallocated to other additional trials.

2.9.3 Resource allocation: optimal number of locations, replications and crop-years

A second metric proposed by Adcock *et al.* (1997) is used to assess the best allocation of the three testing resources based on the precision with which the resources would detect differences among genotypes in testing sites. Components of the GEI are used to calculate the number of locations (L) required at a given Type I ($\alpha = 0.05$) and Type II ($\beta = 0.25$) error probability to detect a specified mean difference between two genotypes for varying number of replications and crop-years:

$$L \geq [2x(t_{\alpha/2} + t_b)^2(\sigma^2_E/jc + (\sigma^2_{R/L})/j + \sigma^2_{GLC}/c)]/d^2 \quad (10)$$

Where, σ^2_E is the variance component for the residual term, the divisors j and c refers to the number of replication and crop years, respectively; $\sigma^2_{R/L}$ is the variance component for the j^{th} replication nested within the l^{th} location; $t_{\alpha/2}$ is the tabular t value ($\alpha = 0.05$); t_b is the t value

associated with committing a type 2 error ($\beta = 0.25$) and d is the difference between genotypic means for the trait expressed as a percentage of that trait's trial mean.

Kimbeng *et al.* (2009) reported that for sucrose yield, the best combination of replications (2), crop-years (4) and locations (4) identified through heritability would reliably detect differences among genotypes if the means differ by at least 20%. To improve the precision with which genotypic differences can be detected, the logistics and resources required favour planting an additional replication or harvesting an extra crop rather than planting a new trial location. Rattey and Kimbeng (2001) in a GEI analysis concluded that six locations (across two series of final assessment trials), each with two replicates and three crop-years, appeared to be the optimum combination of locations, replicates and crop-years to provide an adequate level of discrimination between genotypes in the Burdekin sugarcane breeding programme.

2.10 References

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CHAPTER 3

ADAPTABILITY AND PHENOTYPIC STABILITY OF IMPORTED SUGARCANE VARIETIES IN SWAZILAND

3.1 Abstract

The Swaziland sugar industry (SSI), recognizing the risk associated with reliance on fewer sugarcane (*Saccharum officinarum* L.) varieties compared to the other industries, imported eight Mauritian varieties for performance evaluation under Swaziland conditions in 2002. Four replicated variety trials were set up at two locations (Mhlume, poor draining soils; Simunye, good draining soils) equally split between early and late season harvest. These were tested across five successive crops (plant plus four ratoons). To assess the effect of varieties (G), locations (site x season) (L) and crop-years (C), and their interactions, an analysis of variance (ANOVA) was performed for tons of sucrose per hectare (TSH) and its components, tons cane per hectare (TCH) and sucrose content (Suc% cane). To explain the genotype × environment interaction (GEI), the Additive Main effects and Multiplicative Interactions (AMMI) analysis was conducted for each yield variable. To investigate the adaptability and phenotypic stability of these varieties, the AMMI and GGE (genotype + genotype × environment) biplot analyses were utilized.

The analysis showed that G, L, C and their interactions were significant for all traits except GxLxC for TSH and TCH. The AMMI ANOVA indicated that environments accounted for most variation in all traits. For TSH and Suc% cane, GEI accounted for larger variation than G, while the opposite was true for TCH ($G > GEI$). The biplot analyses characterized the test environments according to harvest seasons, indicating greater seasonal effect on variety performance than soil type effect. These emphasized the need to allocate more resources for testing across seasons as opposed to soil type. On average, early season trials had higher TCH but lower Suc% cane than late season trials. However, late season trials had higher TSH than early season trials. On TSH (the product of value to growers), varieties M1176/77 and M1551/80 were widely adapted across environments, while M1400/86 was specifically adapted to good draining soil. Varieties M1176/77 and M1400/86 produced higher TSH under their recommended conditions. These findings indicate that the SSI stands to benefit from importing Mauritian-bred sugarcane varieties while meeting the objective of diversifying and broadening its commercial varieties.

3.2 Introduction

The sugar industry plays a significant role in the socio-economic status of Swaziland. The industry's annual gross output is 10% of gross domestic product (GDP), and it is a major foreign exchange revenue earner for the economy because of export markets (Crawford, 2014). Moreover, the location of the industry in the poverty-stricken, semi-arid Lowveld gives it an impetus to rural development and poverty reduction initiatives.

Over years, the availability of high sucrose yielding, sustainable, pest and disease resistant varieties proved to be an essential component of productivity and profitability in the Swaziland sugar industry (SSI). With the absence of sugarcane breeding facilities in the country, the industry imports sugarcane varieties from the South African Sugarcane Research Institute (SASRI) (prefixed-N) through a cooperative agreement. These varieties are tested under different growing conditions prevailing within the industry, including soil types and harvesting seasons across successive crops (ratoons). In the past 25 years, 14 South African sugarcane varieties were tested, and eight were approved for cultivation; the rest were not adopted due to reasons including poor performance (yield) and susceptibility to pests and diseases. To broaden and diversify the industry's variety base, eight Mauritian varieties were imported in 2002 for testing in Swaziland. The specific objective was to find varieties that were resistant to pests and diseases, with good ratooning ability, and produce sucrose yields equal to or better than the commercial varieties (Dlamini, 2014).

However, there is no surety that high yielding varieties in Mauritius will repeat such performances under local conditions due to GEI effects. The GEI may be explained as changes in the relative ranking of genotypes or changes in the magnitude of differences between genotypes from one environment to another (Sandhu *et al.*, 2012; Malosetti *et al.*, 2013). These changes limit the identification and selection of superior genotypes (Setimela *et al.*, 2010; Da Silveira *et al.*, 2013). If large, GEI may result in failure to differentiate performance of genotypes across environments, and it can reduce the precision of selection across the environments (Rodríguez *et al.*, 2010). It complicates breeding, testing and selection because of the lack of correlation among genotypes across locations. According to Sandhu *et al.* (2012), differential environmental influences cause cross-over interactions and reduction of heritability, thereby resulting in decreased genetic gain. This GEI phenomenon is well reported

in literature (Comstock and Moll, 1963; Allard and Bradshaw, 1964; Crossa *et al.*, 1991; Rakshit *et al.*, 2012).

One of the most important characteristics of an ideal cultivar is high yield stability under inconsistent environmental conditions (Tekalign, 2007). Yield stability is a measure of the ability of a genotype to maintain relative performance across a wide range of environments (Kamutando *et al.*, 2013). While there can be genotypes that do well across a wide range of conditions (widely adapted genotypes), there are also genotypes that do relatively better than others exclusively under a restricted set of conditions (specifically adapted genotypes) (Malosetti *et al.*, 2013). Phenotypic stability has two concepts, static and dynamic (Becker and Léon, 1988). The static phenotypic stability (also called biological stability) exists when a genotype maintains its performance independently of variations in the environmental conditions. A genotype has dynamic stability if its performance varies with environmental changes in a predictable way. This kind of stability is also called agronomic stability. The success of sugarcane varieties can be attributed to their wider adaptability and stable performance across years (Kumar *et al.*, 2011).

Assessment of the adaptability and stability of a genotype to different environments is important for variety recommendations and it is a requirement in any breeding programme (Ma'ali, 2008). According to Ferreira *et al.* (2006), there are several methods suggested to measure phenotypic stability by modelling GEI: a) Univariate parametric methods including simple and bi-segmented linear regression, variance components mixed models, descriptive statistics, and non-linear regression models; b) Non-parametric methods including variance of genotype rank values; and c) Multivariate methods including AMMI (Additive Main effects and Multiplicative Interactions) analysis and GGE (Genotype + Genotype x Environment) biplot analysis.

The AMMI model is used for initial statistical analysis of yield trials, clarifying GEI, and summarizing the patterns and relationships of genotypes and environments (Akbarpour *et al.*, 2014). These authors suggested that AMMI also improved the accuracy of yield estimates which was equivalent to raising the number of replications from two to five. It has also been shown to be effective in understanding GEI, as well as increasing the precision of making variety recommendations to different target sites, and evaluating test environments (Chimonyo *et al.*, 2014). The method integrates additive main effects and multiplicative components,

extracting first the additive main effects and then using principal components to investigate the GEI (Crossa *et al.*, 1991). Biplot analysis is possibly the most powerful interpretive tool for AMMI models (Kulsum *et al.*, 2013). In the biplot, both genotypes and environments occur on the same scatterplot, and inferences about specific genotype by environment combinations can be made (Crossa *et al.*, 1991). The effectiveness of the AMMI procedure in sugarcane studies has been clearly demonstrated by various authors (Bissessur *et al.*, 2001; Guerra *et al.*, 2009; Rodríguez *et al.*, 2010; Kumar *et al.*, 2011; Ramburan, 2011; 2012; Da Silveira *et al.*, 2013).

GGE biplot is another analytical methodology that was proposed by Yan *et al.* (2000) which graphically portrays GEI patterns in a way that facilitates visual variety evaluation and mega-environment identification. It is useful first in displaying the “which-won-where” pattern of data which assists in identifying high-yielding and stable varieties, and second in identifying discriminating and representative test locations (Dehghani *et al.*, 2006). The term GGE emphasizes the understanding that genotype (G) and genotype by environment interaction (GEI) are the sources of variation that are relevant to genotype evaluation, hence they must be considered concurrently for appropriate genotype and test environment evaluation (Yan and Tinker, 2006). According to Yan *et al.* (2001), the GGE biplots display multi-environmental trial data by plotting the first two principal components (PC1 and PC2) derived from singular value decomposition (SVD) of environment-centered data against each other. The GGE biplot explains a higher proportion of the SS of the GEI and is more informative with regards to environments and variety performance than the AMMI analysis (Sandhu *et al.*, 2012). GGE biplots have been successfully used in sugarcane research to characterise sugarcane varieties and testing sites (Rodríguez *et al.*, 2010; Ramburan and Zhou, 2011; Sandhu *et al.*, 2012; Ramburan, 2012; Klomsa-ard *et al.*, 2013; Luo *et al.*, 2014).

Although the SSI has been importing and testing South African-bred varieties for more than three decades, there is no formal study that was conducted to assess the adaptability and stability of foreign-bred varieties in the country using analytical techniques. Stakeholders have expressed concern that the industry relies on fewer varieties, hence there is a need to release imported varieties at a faster rate to ensure a desirable disposition and reduce the risk of pests and diseases. There is also a need to acquire varieties that are robust all year round. Hence, the objective of this study was to assess the adaptability and phenotypic stability of sugarcane varieties imported from Mauritius Sugarcane Industry Research Institute (MSIRI) relative to

standard commercial varieties, using AMMI and GGE biplot analyses. The results of the study will inform future importations of foreign-bred varieties into Swaziland.

3.3 Materials and methods

3.3.1 Treatments

Table 3.1 shows the eight imported varieties and their respective recommended harvest periods and soil types in Mauritius.

Table 3.1: Recommended harvest time and soil types for the eight varieties imported from Mauritius

Variety	Recommended harvest period [#]	Recommended soil type ⁺					
		B1/B2	F1/F2	H1/H2	L1	L2	P1/P2/P3
R570	Mid-Aug. to Nov.			x	x	x	x
M1176/77	Mid-Aug. to Oct.				x	x	x
M1186/86	Mid-Aug. to Nov.			x	x	x	x
M1246/84*	Aug. to Sept.			x	x	x	x
M1400/86**	Aug. to Sept.	x	x	x	x	x	x
M1551/80	Mid-Jul. to Mid-Sept.				x	x	x
M695/69*	Mid-Jun. to Mid-Aug.			x		x	
M96/82*	Aug. to Sept.			x	x	x	x

x Indicates a soil type where the variety can be planted

* When flowering does not exceed 25%, harvest of M1246/84, M695/69 and M96/82 could be extended to October, Mid-September and October, respectively.

**When flowering does not exceed 40%, harvest of M1400/86 could be extended to end October.

Soil types

B1/B2: Latosolic brown forest; F1/F2: Humic ferruginous latosols; H1/H2: Humic latosols; L1, L2: Low humic latosols; P1/P2/P3: Latosolic reddish prairie.

[#] Information sourced from MSIRI recommendation sheet of April 2008, No. 162

⁺ Information sourced from MSIRI recommendation sheet of April 2008, No. 161

Prior to release as plantlets for bulking-up in Swaziland, the eight varieties were imported from Mauritius as single budded setts for quarantining at SASRI in 1998. This was necessitated by a provision in the SSA – SASRI cooperation agreement which states that prior to importation into Swaziland, all foreign bred sugarcane varieties are to be quarantined at SASRI facilities. The purpose is to safeguard against importation of pest and disease infected sugarcane planting material which might pose a biosecurity threat within the region. In 2002, the varieties were imported from SASRI as plantlets and were planted for seed build-up at SSA trial field S604, located at Simunye. To obtain the required planting material for the variety evaluation programme, the following year (2003) the seedcane from the plantlets was further planted for multiplication in a bulking block within the same trial field.

3.3.2 Experimental design

Between 2004 and 2005, four replicated trials were established at two SSA trial sites located at Mhlume and Simunye. Two trials were set up at Simunye field 604 using a Latin square design, each replicated ten times (100 plots) with gross plot and net plot sizes of 97.5 m² (13.0 m row length) and 49.5 m² (11.0 m row length), respectively. The other two trials established at Mhlume field 428 were laid out as a randomised complete block design replicated eight times (80 plots) with gross plot and net plot sizes of 114.0 m² (19.0 m row length) and 51.0 m² (17.0 m row length), respectively. In all the trials, plot spacing (distance between plots) ranged from 1.0 to 2.0 m while inter-row spacing was maintained at 1.5 m. A 1.0 m end of row effect was provided for in all plots.

3.3.3 Site selection and description

Each trial site represented the major soil characteristics prevalent within the SSI, these being poor draining and good draining. The Simunye trials represented good draining and moderate to well-structured soils (R-set). On the other hand, the Mhlume planted trials represented poor draining and weakly-structured / duplex soils (Z-set). On each site, one trial was set up for harvesting within the early season (April to June) and the other for late season (October to December). In this study, site/soil x harvesting season (crop-year) represented a specific environment. In each location, the eight Mauritian varieties were planted alongside NCo376 and other South African cultivars recommended for these sets of circumstances in the SSI. This information is summarized in Table 3.2.

Table 3.2: General information on the four trials

Trial code	Mauritian varieties	S. African varieties	Harvesting season	Soil type	Location	Planting date
SE	All-8	NCo376, N23	Early season (May)	R-set (<i>Hutton or Shortlands form</i>)	Simunye	12 Feb. 2004
ME	All-8	NCo376, N40	Early season (May)	Z-set (<i>Sterkspruit form</i>)	Mhlume	19 Feb. 2004
SL	All-8	NCo376, N25	Late season (November)	R-set (<i>Hutton or Shortlands form</i>)	Simunye	04 Nov. 2004
ML	All-8	NCo376, N25	Late season (October)	Z-set (<i>Sterkspruit form</i>)	Mhlume	15 Oct. 2005

Soil forms in brackets are South African equivalents (Nixon, 2006)

3.3.4 Crop maintenance

The general management of the trials was carried out by the estates where they were established in collaboration with SSA, observing industry standards. All trials were irrigated by surface drip with emitter spacing of 900 mm. Fertilizer in all crops was applied by the SSA agronomy team following soil analysis results. Composite soil samples were taken in a manner that ensured representativeness of all trial plots either six weeks before harvesting or immediately after harvesting, depending on whether the crop was heavily lodged or not.

3.3.5 Data collection

Yield data was collected at harvesting (12 months cycle) on a plot basis for seven consecutive crops (plant *plus* six ratoons). However, for this study only the first five crops (plant *plus* four ratoons) were considered because variety recommendations in the Swaziland sugar industry are based on variety performances over these period (five years). To remove the extraneous matter, the cane was burnt a day prior to harvesting. On the day of harvesting, the end-row effects were cut and removed from the net plots and placed in a windrow together with cane from the guard rows. The cut cane was topped according to estate practice which is usually below the natural breaking point. Cane from the net rows was also cut and placed in heaps manageable to the grab mounted on a tractor carrying the digital scale for measuring cane weight. After weighing, a total of 16 stalks per plot were sampled at random from within each heap to determine sucrose content (%) at the Royal Swaziland Sugar Corporation (RSSC) laboratory using a polarimeter (Shoonees-Muir *et al.*, 2009), hence sucrose content (%) is also referred to as pol%. For all practical purposes, pol% and sucrose% are synonyms in this study. The collected data was then transformed and expressed on a per hectare basis as follows:

$$\text{Tons cane per ha (TCH)} = \text{weight of cane (kg)} \div \text{net plot area (m}^2\text{)} \times 10 \quad (1)$$

$$\text{Tons sucrose per ha (TSH)} = \text{tons cane per ha} \times \text{sucrose content (\%)} \quad (2)$$

3.3.6 Statistical analysis

Statistical analyses of cane yield (TCH), sucrose yield (TSH) and sucrose content (Suc% cane) per site and across sites were conducted using GenStat[®] 17th Edition statistical software (VSN International, 2015). An ANOVA for each trial was performed on each yield parameter (TCH, TSH and Suc% cane). To compare treatment means, Fisher's protected least significant difference (LSD) test at 5% probability was used. To determine homogeneity of variances for

the yield parameters before combining the data of all sites across years (crop-cycle), Barlett's test was conducted. The test showed homogeneity of variances for the yield parameters, hence combined data analyses were done. The combined ANOVA across sites and crop-cycles enabled estimation of differences between main effects (G), location (L) and crop-cycles (C), and their interactions on yield parameters (Chimonyo *et al.*, 2014).

To explain GEI, data for the yield parameters TCH, TSH and Suc% cane were subjected to AMMI analysis. In the AMMI ANOVA, the trial x crop-cycle (ratoon) combination was considered to constitute the environment. The following AMMI model equation was used:

$$Y_{ij} = \mu + G_i + E_j + \sum \lambda_k \alpha_{ik} \delta_{jk} + R_{ij} + \varepsilon \quad (3)$$

Where, Y_{ij} is the value of the i^{th} genotype in the j^{th} environment; μ is the grand mean; G_i is the deviation of the i^{th} genotype from the grand mean; E_j is the deviation of the j^{th} environment from the grand mean; λ_k is the singular value for principal component (PC) axis k; α_{ik} and δ_{jk} are the PC scores for axis k of the i^{th} genotype and j^{th} environment, respectively; R_{ij} is the residual and ε is the error term (Gauch, 1992).

To graphically explain the GEI and adaptation of the varieties to the different environments, two AMMI biplots for each yield parameter were created. On the first one (AMMI 1 biplot), IPCA 1 scores were plotted against the main effects (varieties and environments), and the second (AMMI 2 biplot) involved plotting IPCA 1 scores against IPCA 2 scores of environments and varieties. With the AMMI 1 biplot, genotypes with IPCA 1 scores outside 1 and -1 were considered specifically adapted to particular environments, while those with IPCA 1 scores closer to zero were deemed stable over all the environments. The ideal variety was one with high productivity and IPCA 1 values close to zero, and an undesirable variety had low productivity associated with low stability (Da Silveira *et al.*, 2013). In the AMMI 2 biplot, the position of the point for varieties was given by the estimates for the genotypic scores, similarly, the point for environments originated from the estimates for the environmental scores (Rashidi *et al.*, 2013). The distance from the biplot origin was indicative of the nature of interaction that was displayed by either varieties over environments or environments over varieties. For the AMMI biplots, environments were constituted by trial site and harvesting season. The data considered for each variety was the mean of the five crops, the drive being that the core interest

in sugarcane growing is the overall sugar yield of a genotype within the comparable crop-cycles.

A second biplot analysis was conducted using the GGE biplot technique for TCH, TSH and Suc% cane. The purpose was to compare the results of the two methods (AMMI and GGE) and to facilitate the process of drawing sound and solid conclusions on this study. The GGE biplot model is:

$$Y_{ij} - E_j = \sum \lambda_k \alpha_{ik} \delta_{jk} + R_{ij} + \varepsilon \quad (4)$$

In the GGE biplot, the PC1 scores of both genotypes and environments were plotted against their respective PC2 scores for each of the yield variables. A polygon was first drawn on varieties located farthest away from the biplot origin in various directions such that all varieties were contained within the polygon. Varieties located on the vertices of the polygon were either the best or the poorest in one or more environments (Yan and Tinker, 2006). Thereafter, perpendicular lines were drawn from the biplot origin such that the biplot was divided into sectors. The perpendicular lines were equality lines between the adjacent varieties on the polygon, which facilitated visual comparison between the varieties. These equality lines divided the biplot into sectors such that the winning variety for each sector was the one located on the respective vertex (Yan and Tinker, 2006).

To quantify and rank genotypes according to yield stability, Purchase (1997; 2000) proposed the AMMI stability value (ASV). The ASV in general terms is the distance from the point of origin in the AMMI 2 biplot (IPCA 1 against IPCA 2) (Purchase, 2000; Chimonyo *et al.*, 2014). The nearer the ASV to zero, the more stable the genotype was considered to be for most environments (Ma'ali, 2008).

$$ASV = \sqrt{\left[\frac{IPCA\ 1\ sum\ of\ squares}{IPCA\ 2\ sum\ of\ squares} (IPCA\ 1\ score)^2 \right] + [IPCA\ 2\ score]^2}$$

Since AMMI and ASV analysis cannot handle unbalanced data, variety NCo376 was considered as the only commercial check as it was planted in all the locations and the last two blocks of the Latin square design (Simunye trials) were omitted for these analyses.

3.4 Results

3.4.1 Performance of varieties across individual locations

Significant differences ($p < 0.05$) were observed between experimental varieties on cane yield (TCH) across all locations (Table 3.3). Variety N25 had significantly higher ($p < 0.05$) TCH than most varieties in all late season trials except M96/82 (ML), M1176/77 (SL) and M1400/86 (SL). Variety M1176/77 had significantly higher ($p < 0.05$) TCH than all varieties in all early season trials. The early season trials had higher average cane yields (SE, 118.9 TCH; ME, 115.0 TCH) compared to the late season trials (SL, 106.7 TCH; ML, 101 TCH). This is attributed to favourable vegetative growing conditions in the early season relative to the late season.

On sucrose content (Suc% cane), there were significant differences ($p < 0.05$) between varieties at all locations (Table 3.4). Varieties N40 and M695/69 had significantly higher ($p < 0.05$) Suc% cane than all varieties at ME and SE, respectively. At ML, varieties M1400/86, M1246/84 and M695/69 had significantly higher ($p < 0.05$) Suc% cane than most varieties while at SL varieties M1551/80 and M1400/86 had significantly higher ($p < 0.05$) Suc% cane than most varieties. Contrary to TCH, late season trials had higher average sucrose content compared to early season trials. This is attributed to that late season harvested cane grow through the cold months, a period when sucrose accumulation in sugarcane stalks is maximized.

Significant differences ($p < 0.05$) were also observed between varieties on sucrose yield (TSH) (Table 3.5). At ME, variety N40 had significantly higher ($p < 0.05$) TSH than most varieties except NCo376 and M1176/77, while at SE variety M1176/77 yielded significantly higher ($p < 0.05$) TSH than most varieties except M1551/80 and M1400/86. In all late season trials, variety N25 had significantly higher ($p < 0.05$) TSH than most varieties except M1400/86, M1176/77 and M1551/80 at SL. Late season trials had higher TSH than their early season equivalents. The higher Suc% cane in the late season sufficiently compensated for the lower TCH during the same season, while the higher TCH in the early season did not result in comparably higher TSH.

Table 3.3: Cane yield results (tons cane per hectare, TCH) averaged over five crop-years

Variety	Locations			
	ME	ML	SE	SL
R 570	114.4 bc	91.7 cd	112.4 de	103.2 d
M1176/77	135.0 a	111.0 a	135.0 a	116.0 ab
M1186/86	113.1 bc	83.2 e	109.6 de	90.2 f
M1246/84	116.4 bc	97.3 bc	116.9 bcd	110.4 bc
M1400/86	111.8 c	102.6 b	123.7 b	112.2 abc
M1551/80	119.0 b	102.5 b	123.7 b	108.2 cd
M695/69	101.6 d	89.9 d	108.5 e	95.9 ef
M96/82	113.9 bc	112.4 a	121.0 bc	109.1 bcd
N23	-	-	115.3 cde	-
N25	-	116.5 a	-	119.3 a
N40	105.4 d	-	-	-
NCo376	119.0 b	102.7 b	122.7 bc	102.9 de
Mean	115.0	101.0	118.9	106.7
LSD (5%)	6.2	6.0	8.3	7.2
CV (%)	12.2	13.5	17.0	21.0

Numbers followed by different letters are statistically different

LSD: least significant difference; CV: coefficient of variation

Table 3.4: Sucrose content results (Suc% cane) averaged over five crop-years

Variety	Locations			
	ME	ML	SE	SL
R 570	12.98 def	17.23 bc	13.92 c	16.11 b
M1176/77	12.50 f	16.31 e	13.73 c	16.13 b
M1186/86	13.09 de	16.74 d	13.85 c	16.03 b
M1246/84	13.00 de	17.39 ab	13.74 c	16.26 b
M1400/86	13.82 bc	17.76 a	14.38 b	17.03 a
M1551/80	13.34 cd	16.91 cd	14.51 b	17.15 a
M695/69	14.10 b	17.49 ab	15.03 a	16.37 b
M96/82	12.62 ef	16.26 e	13.01 d	15.44 c
N23	-	-	14.37 b	-
N25	-	16.91 cd	-	16.04 b
N40	16.19 a	-	-	-
NCo376	13.90 b	16.56 de	14.10 bc	16.10 b
Mean	13.55	16.96	14.06	16.27
LSD (5%)	0.50	0.42	0.45	0.36
CV (%)	8.40	5.60	10.40	7.20

Numbers followed by different letters are statistically different

LSD: least significant difference; CV: coefficient of variation

Table 3.5: Sucrose yield results (tons sucrose per hectare, TSH) averaged over five crop years

Variety	Locations			
	ME	ML	SE	SL
R 570	14.69 de	15.78 e	15.57 de	16.72 cd
M1176/77	16.64 ab	18.05 bc	18.55 a	18.73 ab
M1186/86	14.60 de	13.82 f	15.13 e	14.53 e
M1246/84	15.07 cde	16.89 d	16.15 cde	17.93 bc
M1400/86	15.25 cd	18.2 b	17.89 ab	19.20 a
M1551/80	15.72 bc	17.31 bcd	17.89 ab	18.53 ab
M695/69	14.21 e	15.72 e	16.29 cde	15.76 d
M96/82	14.18 e	18.21 b	15.65 de	16.8 cd
N23	-	-	16.54 cd	-
N25	-	19.63 a	-	19.20 a
N40	17.01a	-	-	-
NCo376	16.51ab	17.01 cd	17.24 bc	16.62 d
Mean	15.388	17.062	16.69	17.401
LSD (5%)	1.005	1.109	1.169	1.213
CV (%)	14.8	14.8	20.5	23.3

Numbers followed by different letters are statistically different

LSD: least significant difference; CV: coefficient of variation

3.4.2 Combined analysis of variance

Results of the combined ANOVA for yield parameters (TSH, TCH and Suc% cane) for nine varieties evaluated over four locations across five crops are presented in Table 3.6. These results exclude the location specific SASRI varieties (N23, N25 and N40) because ANOVA cannot handle unbalanced data. The main effects: genotypes, locations and crop-years; and GEI components: GxL, GxC and LxC showed highly significant differences ($p < 0.001$) for the three yield variables. This implies that sites (soil type x season) and crop-years significantly ($p < 0.001$) influenced the performance of the varieties. The higher interaction effect GxLxC was only significant ($p < 0.001$) for Suc% cane, indicating that ratooning ability was not dependent on location for TCH and TSH.

Table 3.6: Analysis of variance for sucrose yield (TSH), cane yield (TCH) and sucrose content (Suc% cane) of nine varieties tested across four locations

Source of variation	df	TSH		TCH		Suc% cane	
		SS	MS	SS	MS	SS	MS
Block	7	250.9	35.8	8857.5	1265.4	31.6	4.5
Genotype (G)	8	1472.7	184.1***	75686.9	9460.9***	302.2	37.8***
Location (L)	3	1092.8	364.3***	95005.9	31668.6***	3311.2	1103.7***
Crop cycle (C)	4	3005.1	751.3***	230380.5	57595.1***	380.1	95.0***
GxL	24	545.2	22.7***	17849.3	743.7***	92.9	3.9***
GxC	32	584.8	18.3***	23752.3	742.3***	139.7	4.4***
LxC	12	4137.2	344.8***	116868.9	9739.1***	631.8	52.7***
GxLxC	96	604.6	6.3	19269.3	200.7	176.5	1.8***
Residual	1253	9383.2	7.5	357973.6	285.7	1331.7	1.1
Total	1439	21076.6		945644.3		6397.6	

*** significant at $p < 0.001$; df: degrees of freedom; SS: sum of squares; MS: mean of squares

3.4.3 Additive main effects and multiplicative interaction (AMMI) analysis of variance

Table 3.7 presents the AMMI ANOVA for sucrose yield of the nine varieties grown in 20 environments (four locations x five crop-years). The analysis showed that sucrose yield was significantly ($p < 0.001$) affected by genotype (G), environment (E) and GEI. E, G and GEI explained 72.0%, 12.9% and 15.2% of the treatments SS (SS), respectively. The first two interaction principal components axes, IPCA 1 and IPCA 2, of the AMMI model were also highly significant ($p < 0.001$) explaining 45.2% and 20.9% of the total GEI SS, respectively. Combined, the first two IPCAs accounted for 66.1% of the total variation due to GEI.

The analysis showed that E, G and GEI significantly ($p < 0.001$) influenced cane yield, explaining 76.4%, 13.1% and 10.5% of the treatments SS, respectively (Table 3.8). IPCA 1 and IPCA 2 were also significant (IPCA 1 at $p < 0.001$; IPCA 2 at $p < 0.05$) explaining 49.2% and 15.2% of the interactions SS, respectively, giving a pooled total of 64.4%.

Table 3.7: AMMI analysis of variance for sucrose yield (TSH) of nine varieties tested across 20 environments

Source of variation	df	SS	%SS	Explained % of interaction	MS	P-value
Total	1439	21077			14.65	
Treatments	179	11442			63.92	<0.001
Genotypes	8	1473	12.9%		184.09	<0.001
Environments	19	8235	72.0%		433.43	<0.001
Block	140	3048			21.77	<0.001
Interactions	152	1735	15.2%		11.41	<0.001
IPCA 1	26	785	6.9%	45.2%	30.18	<0.001
IPCA 2	24	363	3.2%	20.9%	15.11	<0.001
Residuals	102	587			5.76	0.541
Error	1120	6586			5.88	

df: degrees of freedom; SS: sum of squares; MS: mean of squares

Table 3.8: AMMI analysis of variance for cane yield (TCH) of nine varieties tested across 20 environments

Source of variation	df	SS	%SS	Explained % of interaction	MS	P-value
Total	1439	945644			657	
Treatments	179	578813			3234	<0.001
Genotypes	8	75687	13.1%		9461	<0.001
Environments	19	442255	76.4%		23277	<0.001
Block	140	123058			879	<0.001
Interactions	152	60871	10.5%		400	<0.001
IPCA 1	26	29977	5.2%	49.2%	1153	<0.001
IPCA 2	24	9236	1.6%	15.2%	385	0.013
Residuals	102	21658			212	0.551
Error	1120	243774			218	

df: degrees of freedom; SS: sum of squares; MS: mean of squares

Table 3.9 presents the AMMI ANOVA for sucrose content for the nine varieties tested across the 20 environments. The analysis indicated that E, G and GEI had highly significant ($p < 0.001$) effects on Suc% cane, explaining 85.9%, 6.0% and 8.1% of the treatments SS, respectively. The IPCA 1 and IPCA 2 were also highly significant ($p < 0.001$), explaining 35.0% and 24.0% of the total GEI SS, respectively, with a combined total of 59.0%.

Table 3.9: AMMI analysis of variance for sucrose content (Suc% cane) of nine varieties tested across 20 environments

Source of variation	df	SS	%SS	Explained % of interaction	MS	P-value
Total	1439	6398			4.45	
Treatments	179	5034			28.12	<0.001
Genotypes	8	302	6.0%		37.77	<0.001
Environments	19	4323	85.9%		227.53	<0.001
Block	140	282			2.02	<0.001
Interactions	152	409	8.1%		2.69	<0.001
IPCA 1	26	143	2.8%	35.0%	5.51	<0.001
IPCA 2	24	98	1.9%	24.0%	4.07	<0.001
Residuals	102	168			1.65	<0.001
Error	1120	1081			0.97	

df: degrees of freedom; SS: sum of squares; MS: mean of squares

The significant GEI for all three traits (TSH, TCH and Suc% cane) necessitated the estimation of phenotypic stability and adaptability.

3.4.4 Mean performance and stability of varieties

3.4.4.1 AMMI 1 biplot model

The AMMI 1 biplot involves plotting main effects against their respective IPCA1 scores. Figures 3.1, 3.2 and 3.3 present the AMMI 1 biplots for TSH, TCH and Suc% cane, respectively, of the nine varieties and the four environments. These biplots give a direct measure of the yield potential and the stability of varieties being studied (McDermott and Coe, 2012). Varieties distributed within quadrants B and D of the biplot had average yields above the grand mean, while those in quadrants A and C had average yields below the grand mean. Varieties close to the point of origin of the IPCA 1 had a smaller interaction with most of the environments, and as such were more stable on that particular trait. On the other hand, varieties falling beyond 1 and -1 had a larger GEI and were generally considered unstable. Likewise, environments were interpreted in a similar manner as varieties. Environments with IPCA 1 scores closer to zero had lower interaction with varieties, while those with scores beyond 1 and -1 distinguished varieties more effectively.

For TSH, most varieties had IPCA 1 scores within the 1 and -1 range, except M1186/86 (1.04) (Figure 3.1). Variety M96/82 had a larger negative IPCA 1 score of -0.93. Varieties M1176/77

(0.07) and M1551/80 (-0.09) had IPCA 1 scores closer to zero and average TSH above the grand mean. Variety R570 (0.07) had an IPCA 1 score closer to zero, but had TSH lower than the grand mean. Other varieties that had IPCA 1 scores less than ± 1 were M695/69 (0.24) and M1286/84 (-0.34). Varieties NCo376 (0.60) and M1400/86 (-0.68) had higher IPCA 1 scores which were less than ± 1 , and their TSH were above the grand mean. Both Mhlume test locations (ME, 1.15 and ML, -1.03) had IPCA 1 scores above ± 1 , while the Simunye test locations (SE, 0.47 and SL, -0.59) had IPCA 1 scores below ± 1 . Only the ME location had sucrose yield below the locations grand mean, showing low potential conditions for TSH.

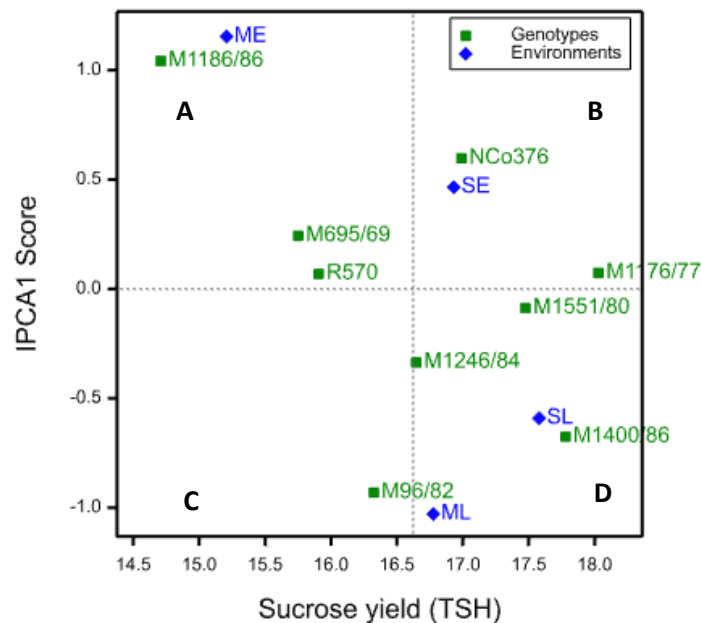


Figure 3.1: AMMI 1 biplot IPCA 1 scores for nine varieties and four environments plotted against sucrose yields (TSH) averaged over five crop-years for both genotypes and environments

For TCH, M1246/84 (0.03), M1551/80 (-0.04) and NCo376 (0.19) had IPCA 1 scores very close to the point of origin, while M1186/86 (2.38), M96/82 (-2.37), M1400/86 (-1.57) and M1176/77 (1.48) had IPCA 1 scores greater than ± 1 (Figure 3.2). Varieties R570 (0.80) and M695/69 (-0.91) also had larger IPCA 1 scores close to ± 1 . Environment SE (0.15) had an IPCA 1 score closer to zero, while the Mhlume locations (ME, 3.24 and ML, -2.46) had very

large IPCA 1 scores. Environment SL (-0.94) had a larger IPCA 1 score very close to -1. The late-season harvested trials had average cane yield below the grand mean.

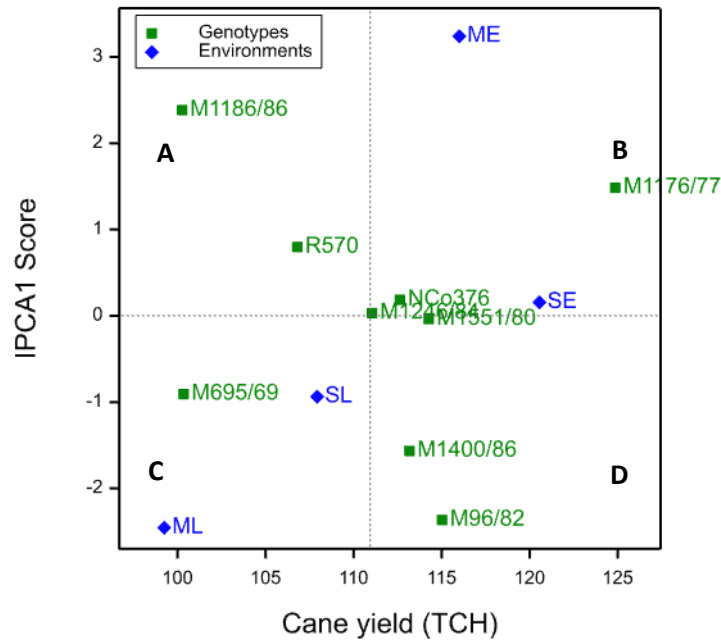


Figure 3.2: AMMI 1 biplot IPCA 1 scores for nine varieties and four environments plotted against cane yields (TCH) averaged over five crop-years for both genotypes and environments

The AMMI 1 biplot for sucrose content (Suc% cane) is presented in Figure 3.3. All varieties had IPCA 1 scores below ± 1 , and five of the nine varieties (M1246/84, R570, M1186/86, M1176/77 and M96/82) had average Suc% cane below the grand mean. Varieties 96/82 (-0.003) and M1186/86 (-0.068) had IPCA 1 scores closer to the point of origin indicating less interactions with environments for this trait. The commercial check NCo376 was the furthest from zero with an IPCA 1 score of -0.717. The early season trials had sucrose content below the grand mean, while the late season trials had sucrose content above the grand mean.

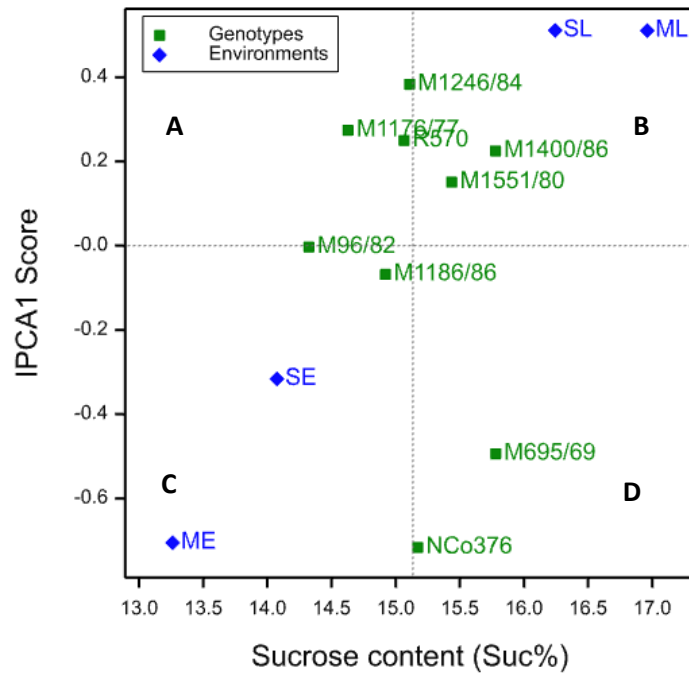


Figure 3.3: AMMI 1 biplot IPCA 1 scores for nine varieties and four environments plotted against sucrose content (Suc% cane) averaged over five crop-years for both genotypes and environments

A common observation in all three AMMI 1 biplots (Figures 3.1, 3.2 and 3.3) was that environments tended to cluster more according to harvesting seasons as opposed to locations (soil type). This implies that the seasonal effect was much stronger than the soil type effect on the performance of these varieties.

3.4.4.2 GGE biplot analysis

The concept of high stability is meaningful only when associated with high mean performance, as a result a stable variety is desirable only when it has a high mean performance (Yan and Tinker, 2006). Figures 3.4, 3.5 and 3.6 present the GGE biplot analysis showing mean performance and stability of the varieties for TSH, TCH and Suc% cane, respectively. The single arrowed line in these figures is the average environment coordination (AEC) abscissa (or AEA) and it points to higher mean yield across environments. The line running perpendicular to the AEC abscissa is the AEC ordinate and it points to greater variability (poor stability) in either direction (Yan and Tinker, 2006). Unlike the AMMI 1 biplots which plots

IPCA 1 scores against main effects, the AEC view of the GGE biplot plots the PC1 scores against their respective PC2 scores.

On TSH, the varieties ranked in the order M1176/77 > M1400/86 > M1551/80 > NCo376 > M1246/84 > M96/82 > R570 > M695/69 > M1186/86 on mean performances (Figure 3.4). M1551/80 was the most stable variety on this trait (since it was closest to the AEA), and had average TSH above the grand mean. M1176/77 and M1400/86 had higher mean TSH, and were oriented towards the early and late season environments, respectively. Commercial check NCo376 was specifically adapted to early environments while M96/82 was specifically adapted to the ML environment. R570 and M695/69 were stable in their performances, however, they had average TSH lower than the grand mean. Variety M1186/86 was unstable and had the least mean TSH. The ranking of the varieties and the grouping of the environments according to harvesting seasons were consistent with the results of AMMI 1 biplot (Figure 3.1).

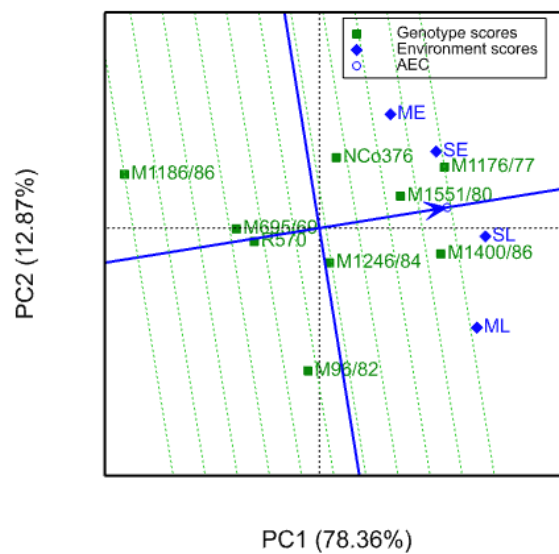


Figure 3.4: The average environment coordination (AEC) view showing mean performance and stability of nine sugarcane varieties tested in four environments averaged over five crop-years on sucrose yield (TSH)

Variety M1176/77 had the highest mean TCH followed by M96/82, M1551/80, M1400/86 and NCo376 (Figure 3.5). M1246/84 was located on the biplot origin, indicating that it was stable and had average TCH similar to the grand mean. R570, M1186/86 and M695/69 were the poorest performing on TCH with an average yield below the grand mean. M1551/80 and

NCo376 were stable, and had average TCH above the grand mean. M1176/77 was specifically adapted to the early season environments, while M1400/86 and M96/82 were exclusively adapted to the late season environments. The Simunye trials were closer to the AEA, indicating that they are ideal for identifying widely adapted varieties compared to the Mhlume trials. The ranking of the varieties in this biplot was similar to that of AMMI 1 biplot (Figure 3.2).

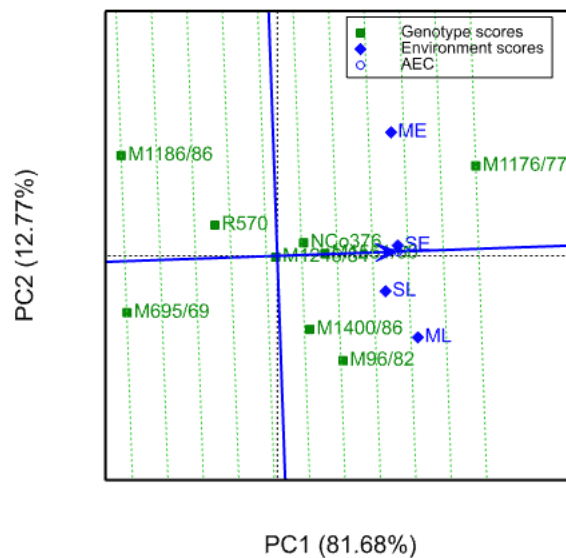


Figure 3.5: The average environment coordination (AEC) view showing mean performance and stability of nine sugarcane varieties tested in four environments averaged over five crop-years on cane yield (TCH)

Figure 3.6 shows the mean performance and stability of varieties over the four environments on Suc% cane. M695/69 and M1400/86 had the highest mean Suc% cane followed by M1551/80 and NCo376. M1246/84, R570, M1186/86, M1176/77 and M96/82 had the lowest average Suc% cane which was below the grand mean. M1551/80, while inclined towards the late season trials, was the closest to the AEA, implying that it was the most stable on this trait. M1400/86 and M695/69 were each specifically adapted to the late and early season environments, respectively. NCo376 was specifically adapted to the ME environment. The ranking of varieties and the tendency of environments to group according to harvesting seasons on Suc% cane observed in the AMMI biplots were observed in the GGE biplot as well.

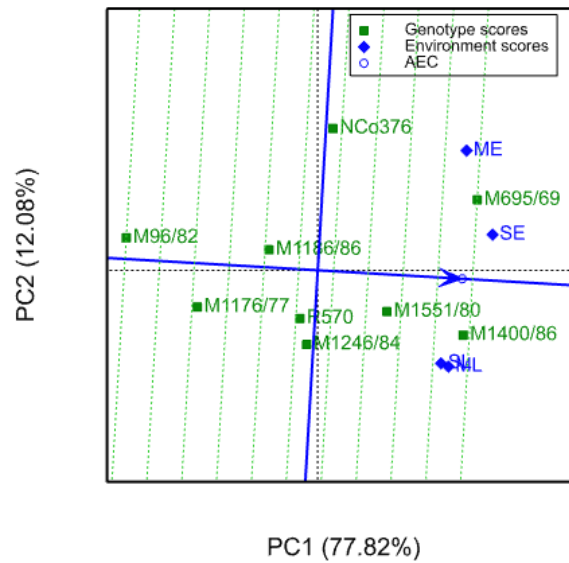


Figure 3.6: The average environment coordination (AEC) view showing mean performance and stability of nine sugarcane varieties tested in four environments averaged over five crop-years on sucrose content (Suc% cane)

3.4.4.3 AMMI stability value

A third technique, the AMMI stability value (ASV), was also used to estimate the stability of varieties in this study (Table 3.10). By interpretation, an ASV closer to zero indicates that a variety is more stable in most environments. On the other hand, environments with an ASV closer to zero are less discriminating, hence they provide very little information about the varieties.

For TSH, three varieties had ASV closer to zero, hence they were stable in the order M1176/77 > R570 > M695/69, and the rest of the varieties M96/82 > M1186/86 > M1400/86 > NCo376 > M1551/80 > M1246/84, in sequence, were the most unstable. On TCH, only one variety (M1551/80, ASV = 0.052) was stable while the rest had ASV greater than one, and varieties M96/82 > M1186/86 > M1400/86 > M1176/77 were the most unstable with ASV indices of 3.339, 3.056, 2.251 and 2.166, respectively. For Suc% cane, variety M1186/86 (0.090) was the most stable since it had ASV closest to zero. NCo376 was the most unstable as it had the largest ASV of 0.807 on this trait. SE was the most indiscriminate environment for all three phenotypic traits. For TSH, the late season environments were more discriminating than the early season

environments. For TCH, the order of discrimination was SL > ML > ME > SE. For Suc% cane, the order of discrimination was ML > SL > ME > SE.

Table 3.10: AMMI stability values (ASV) for varieties and environments

Genotype	ASV			Environment	ASV		
	TSH	TCH	Suc% cane		TSH	TCH	Suc% cane
M1176/77	0.119	2.166	0.514	ME	0.791	1.184	0.581
M1186/86	1.329	3.056	0.090	ML	1.054	1.829	0.797
M1246/84	0.509	1.855	0.571	SE	0.369	0.706	0.299
M1400/86	1.005	2.251	0.259	SL	1.029	2.751	0.755
M1551/80	0.497	0.052	0.661	TSH: tons sucrose per hectare			
M695/69	0.315	1.157	0.584	TCH: tons cane per ha			
M96/82	1.502	3.339	0.171	Suc% cane: sucrose content			
NCo376	0.954	1.266	0.807				
R570	0.247	1.542	0.413				

Table 3.11 presents the AMMI variety recommendations for each environment characterised according to yield variables (TSH, TCH and Suc% cane). For TSH and TCH, M1176/77 was the most predominant followed by M1400/86, M96/82, M1551/80 and NCo376 (commercial check). On Suc% cane, M695/69 was the leading variety followed by M1400/86, M1551/80 and NCo376.

Table 3.11: Table showing top four AMMI variety selections per environment

Sucrose yield (tons sucrose per ha, TSH)					
Environment	Environment AMMI 2 Score	1	2	3	4
ME	1.154	M1176/77	NCo376	M1551/80	M1400/86
ML	-1.029	M96/82	M1400/86	M1176/77	M1551/80
SE	0.465	M1176/77	M1400/86	M1551/80	NCo376
SL	-0.591	M1400/86	M1176/77	M1551/80	M1246/84
Cane Yield (tons cane per ha, TCH)					
ME	3.240	M1176/77	M1551/80	NCo376	M1246/84
ML	-2.456	M96/82	M1176/77	M1400/86	M1551/80
SE	0.155	M1176/77	M96/82	M1551/80	NCo376
SL	-0.938	M1176/77	M1400/86	M1246/84	M1551/80
Sucrose content (Suc% cane)					
ME	-0.705	M695/69	NCo376	M1400/86	M1551/80
ML	0.511	M1400/86	M695/69	M1246/84	R570
SE	-0.317	M695/69	M1400/86	M1551/80	NCo376
SL	0.511	M1551/80	M1400/86	M695/69	M1246/84

3.4.5 Performance of varieties in specific environments

3.4.5.1 AMMI 2 biplot model

The AMMI 2 biplots were created by plotting IPCA 1 scores against IPCA 2 scores for both varieties and environments. By interpretation, the performance of a variety in an environment is better than average if the angle between its vector and the environment's vector is less than 90° (acute angle), poorer than average if the angle is greater than 90° (obtuse angle) and near average if the angle is 90° (right angle) (Yan and Tinker, 2006). A variety closer to the point of origin was less affected by its interaction with the environment.

For TSH, the AMMI 2 biplot explained 88.4% of the total GEI (Figure 3.7). M1186/86 was positively correlated with both early harvested environments, while NCo376 was positively correlated with ME. M1176/77, M695/69 and R570 was closest to the point of origin indicating that they had minimum interaction with the environments compared to other varieties. M1400/86, M1551/80 and M1246/84 were positively associated with the SL environment. M96/82 was exclusively associated with the ML conditions.

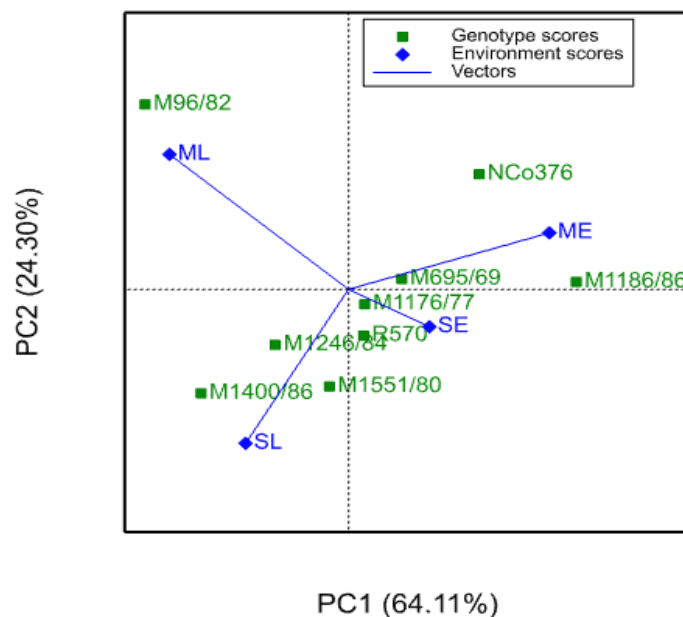


Figure 3.7: AMMI 2 biplot for IPCA 1 against IPCA 2 scores for nine sugarcane varieties and four environments averaged over five crop-years on sucrose yield (TSH)

The AMMI 2 biplot for TCH explained 94.0% of the total GEI (Figure 3.8). M1551/80 was located at the point of origin implying that it was less responsive to environmental changes on TCH. M96/82, M1186/86, NCo376 and M1246/84 were positively associated with ML, ME, SE and SL environments, respectively. M1400/86 and M695/69 were positively associated with late season environments, while M1176/77 was positively correlated with early season environments. R570 was positively correlated with both ME and SL.

The AMMI 2 biplot for Suc% cane accounted for 88.7% of the total GEI (Figure 3.9). M1186/86 was the closest to the biplot origin, indicating that it was less interactive with environment on Suc% cane. Varieties M1246/84 and R570 were positively associated with ML while M1176/77 and M1551/80 were positively associated with SL. M695/69 and NCo376 were positively correlated with early season environments. M1400/86 was positively associated with late season environments. M96/82 was positively associated with Mhlume environments.

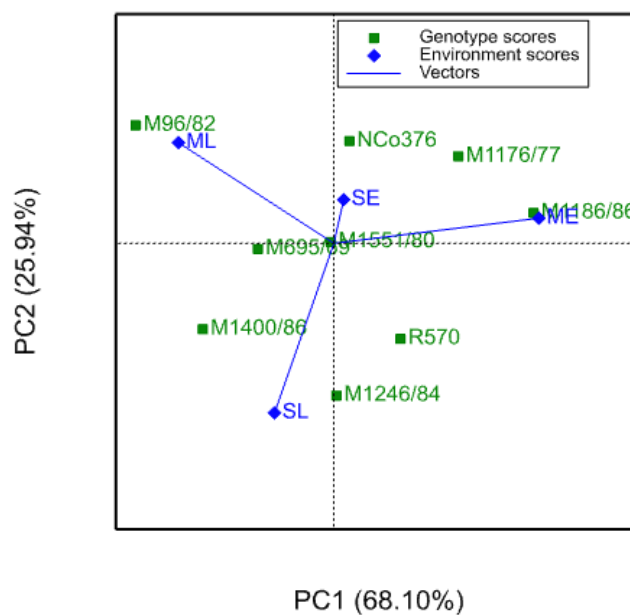


Figure 3.8: AMMI 2 biplot for IPCA 1 against IPCA 2 scores for nine sugarcane varieties and four environments averaged over five crop-years for cane yield (TCH)

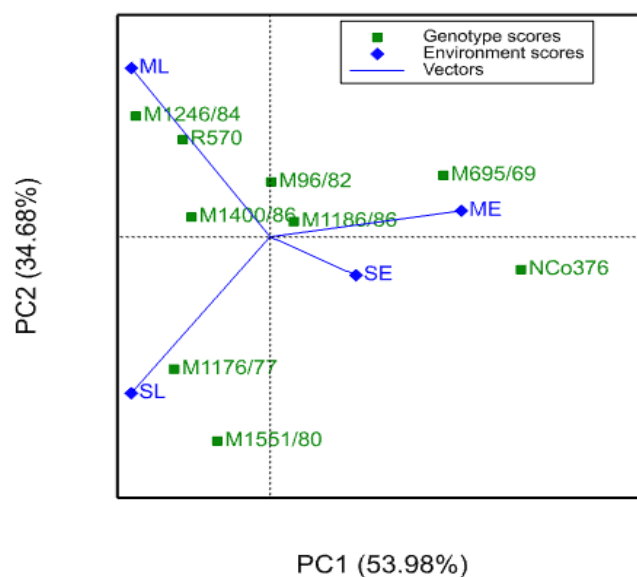


Figure 3.9: AMMI 2 biplot for IPCA 1 against IPCA 2 scores for nine sugarcane varieties and four environments averaged over five crop-year for sucrose content (Suc% cane)

3.4.5.2 GGE biplot analysis

Similar to the AMMI 2 biplot, environment vectors were drawn from the biplot origin so that the performance of varieties in each environment can be visualized. The interpretation is similar to that of the AMMI 2 biplot.

The TSH GGE biplot accounted for 91.2% of the total interaction (Figure 3.10). An equivalent AMMI 2 biplot explained 88.4% of the variation due to GEI (Figure 3.7). Varieties M1176/77, M1551/80 and NCo376 were closely associated with the early season environments, and varieties M1400/86 and M1246/84 were positively related with late season environments. M96/82 was specifically adapted to ML environment, but negatively correlated with the early season environment. While the AMMI 2 biplot linked M1186/86 with ME, and R570 with both SE and SL, the GGE biplot showed poor associations between these varieties and the environments. As previously observed, the relationships between environments was much stronger on seasons than soil types even on this biplot.

The TCH GGE biplot explained 94.5% of the total variation due to GEI (Figure 3.11), a variance almost similar to that described by a comparable AMMI 2 biplot (94.0%) (Figure 3.8). Variety M1176/77 was closely related to early season environments, and M96/82 and

M1400/86 were closely related to late season environments. M1246/84, NCo376 and M1551/80 were very close to the biplot origin indicating that their performance was stable across environments. M1186/86, R570 and M695/69 performed poorly in all environments. Even for TCH, the environments tended to group more closely according to their harvesting seasons.

The Suc% cane GGE biplot accounted for 89.9% of the GEI effect (Figure 3.12), while a similar AMMI 2 biplot explained 88.7%. Varieties NCo376 and M695/69 were closely associated with early season environments, while M1400/86, M1551/80, M1246/84 and R570 were positively associated with the late season environments. Varieties M96/82, M1176/77 and M1186/86 were negatively correlated with all the environments on Suc% cane. However, the AMMI 2 biplot (Figure 3.9) showed a positive correlation between M1176/77 and SL; M96/82 and ML; and, M1186/86 and ME. The relationship observed between environments in previous biplots was visible even in this GGE biplot (Figure 3.12). The late season environments (ML and SL) were strongly correlated as shown by their vectors running over each other. This implied that Suc% cane observed at ML for a particular variety accurately predicts performance at SL, and the other way round as well. The AMMI 2 biplot did not show such a strong relationship between these environments (Figure 3.9). The early season environments were also positively related.

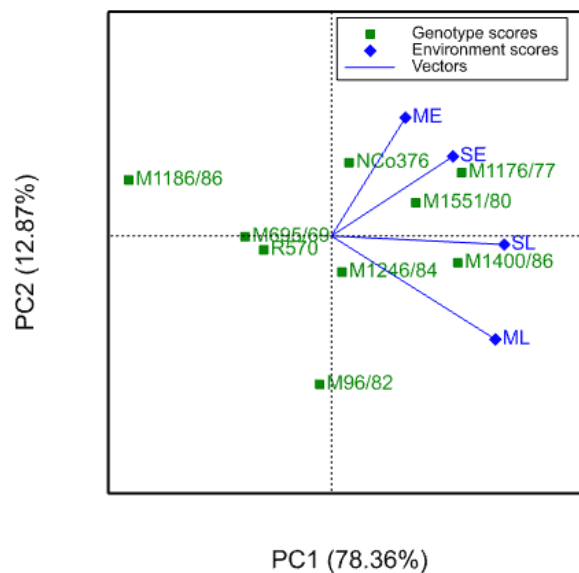


Figure 3.10: GGE biplot showing the performance of nine sugarcane varieties tested in four environments averaged over five crop-years on sucrose yield (TSH)

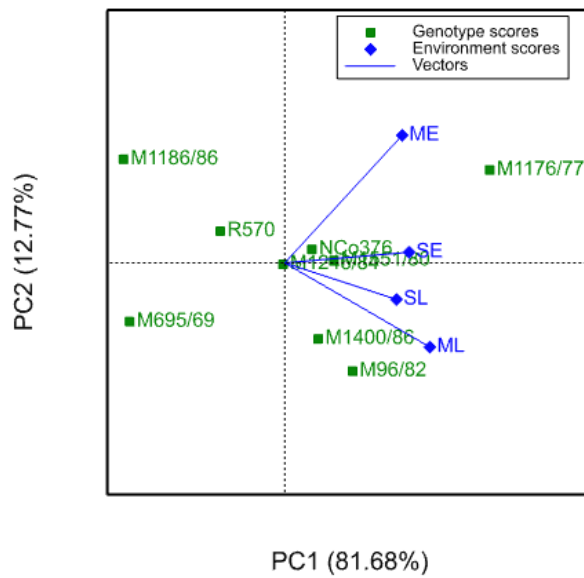


Figure 3.11: GGE biplot showing the performance of nine sugarcane varieties tested in four environments averaged over five crop-years on cane yield (TCH)

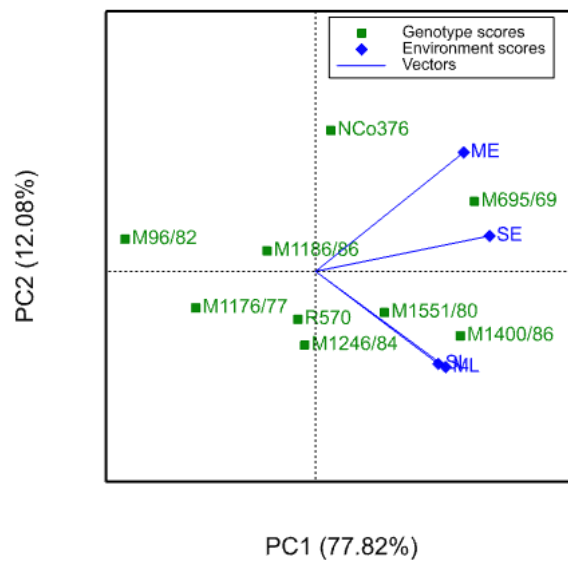


Figure 3.12: GGE biplot showing the performance of nine sugarcane varieties tested in four environments averaged over five crop-years on Suc% cane

3.4.6 Mega-environment identification

The datasets used in this study were unbalanced in that certain varieties adopted by the Swaziland sugar industry for specific conditions were included only in trials under those

conditions (refer to Table 3.2). For example, N25 is a late season variety in the industry, hence it was included only in late season trials (ML and SL). Since AMMI analysis is not able to handle unbalanced datasets, only the GGE biplot analysis was used to identify mega-environments (MGE) and the adaptiveness of the 12 varieties in a graphical form.

One of the most helpful characters of the GGE biplots is the ability to show the ‘which-won-where’ pattern of a GEI dataset (Yan and Tinker, 2006). Figures 3.13, 3.14 and 3.15 show the MGEs polygon view of GGE biplots for TSH, TCH and Suc% cane, respectively. Each biplot was divided into sectors representing different MGEs. Environments within the same MGE or sector have similar effects on the performance of varieties, as a result they are considered as a homogenous group (Ramburan and Zhou, 2011). Similarly, varieties located within the same MGE have similar responses to that particular sector. A variety located on the vertex of a sector performed better than other varieties in that MGE.

For TSH, the GGE biplot explained 94.9% of the total variation of the environment-centered two-way (GEI) table (Table 3.13). The biplot was divided into five sectors, and early season environments (ME and SE) were clustered in one MGE while the late season environments (ML and SL) were also clustered in one MGE. This pattern suggests that the target environment consists of two different MGEs, and that different varieties may be selected and deployed for each (Yan and Tinker, 2006). This observation is consistent with results presented in previous sections. Variety N40 had the highest average TSH followed closely by M1176/77 in the early season MGE, and N25 had the highest TSH followed by M1400/86 in the late season MGE. M1551/80 was located in the early season MGE, while the other varieties (M96/82, M1246/84, R570, N23, M695/69, M1186/86 and NCo376) were not associated with these MGE.

The TCH GGE biplot accounted for 96.0% of the two-way GEI (Figure 3.14). Similar to the TSH GGE biplot, this polygon was divided into five sectors, and the test environments were grouped into two different MGEs, early season and late season. Variety M1176/77 had the highest mean TCH in the early-season environment, while N25 had the highest mean TCH in the late-season environment. NCo376 and M1551/80 were located in the early season MGE. M1246/84 was located in the biplot origin indicating stable performance across MGE on TCH. All the other varieties were located in separate sectors that were not associated with the identified MGEs.

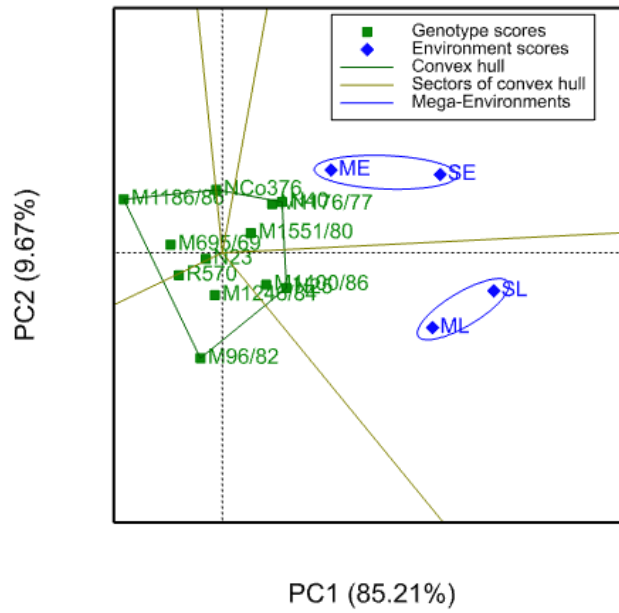


Figure 3.13: The which-won-where polygon view of the GGE biplot showing the performance of 10 sugarcane varieties tested across four environments averaged over five crop-years on sucrose yield (TSH)

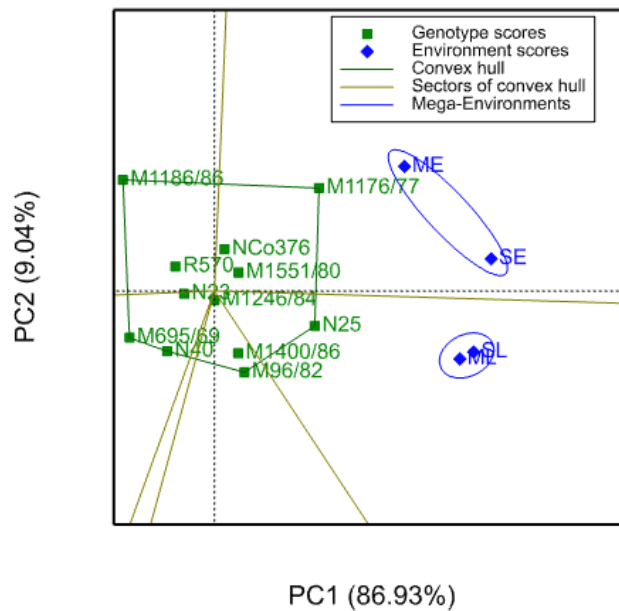


Figure 3.14: The which-won-where polygon view of the GGE biplot showing the performance of 10 sugarcane varieties tested across four environments averaged over five crop-years on cane yield (TCH)

For Suc% cane, the GGE biplot explained 95.6% of the total variation of the environment-centered two-way table of GEI means (Figure 3.15). In this biplot, even though a strong season association between environments was observed, all environments fell in one MGE as opposed to the two for TCH and TSH. This indicates that the variation across harvesting seasons for Suc% cane was not as large as it was for TCH and TSH. Variety N40 had the largest Suc% cane within this MGE followed by M695/69. Variety N23 was positioned at the biplot origin indicating that it was stable on Suc% cane across environments.

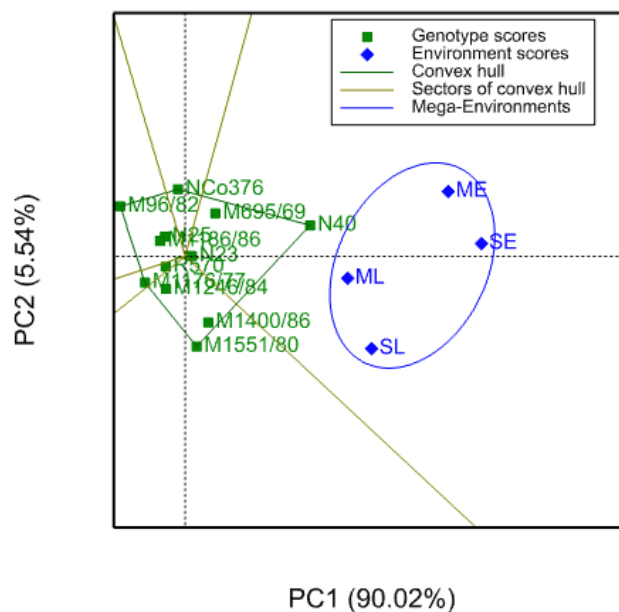


Figure 3.15: The which-won-where polygon view of the GGE biplot showing the performance of 10 sugarcane varieties tested across four environments averaged over five crop-years on sucrose content (Suc% cane)

3.5 Discussion

Results of this study provided valuable insight on the performance of Mauritian (M) varieties in different environmental conditions in Swaziland. These varieties were planted alongside standard industry (N) varieties imported from South Africa. On sucrose yield (the product of value to growers), the individual trial analysis, indicated that standard variety N40 outperformed all varieties under ME trial but was not statistically different from M1176/77. Late season, standard variety N25 outperformed all varieties under ML conditions but

performed similar to M1400/86 under SL conditions and was not significantly different from M1176/77 and M1551/80. Under SE conditions, M1176/77, M1400/86 and M1551/80 out-yielded standard varieties N23 and NCo376.

Results of the combined ANOVA indicated that environment, varieties and GEI components (genotype x location, GxL and genotype x crop-years, GxC) were highly significant for all three variables (TSH, TCH and Suc% cane) (Table 3.6). Genotype x location x crop-years (GxLxC) was significant for Suc% cane, but not TSH and TCH. Significant varietal effect was an indication that a wide range of diversity existed among the test varieties. The magnitude of variability present within the genetic material is of prime importance for a breeder to initiate an effective selection programme (Chaudhary, 2001). Significant environmental effects indicated distinctness in the different test environments. As a result, these environments represented a wide range of agro-climatic and soil conditions for assessing the performance and stability of the tested sugarcane varieties (Kassa *et al.*, 2006). Significant GxL and GxC showed that there was a change in performance ranking of varieties across locations and crop-years, respectively. This highlights that there is an opportunity of identifying varieties with higher ratooning ability suited to locations (Zhou *et al.*, 2012). The non-significance of GxLxC for TSH and TCH indicated that ratooning ability for these traits was not dependent on the location at which varieties were tested.

Environments accounted for a large portion of the variability for all three traits (TSH, 72.0%; TCH, 76.4%; and Suc% cane, 85.9%) as shown in Tables 3.7, 3.8 and 3.9. Similar results were reported by Ramburan (2011). Gauch and Zobel (1996) alleged that in normal METs, the environment accounts for 80% of the total yield variation, while variety and GEI each account for about 10%. In plant breeding, large environmental effects tends to stifle genetic improvement since heritability is severely reduced (Sandhu *et al.*, 2012). For TSH and Suc% cane, the GEI effect was larger than the varietal effect, while the opposite was true for TCH. The larger GEI for Suc% cane than varietal effect may be attributed to the fact that environments in this study consisted largely of early and late seasons which are known to affect sucrose accumulation in cane. The higher GEI effect on TSH suggests that Suc% cane had a larger effect on this secondary trait compared to TCH, since TSH is a product of both TCH and Suc% cane. The smaller GEI effect on TCH implied that varieties were relatively stable for this trait across the test environments.

The ANOVA components give valuable information on the contribution of different sources of variation towards GEI, however, it does not provide detailed information on the performance of each variety over a series of environments. The analysis of adaptability and phenotypic stability becomes very important in order to identify and recommend superior varieties in different environments (Eberhart and Russell, 1966; Tiawari *et al.*, 2011). In this study, the AMMI and GGE biplot analyses were employed to investigate the adaptability and stability of the imported varieties under Swaziland conditions. Biplots are widely used to investigate patterns in GEI, since they can help identify interesting varieties that are adapted to particular environments, and to characterise environments in groups (Malosetti *et al.*, 2013). For plant breeders, it is often difficult to determine the pattern of genetic response of different varieties across environments without the assistance of graphical techniques (Yan *et al.*, 2001). The benefit of using biplots was that the target environments were zoned into homogenous ecosystems called mega-environments (MGEs). The portioning of the target environments into MGEs was desirable because it minimises the influence of GEI and facilitates efficient breeding and in understanding patterns of the GEI. Precise recommendation of varieties for general and specific adaption requires clear understanding of the real pattern of GEI (Ahmadi *et al.*, 2012)

The general observation that environments tended to group according to harvest seasons compared to soil types was evident in all biplots (AMMI and GGE) including the 'which-won-where' GGE biplots. This emphasized that testing across seasons was more important than testing across locations. Similar findings were reported by Ramburan (2011) and Zhou (2015a). These authors proposed that establishing early and late season breeding programmes for the irrigated region would enhance genetic gains for yield and quality in each season. As a result, sugarcane varieties are classified according to their optimum harvesting seasons in most industries, that is: early, mid- and late season varieties. At present, in most sugar industries, more resources are used to test varieties across soil types while very few are allocated for testing across seasons. These therefore emphasize that some resources will have to be redirected towards setting up mid-season trials in addition to early and late trials as practised in some sugarcane industries such as Mauritius (Bissessur *et al.*, 2007), Australia (Di Bella *et al.*, 2008) and Malawi (Zhou *et al.*, 2012) to peg varieties at their most optimal production times.

On TSH, the AMMI analysis and the ASV technique identified the top performing M1176/77 as the most stable and widely adapted variety across the targeted environment. The GGE biplots indicated that the variety is closely associated with early season environments, however, it was positively correlated with all the test environments. An added advantage for M1176/77 is its classification as drought tolerant (Bissessur *et al.*, 2007). All three analytical techniques identified M1551/80 as the second most stable and widely adapted variety. An ideal variety is one that has a high mean yield performance and is highly stable (Ferreira *et al.*, 2006). Variety M1176/77 had consistently higher TSH across all environments, while M1551/80 was higher than the commercial variety (NCo376) in three of the four trials. All three analytical methods consistently indicated that M1400/86, NCo376 and M96/82 were unstable, and had specific adaptation to different environments. M1400/86 which performed continuously above the commercial variety was specifically adapted to the S (Simunye) trials. NCo376, the commercial variety, was specifically adapted to early season trials, while M96/82 was specifically adapted to ML conditions. M96/82 significantly outperformed NCo376 only in one (ML) of the four environments (Table 3.3). Varieties R570 and M695/60 were stable in all three analyses but their average TSH were below that of the commercial variety and the grand mean, hence they are not recommended for commercial cultivation.

On TCH, all three analyses (AMMI, ASV and GGE) identified M1551/80 as a stable variety. However, the GGE biplot also identified M1246/84 and NCo376 as stable varieties while the AMMI biplot and ASV indicated that they are not stable. The AMMI biplot showed that M1246/84 and NCo376 were specifically adapted to SL and SE, respectively. M1246/84 had an average TCH equal to the grand mean but less than the NCo376 (commercial check). NCo376 had an average TCH above the grand mean. M1551/80 had a higher average TCH than NCo376. Both AMMI and GGE biplots indicated that M1176/77 and M1400/86 were specifically adapted to early and late season environments, respectively. The AMMI identified M96/82 to be specifically adapted to ML, whereas the GGE showed that it was adapted to both late season conditions (ML and SL). All three varieties (M1176/77, M1400/86 and M96/82) had an average TCH above NCo376 in all four trials. While the GGE biplot indicated that varieties M1186/86, R570 and M695/69 (which had TCH below grand mean) were not associated with any environment, the AMMI biplot indicated that they had specific adaptation (M1186/86, ME; R570, SL and ME; and, M695/69, SL and ML).

On Suc% cane, M1186/86 was stable but had an average sucrose content below that of the grand mean and commercial variety. All three analyses were in agreement with this conclusion. Both AMMI and GGE biplots showed that NCo376 and M695/69 were specifically adapted to early season environments, while M1400/86 was specifically adapted to late season conditions. The GGE biplot indicated that M1551/86 was specifically adapted to late environments, whereas the AMMI biplot showed that it is adapted only to SL. NCo376 had an average Suc% cane slightly above the grand mean, whereas varieties M695/69, M1400/86 and M1551/80 had a higher average Suc% cane than NCo376. While the GGE biplot identified varieties M96/82, M1176/77, R570 and M1246/84 (which had Suc% cane below grand mean) to be associated with no particular environment, the AMMI biplot showed that they has specific adaptation (M96/82, ML and ME; M1176/77, SL; R570 and M1246/84, ML).

While there were some similarities between the results of the AMMI and GGE biplot techniques, some differences were reported. The GGE biplot explained a higher proportion of the GEI SS than the AMMI biplot in this study. For an example, with TSH, the GGE biplot explained 91.2% (Figure 3.10) while an equivalent AMMI biplot explained 88.4% (Figure 3.7) of the variation attributed to GEI. This is in agreement with Yan *et al.* (2007)'s assertion that the AMMI biplot always explains less GEI than the GGE biplot. The reason for this difference is that the AMMI analysis separates G from GEI first and then put them together again, whereas the GGE biplot analysis deals with G+GEI directly (Yan *et al.*, 2007). Malosetti *et al.* (2013) reported that the GGE biplots approximate overall performance (G+GEI), while the AMMI biplots approximate only the GEI part of the phenotype. As a result, Sandhu *et al.* (2012) argued that the GGE biplot analysis is more informative with regards to environments and variety performance than the AMMI analysis.

In a study working with advanced lines of barley, Ahmadi *et al.* (2012) concluded that there were no differences between the AMMI and GGE biplot analyses. Similarly, Rodríguez *et al.* (2010) reported that both methods were similar in showing genotype performance across environments, with differences in the contribution explained in terms GEI variance (GGE>AMMI1). Ngeve and Bouwkamp (1993) suggested that using more than one statistical stability procedure may aid in identifying successful varieties. Yan and Hunt (2001) submitted that although strategies may differ in overall appropriateness, different methods usually lead to the same or similar conclusions for a given dataset. These authors highlighted that it is the quality of the data rather than the method of analysis that is more limiting to the understanding

of GEI. It is recommended that future multi-environmental studies covering a larger number of varieties and environments than this study investigate the main factors giving rise to the differences between the AMMI and GGE biplot analyses. This will largely assist in proper interpretation and drawing of valid conclusions from these analyses taking into consideration the strengths and weaknesses of each.

Early harvested trials had higher TCH than their equivalent late harvested trials. Similar results were reported by Zhou (2004) and Ramburan (2011). For early harvested cane, the period of rapid stalk elongation coincides with the optimum summer conditions which favour cane growth, while for late season the lower TCH reflects the restriction on growth due to low winter temperature (Sweet and Patel, 1985). The summer conditions include high rainfall, radiation and temperatures. However, these conditions favouring high TCH in summer reduce the storage of sucrose on sugarcane stalks. On the contrary, a 12-month old sugarcane crop ratooned (harvested) in winter and spring allocates lower fractions of biomass to foliage and more to sucrose than crops ratooned in autumn and summer (Donaldson, 2013). This eventually leads to higher Suc% cane in the sugarcane stalks during the winter and spring seasons due to the natural ripening. As a result, in this study early harvested trials had lower Suc% cane compared to their late season equivalents. The higher TSH on late season trials implied that the higher Suc% cane on these trials had an overriding effect compared to the TCH. Similar findings were reported by Ramburan (2011).

In recent years, the expansion in area under sugarcane necessitated an extension of the harvesting period. The choice is either to start harvesting earlier or finish harvesting later than usual. An earlier start appeared to be a better proposition because the higher yields of earlier ratooned cane were expected to outweigh the disadvantages of lower sugar content due to an earlier start of crushing. However, the findings of this study did not support this conclusion since late harvested trials outperformed early harvested trials on TSH. On the other hand, stretching the harvesting period by extending the finishing time presents enormous challenges due to heavy rains at this time. As a result, growers in most sugar industries have adopted chemical ripening as a management tool to intentionally induce sugarcane ripening early season when environmental conditions hinder sucrose accumulation.

The significant yield differences between varieties within the same harvesting time in this study indicated the importance of testing varieties for optimum production times to achieve

maximum productivity throughout the harvest window. Hence, a grower should understand a variety's performance especially for sucrose content at various times throughout the harvest season to maximise productivity (Di Bella *et al.*, 2008). Gilbert *et al.* (2006) suggested that grower harvest strategies should incorporate harvesting early maturing varieties at the start of the crushing season because they are higher in sucrose content than the late maturing varieties. The uncertainty about the adaptability of released commercial varieties to time of harvest, and the reasons behind the production differences between early and late season harvests remain unclear (Ramburan, 2011). As a result, future studies will have to attempt to explain and interpret variety x season interactions in relation to genetic and environmental covariates to inform future breeding and variety recommendation strategies.

3.6 Conclusions

The main objective of this study was to assess the phenotypic stability and adaptability of Mauritian bred sugarcane varieties under Swaziland conditions. Varieties M1176/77 and M1551/80 proved to be widely adapted, hence they are recommended for commercial cultivation under all conditions. M1176/77 produced higher sucrose yield than M1551/80 in all conditions. M1400/86 was specifically adapted to high potential soil conditions both early and late harvesting seasons, as such this variety is recommended for propagation under these conditions. Currently, standard variety N25, the most popular cultivar in the industry occupies close to 50% of the total area under sugarcane. With the advent of new pests and diseases in the industry such as yellow sugarcane aphids (*Sipha flava* F.) and Tawny rust (*Puccinia fulvous* sp.), having one variety occupying such a large area presents a biosecurity threat to the industry. It is envisaged that the commercialisation of these varieties will mitigate this risk as M1176/77 and M1400/86 are good alternatives to N25. The testing environments were characterised according to harvest seasons, indicating greater variety x season interactions compared to variety x soil interactions. Since test sites in the industry are categorised according to soil types (poor, moderate and good draining), this study indicated that the number of trials across soil types may be reduced and consideration be given to increase testing across seasons (early, mid- and late). This will assist in identifying varieties suited to the different harvest seasons and in refining the industry's variety recommendation.

3.7 References

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CHAPTER 4

GENOTYPE BY ENVIRONMENT INTERACTIONS AND RESOURCE USE OPTIMIZATION IN SWAZILAND SUGARCANE TRIALS

4.1 Abstract

Multi-environmental trials (METs) are conducted to identify superior varieties adapted to broad and specific environments to inform variety recommendation programmes. However, METs are laborious and they demand more land, time and financing. The Swaziland Sugar Association (SSA) imports sugarcane varieties from the South African Sugarcane Research Institute (SASRI) and Mauritius Sugar Industry Research Institute (MSIRI) for testing and possible release as commercial varieties. The objectives of this study were to establish environmental effects on the performance of varieties and to determine the optimum combinations of locations, replications and crop-years required to provide a desired level of discrimination among varieties in the variety evaluation programme of SSA. Yield (cane [TCH] and sucrose [TSH]) and quality (sucrose [Suc% cane] and fibre [Fibre% cane] contents) data from four trials established in two locations across two seasons and replicated eight times were collected from five successive harvests (plant *plus* four ratoons). These data were analysed using the Mixed Model of GenStat[®] 17th Edition software to estimate variance components which are used to calculate broad sense heritabilities (BSH). The BSH was used to model the optimum number of locations, replications and crop-years resources.

The genotype (G) main effect was significant ($p=0.01$) and it was the largest source of variation for all the traits of interest. On the GEI variance components, GxL and GxC were significant ($p=0.01$) for TCH, TSH and Suc% cane. For TCH, GxC was larger than GxL while for TSH, GxL was larger than GxC, implying that testing across years was important for TCH while testing over many locations was important for TSH. For the quality traits, GxLxC was the largest source of variation, indicating the influence of locations on the ratooning ability of test varieties. All traits had high BSH values in the order: Fibre% cane > TCH > Suc% cane > TSH. The optimum combination of resources required to provide adequate discriminating ability among varieties was four trial locations, replicated four times and data collected over four successive crops. These findings indicate savings of four replications and a crop-year which can be effectively used to test more varieties and accelerate the speed at which varieties are released for commercial cultivation.

4.2 Introduction

Since the late 1980s, a major function of the Technical Services of the Swaziland Sugar Association (SSA) has been to import newly released smut (*Ustilago scitaminea*) tolerant irrigated sugarcane varieties and evaluate their performance in the Swaziland Lowveld (Butler, 2001). The performance and selection of these varieties is dependent upon the environmental conditions under which they are tested. The effects of variety and environment are not independent. The phenotypic response to a change in environment is not the same for all varieties, hence, phenotypic expression of a genotype depends on environment (Comstock and Moll, 1963). This change in varietal performance across different environments is called genotype by environment interaction (GEI). GEI is widely known to reduce the correlation between phenotype and genotype thus invalidating any possible genotypic inferences between different environments.

To accommodate the effect of GEI, SSA conducts a series of replicated trials across different soil types and harvesting seasons to assess the performance of newly imported sugarcane varieties. Multi-environmental trials (MET) are conducted for several crops world-wide to identify superior varieties adapted to broad and/or specific environments to facilitate effective variety recommendations. Apart from identifying superior varieties suited to particular environments, METs also provide information on the nature of the target environments, and how they should be subdivided for further selection and evaluation (Ramburan and Zhou, 2011). According to Crossa (1990), METs play an important role in plant breeding and agronomic research, and as such data from these trials have three main objectives: a) to accurately estimate and predict yield based on limited experimental data; b) to determine yield stability and the pattern of response of varieties across environments; and c) to provide reliable guidance for selecting the best varieties or agronomic treatments for planting in future years and at new sites.

However, conducting METs requires more labour, time and financing. For sugarcane, more resources are required because plots sizes for METs are generally large, crop duration is long (≥ 12.0 months) and includes the plant and several ratoon crops (\geq five crops) (Klomsa-ard *et al.*, 2013). With the current reforms and price volatility in sugar markets, and increasing production costs in the face of plateauing yield levels, sugarcane and sugar producers are compelled to review spending patterns. In such instances budget allocation for research and

development (R&D) is severely reduced. According to Flaherty and Carmichael (2014), agricultural R&D spending as a share of agricultural gross domestic products (GDP) continues to be quite high in small countries because research infrastructure and staffing are expensive given the comparatively small size of the agricultural sector.

A quantitative measure which provides information about the correlation between genotypic variance and phenotypic variance is heritability (Dabholkar, 1999). The concept of heritability originated as an attempt to describe whether differences actually observed between individuals rose from the differences in genetic makeup between the individuals, or resulted from different environmental forces (Hanson, 1963). Heritability measures the relative degree to which a character is transmitted from parent to progeny (Bora *et al.*, 2014). The ability to detect significant differences between means of varieties in an experiment depends on the variance associated with the means (Zhou *et al.*, 2012). The analysis of variance components (VCs) is one of the procedures used to estimate heritability (Ramburan and Zhou, 2011). VCs analyses have been used widely to evaluate the relative contributions of relevant terms to variability and to assess the precision of trial networks to optimise resources (Ceretta and van Eeuwijk, 2008).

Brown and Glaz (2001) used VCs to assess the allocation of resources (replications, harvests and planting years) in the final selection stage of Florida Canal Point sugarcane breeding programme. Rattey and Kimbeng (2001) and Kimbeng *et al.* (2009) employed VCs to determine the most appropriate combinations of locations, replicates and crop-years to provide an adequate level of differentiating between sugarcane genotypes in the final assessment trials for the Burdekin district in Australia and Rio Grande Valley Region of Texas, respectively. Zhou *et al.* (2012) utilised VCs to establish the optimum number of replications and crop-years that provide adequate discriminating ability for trials at Dwangwa Sugar Estate in Malawi. Swallow and Wehner (1989) working on cucumber trials, used VCs to ascertain how experimental resources can be allocated over years, seasons, locations, and replications to furnish as much information as possible in a cost effective manner. VC analysis was conducted at Ohio State University to determine how best to allocate resources among environments, replications, and subsamples (heads per plot) in *Fusarium* head blight (FHB) screening nurseries of wheat (Campbell and Lipps, 1998). Issa (2009) used VCs to predict the optimum allocation of replications, locations and years in maize yield in Ethiopia.

In Swaziland, all of the sugarcane is grown under irrigation and the crop is traditionally planted and harvested over five crops (plant and four ratoon crops) on average. In the variety evaluation programme (VEP) of SSA, trials are conducted in three main areas (Mhlume, Simunye and Big Bend) representing the major soils of the industry. Ideally, the trials should be equally spread over three seasons (early: April to July; mid; August to September; and, late: October to November) per area, however, due to resource scarcity this does not happen. Replications per trial normally range from four to eight, while crop-years/ratoons range between five and seven years. Apart from the work done by Zhou *et al.* (2012), there is no evidence of any documented study done by sugarcane growing industries to ascertain the optimum combination of these resources (locations, replications and crop-years) required to provide sufficient level of discrimination between test varieties in post-release variety trials. Hence this study sought to close this knowledge gap by utilising VCs and heritability. It is envisaged that the results thereof will inform SSA and post-release variety testing strategies worldwide.

4.3 Materials and methods

4.3.1 Experimental design

Data used in this study were obtained from SSA trials established for the purpose of identifying high sucrose yielding, pests and diseases resistant Mauritian varieties that are well-adapted to Swaziland conditions. Between 2004 and 2005, four replicated trials were established at two SSA trial sites located at Mhlume and Simunye. Two trials were set up at Simunye field S604 under a Latin square design, each replicated ten times (100 plots) with gross plot and net plot sizes of 97.5 m² (13.0 m row length) and 49.5 m² (11.0 m row length), respectively. The other two trials established at Mhlume field M428 were laid out as a randomised complete block design replicated eight times (80 plots) with gross plot and net plot sizes of 114.0 m² (19.0 m row length) and 51.0 m² (17.0 m row length), respectively. In all trials, plot spacing (distance between plots) ranged from 1.0 to 2.0 m while inter-row spacing was maintained at 1.5 m. A 1.0 m end of row effect was provided for in all plots. To have balanced data for analysis, the last two replicates for the Simunye trials were ignored, hence, only eight replicates were considered.

4.3.2 Site selection and description

Each trial site represented the major soil characteristics prevalent within the SSI, these being poor draining and good draining. The Simunye trials represented good draining and moderate

to well-structured soils (R-set). On the other hand, the Mhlume planted trials represented poor draining and weakly-structured / duplex soils (Z-set). On each site, one trial was set up for harvesting within the early season (April to June) and the other in the late season (October to December), and they were established side-by-side within the same soil type. In this study, trial site/soil type x harvesting season (crop-year) represented a specific location. In each location, eight Mauritian varieties (R570, M1176/77, M1186/86, M1246/84, M1400/86, M1551/80, M695/69 and M96/82) were planted alongside South African variety NCo376. All trials were managed as per standard estate practices including fertiliser applications, irrigation and weed control.

4.3.3 Data collection

Yield data were collected at harvesting (12 months cycle) on a plot basis for seven consecutive crops (plant *plus* six ratoons). However, for this study only the first five crops (plant *plus* four ratoons) were considered because for a majority of the poor draining soils maintaining the crop number beyond five becomes unsustainable due to heavily declined yields. To remove the extraneous matter, the cane was burnt a day prior to harvesting. On the day of harvesting, the end-row effects were cut and removed from the net plots and placed in a windrow together with cane from the guard rows. The cut cane was topped according to estate practice which is usually below the natural breaking point. Cane from the net rows was also cut and placed in heaps manageable to the grab mounted on a tractor carrying the digital scale for measuring cane weight. After weighing, a total of 16 stalks per plot were sampled at random from within each heap to determine sucrose content (Suc% cane) and fibre content (Fibre% cane) at the laboratory using standard protocol explained by Shoonees-Muir *et al.* (2009). The secondary trait, tons sucrose per hectare (TSH), was calculated as the product of tons cane per hectare (TCH) and Suc% cane.

4.3.4 Data analysis

All the experimental design factors were considered to be random in this analysis. The sugarcane varieties, although chosen, were considered to represent a random sample of several other varieties that could be tested in the SSA VEP. The same applied to environments, although they were selected to represent sugarcane growing areas differing in soil characteristics plus harvest seasons, together with crop-years they represent a random sample of sugarcane growing environments in Swaziland. To establish the genetic correlations among

the test environments (two locations x two harvest-seasons x five crop-years), the data were subjected to a principal component analysis (PCA) for ease of interpretation. The PCAs showed that correlations were generally higher among crop-years within the same location compared to crop-years across locations, however, the correlation between the crop-years within the same location were not in any particular order (data not shown), hence favouring the analytical approach explained below. A combined ANOVA for the first five crops (plant *plus* four ratoons) data was performed for tons cane per ha (TCH), sucrose content (Suc% cane), tons sucrose per ha (TSH) and fibre content (Fibre% cane) using a random model proposed by Rattey and Kimbeng (2001) and Kimbeng *et al.* (2009):

$$Y_{ijkl} = \mu + L_l + R(L)_{kl} + G_i + GL_{il} + GR(L)_{ikl} + C_j + LC_{jl} + CR(L)_{jkl} + GC_{ij} + GLC_{ijl} + E_{ijkl} \quad (1)$$

Where, Y_{ijkl} is the observation for genotype i , in crop-year j , in rep k nested within location l ; μ is the overall mean; L_l is the effect of the l th location; $R(L)_{kl}$ is the effect of the k th rep nested within the l th location (Error 1); G_i is the effect of the i th genotype; GL_{il} is the interaction effect between the i th genotype and l th location; $GR(L)_{ikl}$ is interaction effect between the i th genotype and the k th rep nested within the l th location (Error 2); C_j is the effect of the j th crop-year; LC_{jl} is the interaction effect between the l th location with the j th crop-year; $CR(L)_{jkl}$ is the interaction effect between the j th crop-year and the k th rep nested within the l th location (Error 3); GC_{ij} is the interaction effect between the i th genotype and j th crop-year; GLC_{ijl} is the interaction effect between the i th genotype, l th location and j th crop-year; and E_{ijkl} is the residual term (Error 4). All significant differences were evaluated at $p \leq 0.05$. The model is defined to split all variance components so that they are separated as much as possible, thereby allowing for an evaluation of the variance components of interest (Ramburan and Zhou, 2011).

The data were analysed using the Mixed Model (Restricted Maximum Likelihood, REML) of GenStat® 17th Edition statistical software (VSN International, 2015). Variance components with their approximate standard errors were estimated from this analysis. The variance components were used to estimate the variance of a genotype mean (V_k) for different combinations of locations, replications and crop-years within a location (Fehr, 1987):

$$V_k = \frac{\sigma_E^2}{rlc} + \frac{\sigma_{GLC}^2}{lc} + \frac{\sigma_{GL}^2}{l} + \frac{\sigma_{GC}^2}{c} \quad (2)$$

Where, σ_E^2 is the variance component for the residual term in the model; σ_{GLC}^2 is the variance component for the interaction between genotype, location and crop-year; σ_{GL}^2 is the variance component for the interaction between genotype and location; σ_{GC}^2 is the variance component for the interaction between genotype and crop-year; and, r, l and c are numbers of replications per trial, locations and crop-years, respectively.

From the above equation, it was possible to calculate broad sense heritability (BSH) or genetic repeatability (h^2):

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + V_k} \quad (3)$$

Where, σ_G^2 is the variance component for the main effect of genotype. Using equations 1 and 2, the number of replications was increased from one to eight, the number of crop-years was increased from plant to seven ratoons, and the number of locations was increased from one to eight for each yield variable to model the changes in BSH (Zhou *et al.*, 2012). To determine the optimum number of each resource (replications, crop-years and locations), the change in BSH due to a change in the number of each resource was portrayed in graphical format.

4.4 Results

The variance component of the genotype main effects (G) was significant ($p=0.01$) and it was the largest source of variance for all the four traits (Table 4.1). The variance components GxL and GxC were significant ($p=0.01$) for TCH, TSH and Suc% cane, but were not significant ($p=0.05$) for Fibre% cane. The variance component GxLxC was significant for Suc% cane ($p=0.01$) but it was not statistically significant ($p=0.05$) for TCH, TSH and Fibre% cane. Fibre% cane had the highest BSH (91.91), followed by TCH (84.81) while Suc% cane (82.90) and TSH (79.90) had the least BSH in sequence.

Among the GEI variance components, GxC and GxL were the largest sources of variance for TCH and TSH, respectively, compared to GxLxC (Table 4.2). This finding is different from that of Kimbeng *et al.* (2009) who reported that GxLxC accounted for the largest source of the GEI variance for these traits (TCH and TSH). For TCH, variance component GxC was larger than GxL, implying that testing over years was more important for this trait than testing over

locations, and the opposite was true for TSH. Kimbeng *et al.* (2009) reported that for both TCH and TSH, it was important to test for many years (ratooning ability) than over locations.

Table 4.1: Variance components (VC) \pm standard error (S.E) and broad sense heritabilities (BSH) for tons of cane per hectare (TCH), tons sucrose per hectare (TSH), sucrose content (Suc% cane) and fibre content (Fibre% cane)

Effect	TCH		TSH		Suc% cane		Fibre% cane	
	VC	S.E	VC	S.E	VC	S.E	VC	S.E
G	50.00	$\pm 29.6^{**}$	0.903	$\pm 0.578^{**}$	0.194	$\pm 0.118^{**}$	0.562	$\pm 0.296^{**}$
GxL	13.60	$\pm 5.40^{**}$	0.410	$\pm 0.166^{**}$	0.051	$\pm 0.029^{**}$	0.023	± 0.048
GxC	16.90	$\pm 5.90^{**}$	0.374	$\pm 0.146^{**}$	0.079	$\pm 0.035^{**}$	0.058	± 0.035
GxLxC	9.20	± 3.90	0.107	± 0.120	0.098	$\pm 0.034^{**}$	0.068	± 0.096
BSH	84.81		79.90		82.90		91.91	

*significant at $p=0.05$ **significant at $p=0.01$ G: genotype; GxL: genotype by location; GxC: genotype by crop-years; GxLxC: genotype by location x crop-years

For the quality traits (Suc% cane and Fibre% cane), GxLxC was the largest source of variance compared to GxC and GxL, implying that locations influenced the ratooning ability of the test cultivars (Table 4.2). The GxLxC:G ratio (0.51) was disproportionately larger for Suc% cane than for the other traits. This was so because locations in this study comprised of soil type and harvesting season, and it is known that cane quality is largely influenced by season of harvest. Similar results were reported by Zhou *et al.* (2012) that genotype x season x crop-year (GxSxY) was the largest variance component for quality traits compared to GxS and GxC. On the contrary, Kimbeng *et al.* (2009) reported that for Suc% cane, GxC was the largest source of variance followed by GxLxC and GxL, in sequence. In this study, testing cultivars over years (GxC) was more important for the quality traits than testing over locations (GxL). The GEI:G ratio for Suc% cane was the highest, implying that GEI was larger for this trait compared to the others. The lower GEI:G ratio for Fibre% cane indicated that fibre content was more dependent on genotype than environment hence the large BSH value (Table 4.1).

In this study, to assess the relative importance of various combinations of three resources (replications, crop-years and location) in estimating a genotype mean, the broad sense heritability (BSH) was used. By altering the number of each resource in equation 2, it was possible to determine the resources that would be required to detect repeatable differences among the varieties (Kimbeng *et al.*, 2009), and these were portrayed in graphical form. The multiple curves in each graph show the change in BSH as the combination of resources is

altered. The ideal curve is the one where the change in BSH is very minimal when an additional resource is added, and it is identified by the relatively close proximity with the next curve.

Table 4.2: Variance components as a proportion of the genotype main effect for sugarcane yields (tons cane per hectare, TCH; tons sucrose per hectare, TSH) and quality traits (sucrose content, Suc% cane; fibre content, Fibre% cane)

Trait	GEI	GEI:G	GxL:G	GxC:G	GxLxC:G	GEI ranking
TCH	39.70	0.79(100)	0.27(34)	0.34(43)	0.18(23)	GxC>GxL>GxLxC
TSH	0.89	0.99(100)	0.45(46)	0.41(42)	0.12(12)	GxL>GxC>GxLxC
Suc%	0.23	1.18(100)	0.26(22)	0.41(35)	0.51(43)	GxLxC>GxC>GxL
Fibre%	0.15	0.27(100)	0.04(15)	0.10(39)	0.12(46)	GxLxC>GxC>GxL

Values are expressed as a ration (or percent); GEI: genotype by environment interaction; GxL: genotype by location; GxC: genotype by crop-years; GxLxC: genotype by location x crop-years

For all the traits, the optimum number of replications was determined by plotting the change in BSH with increasing number of replications from one to eight. For TCH, increasing replications beyond four did little to increase the BSH values (Figure 4.1). This was consistent in all the four crops (plant and three ratoon crops) shown. For TSH, there were marginal increases in BSH values beyond replication five for the two younger crops (plant and first ratoon crops), while marginal increases in BSH values beyond four were observed for the second and third ratoon crops (two older crops) (Figure 4.2). For Suc% cane, there were marginal increases in BSH values beyond replications four and three for the two younger crops and two older crops, respectively (Figure 4.3). For Fibre% cane, increasing replications beyond four did little to increase BSH values (Figure 4.4). This observation was consistent across all the crops (plant and three ratoon crops). On average, the optimum number of replications required to detect differences among cultivars was four. A general observation in all the traits was that increasing location number beyond six and ratoon crops to two or three provided an opportunity to reduce the replication numbers by one unit.

To determine optimum test locations, the trends in BSH were plotted from plant to seventh ratoon for all four sugarcane traits. Increasing the number of locations beyond four resulted in very minimal increments in the BSH values for all the traits (TCH: Figure 4.5; TSH: Figure 4.6; Suc% cane: Figure 4.7; and Fibre% cane: Figure 4.8). This trend was observed consistently over all the four crop-years. Therefore, the four test locations used in this study was adequate

to discriminate between genotypic means. To establish the optimum number of crop-years, the trends in BSH were also plotted from one to eight. Increasing crop-years beyond the third ratoon (fourth crop-year) resulted in marginal increases in BSH values for all the four sugarcane traits (TCH: Figure 4.9; TSH: Figure 4.10; Suc% cane: Figure 4.11; and Fibre% cane: Figure 4.12). These results indicate that four crop-years were sufficient to detect repeatable differences among the sugarcane varieties.

To further identify the optimum number of replications, locations and crop-years, an evaluation of the unit increase in BSH values was done for all four traits as shown in Table 4.3. For each of the resources across all four traits, there were very minimal gains in BSH values of two units or less when crop-years, replications and locations were below four.

Table 4.3: Broad sense heritability (unit increase in BSH with additional resources in parentheses) for every additional resource while other resources were kept unchanged

No.	Locations				Replications				Crop-Years			
	TCH	TSH	Suc %	Fibre %	TCH	TSH	Suc %	Fibre %	TCH	TSH	Suc %	Fibre %
1	66	57	63	77	70	63	69	69	62	55	57	71
2	77	70	75	87	78	72	76	81	74	69	71	83
	(11)	(13)	(12)	(10)	(8)	(9)	(7)	(12)	(12)	(14)	(13)	(12)
3	82	76	80	90	81	75	79	85	80	74	77	88
	(5)	(6)	(5)	(3)	(3)	(3)	(3)	(4)	(6)	(5)	(6)	(5)
4	85	80	83	92	82	77	81	88	83	78	81	90
	(3)	(4)	(3)	(2)	(1)	(2)	(2)	(3)	(3)	(4)	(4)	(2)
5	86	82	85	93	83	78	82	89	85	80	83	92
	(1)	(2)	(2)	(1)	(1)	(1)	(1)	(1)	(2)	(2)	(2)	(2)
6	88	84	86	94	86	79	82	90	86	81	85	93
	(1)	(2)	(1)	(1)	(1)	(1)	(0)	(1)	(1)	(1)	(2)	(1)
7	88	85	87	94	84	79	83	91	87	82	86	94
	(0)	(1)	(1)	(0)	(0)	(0)	(1)	(1)	(1)	(1)	(1)	(1)
8	89	86	87	94	85	80	83	92	88	83	87	94
	(1)	(1)	(1)	(0)	(0)	(1)	(0)	(1)	(1)	(1)	(1)	(0)

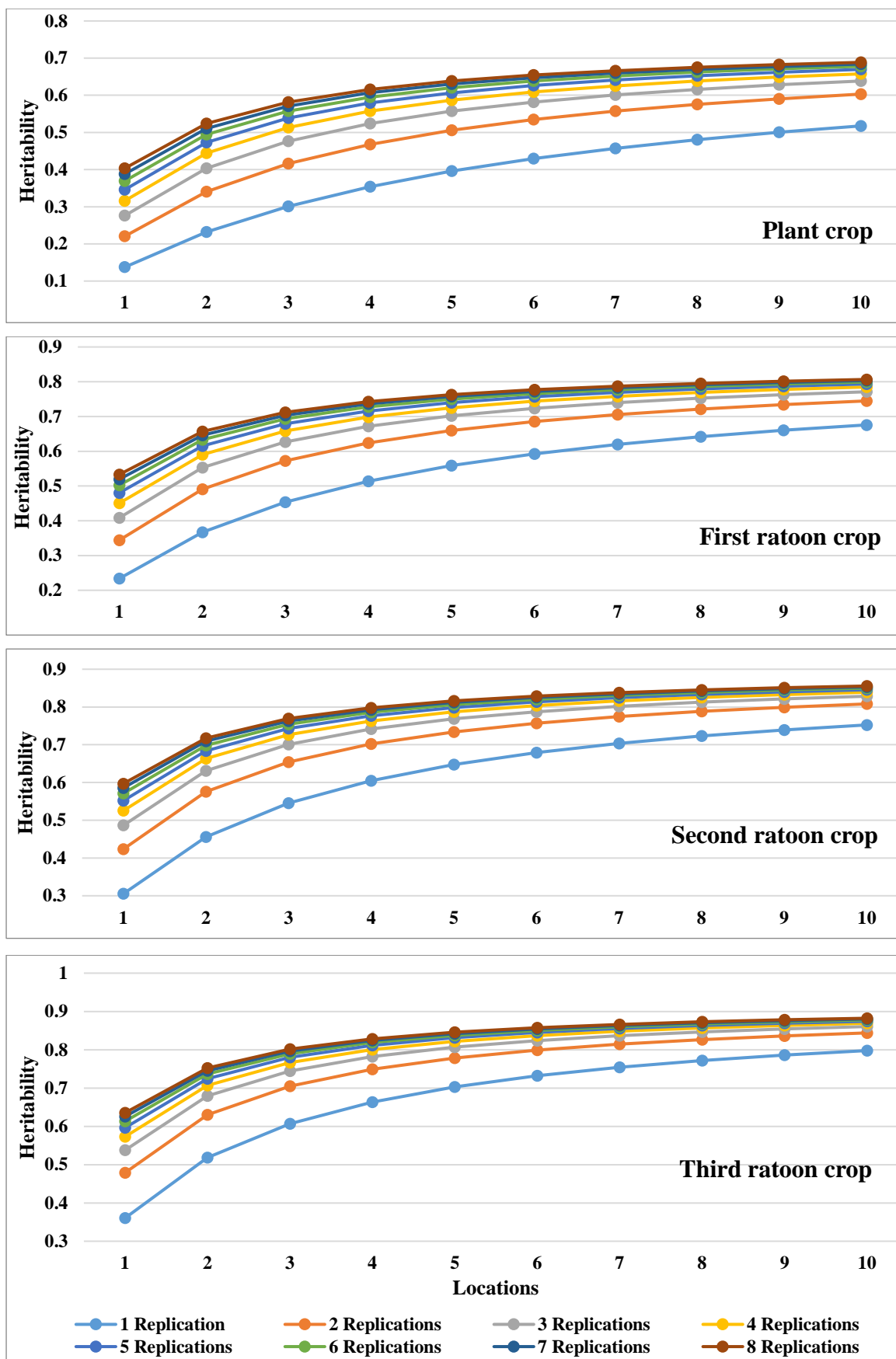


Figure 4.1: Replication effect on the broad sense heritability values for tons cane per hectare

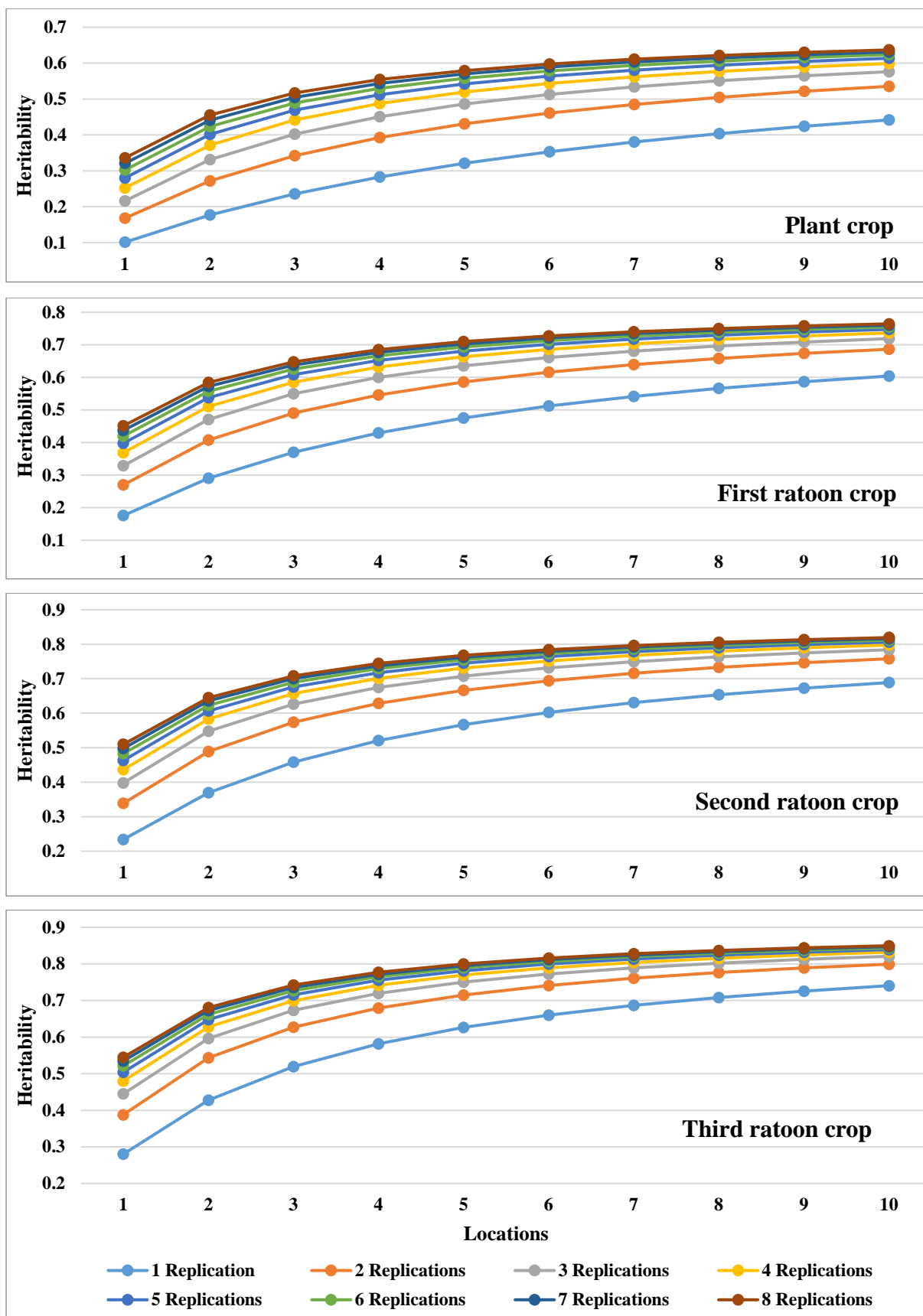


Figure 4.2: Replication effect on the broad sense heritability values for tons sucrose per hectare

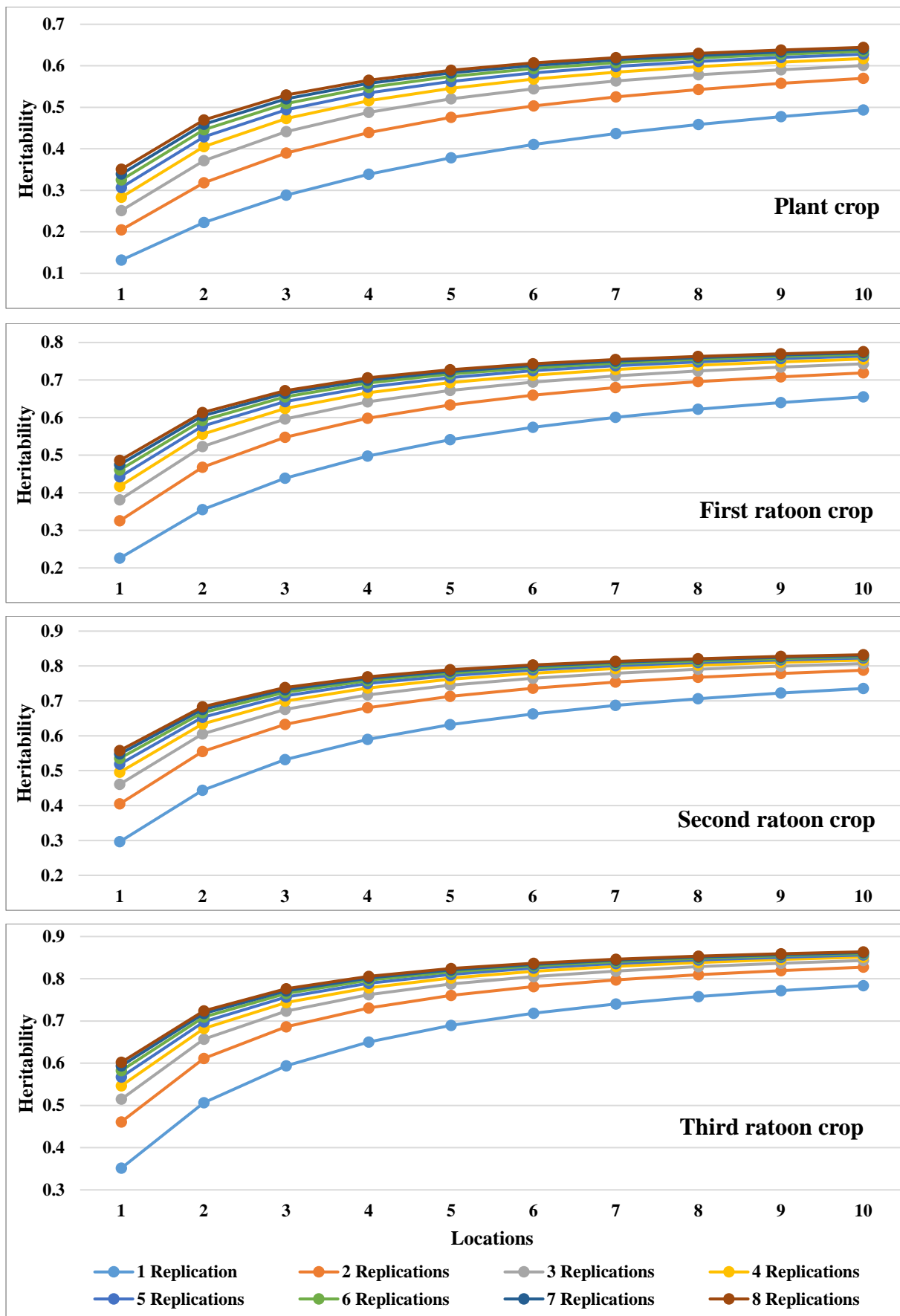


Figure 4.3: Replication effect on the broad sense heritability values for Suc% cane

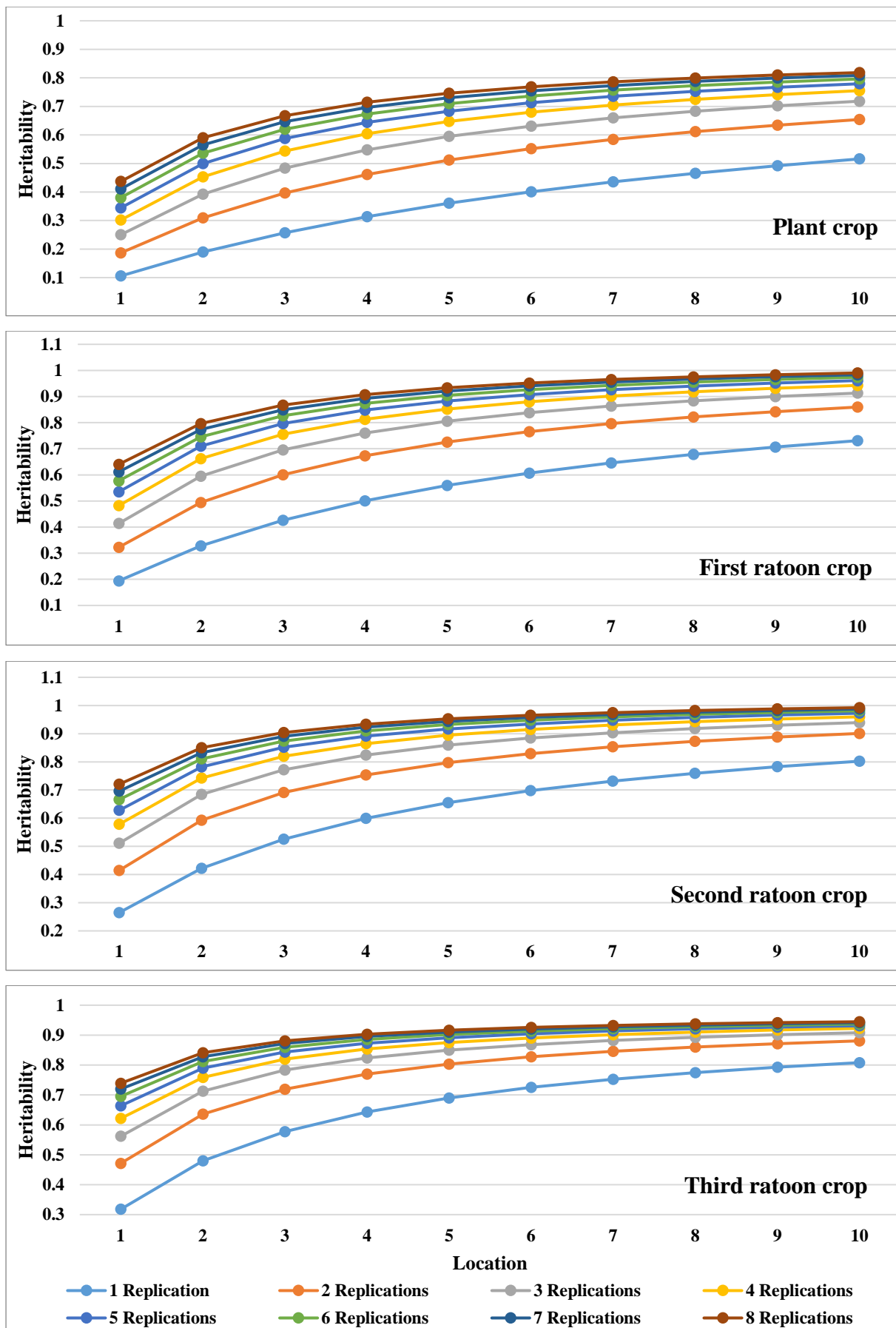


Figure 4.4: Replication effect on the broad sense heritability values for Fibre% cane

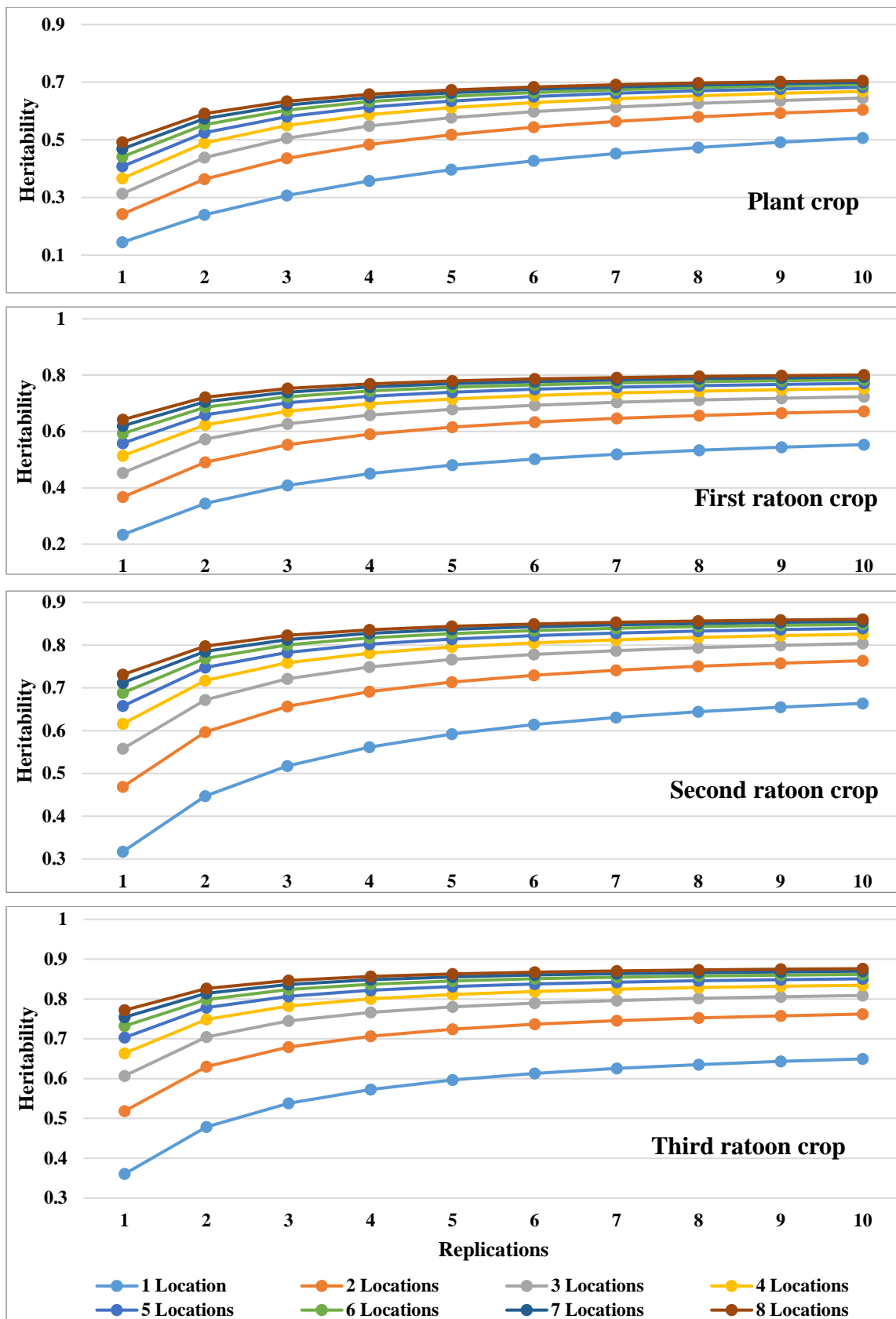


Figure 4.5: Location effect on the broad sense heritability values for tons cane per hectare (TCH)

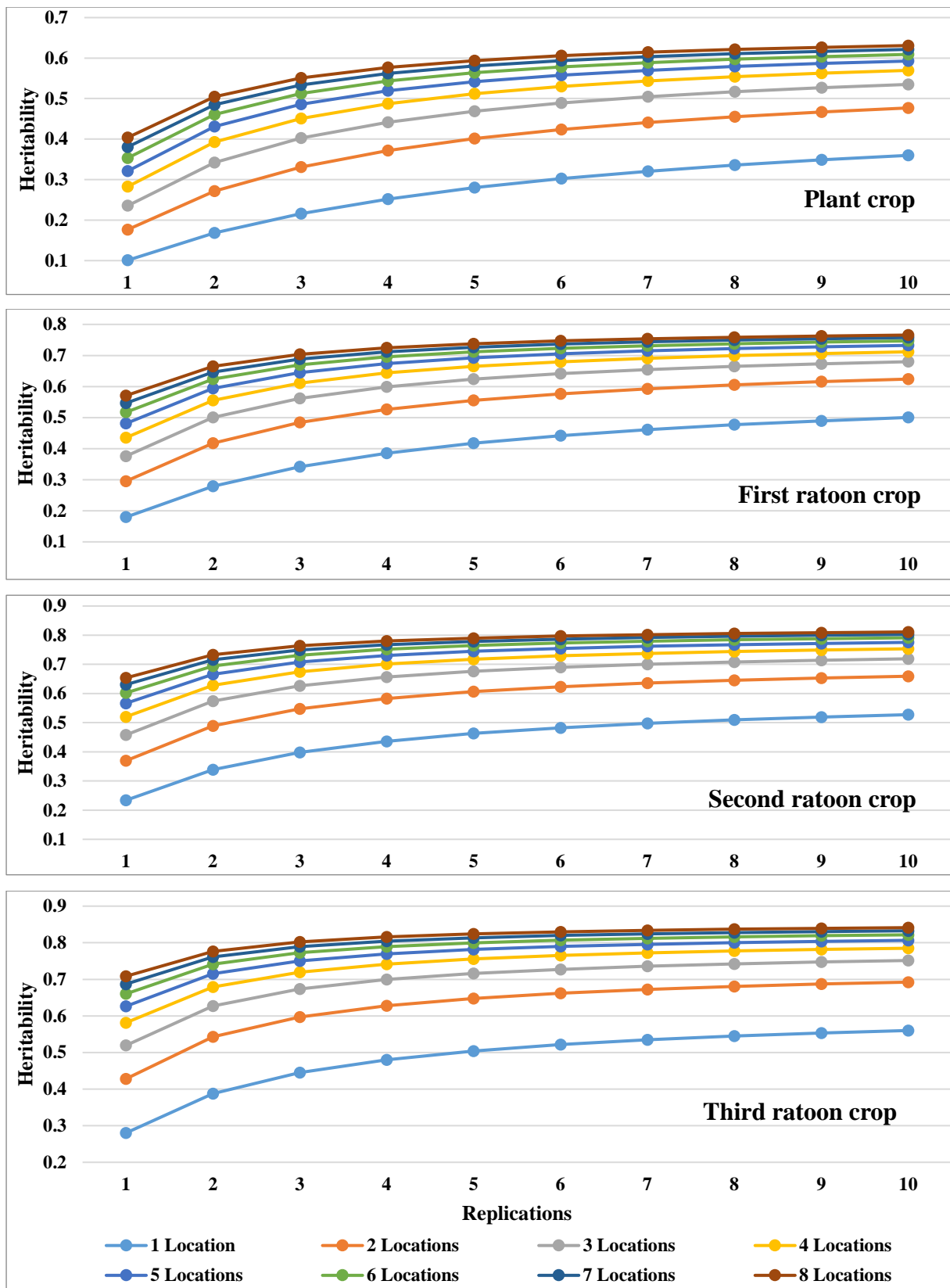


Figure 4.6: Location effect on the broad sense heritability values for tons of sucrose per hectare (TSH)

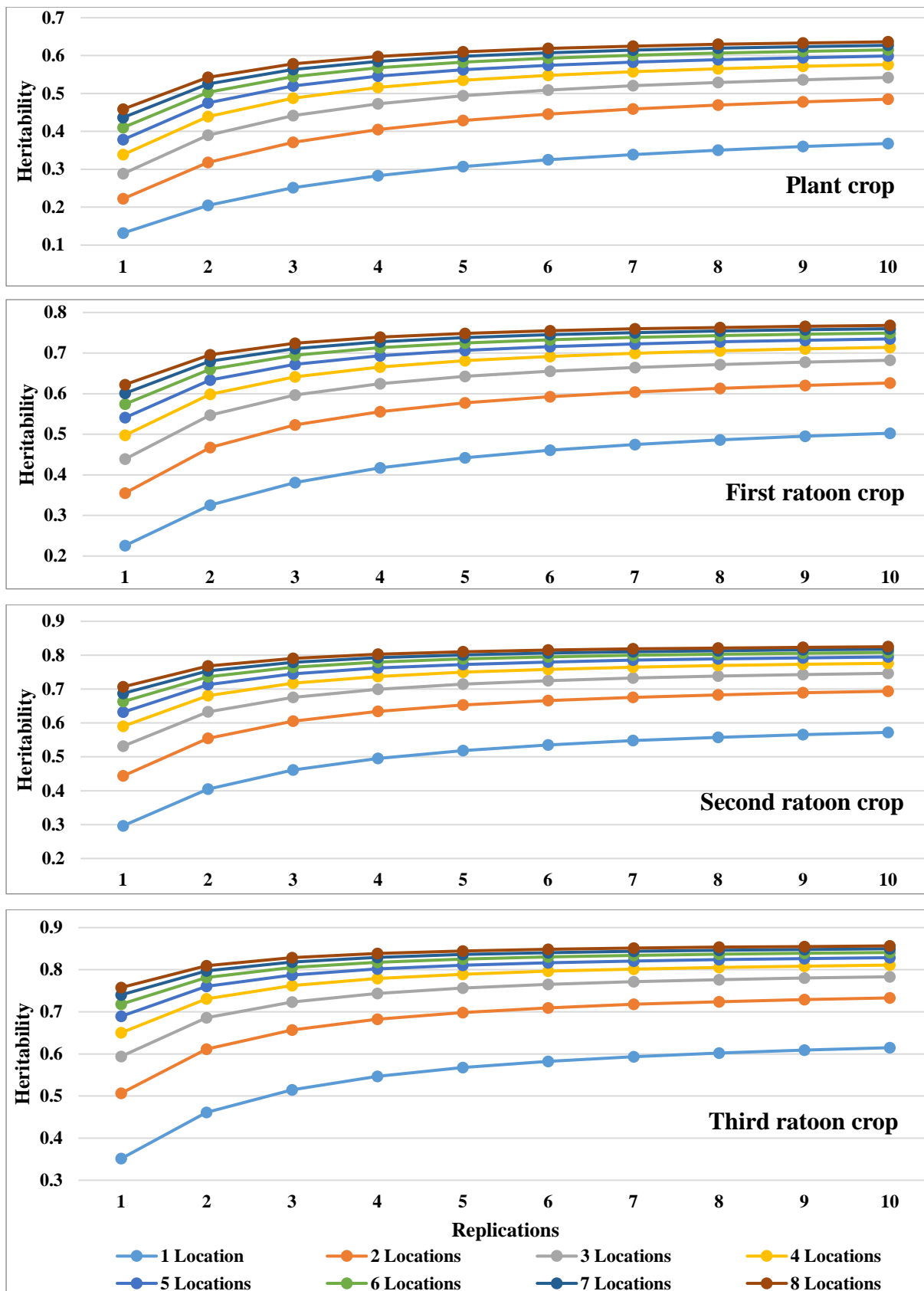


Figure 4.7: Location effect on the broad sense heritability values for Suc% cane

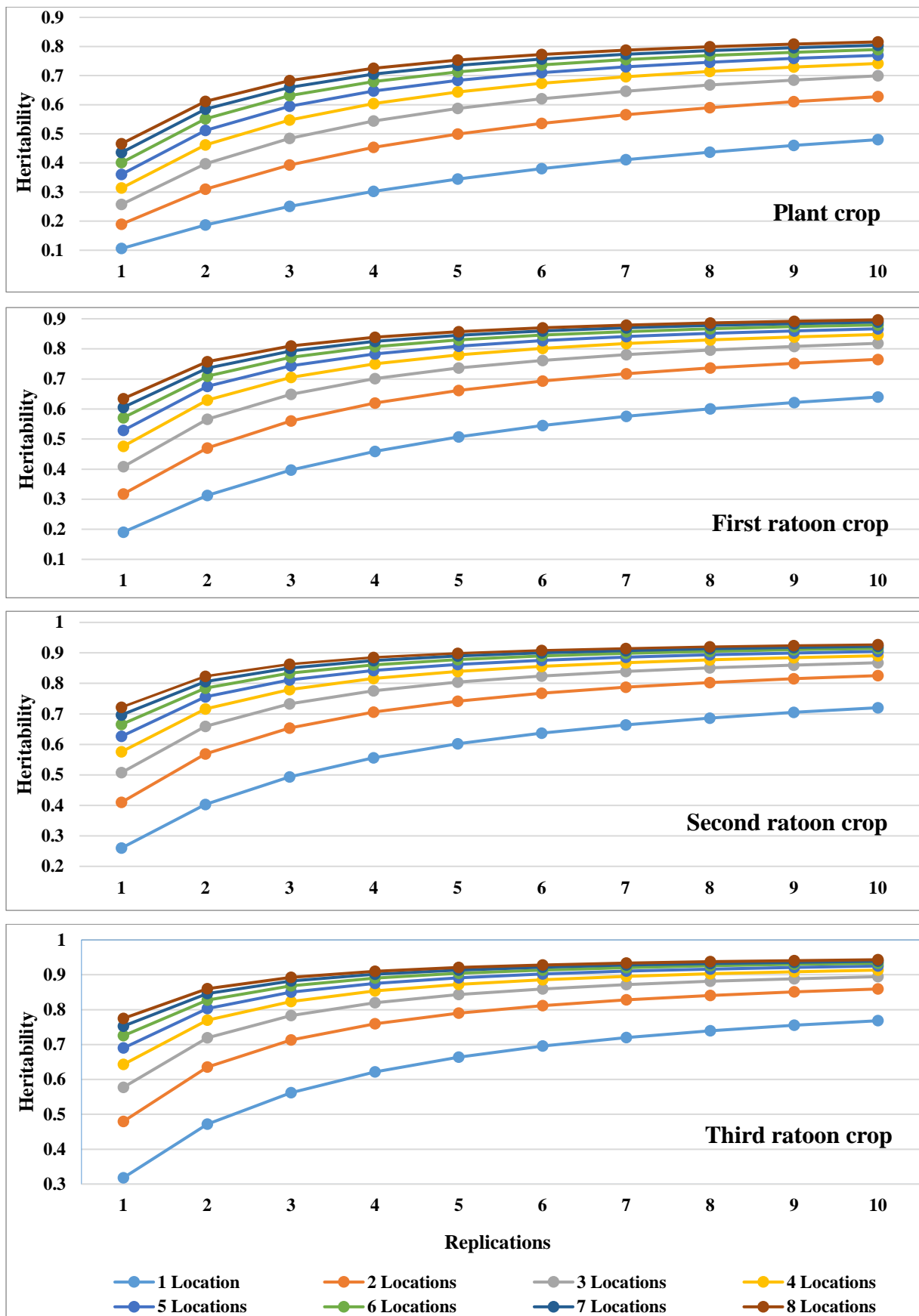


Figure 4.8: Location effect on the broad sense heritability values for Fibre% cane

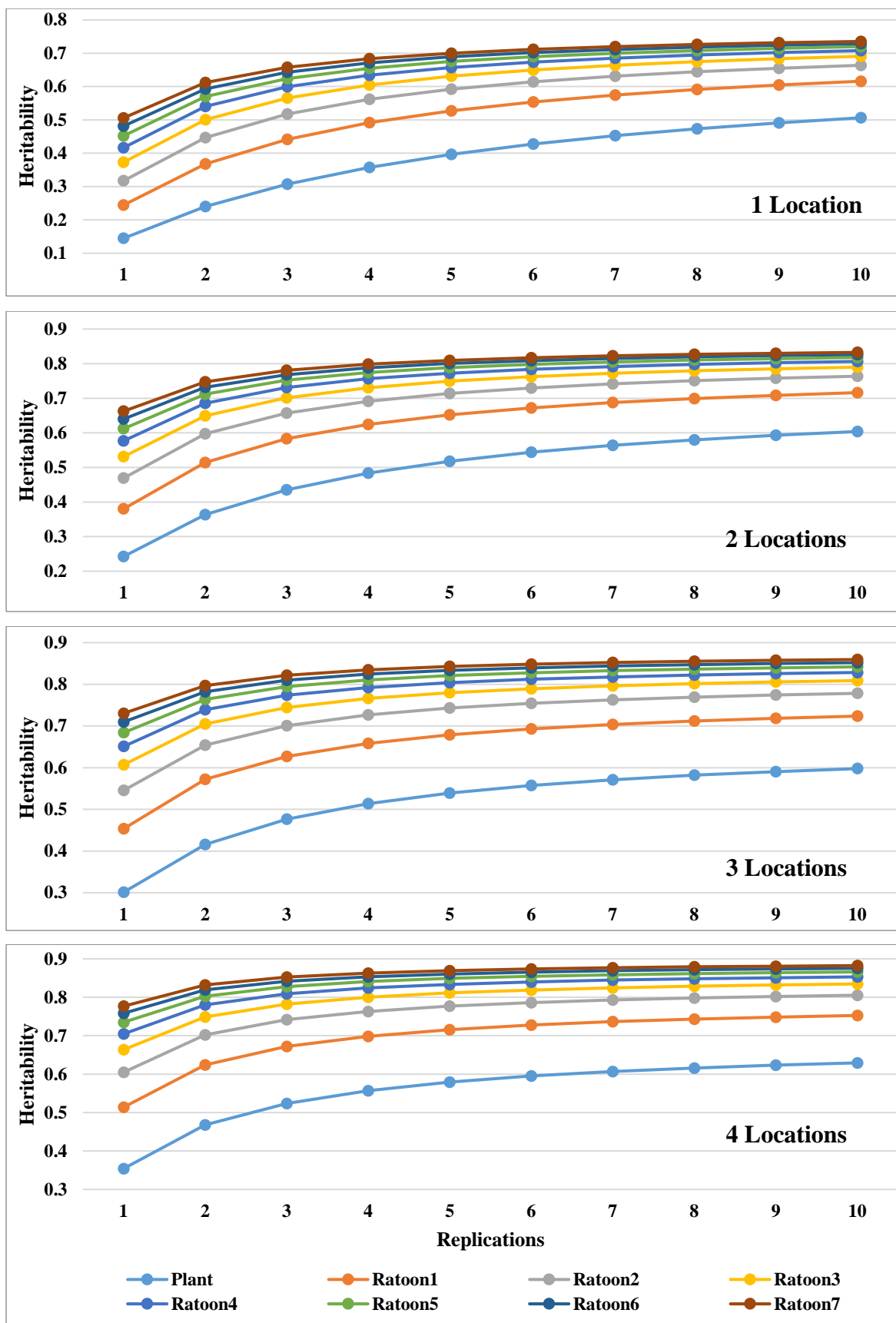


Figure 4.9: Crop-year effect on the broad sense heritability values for tons cane per hectare (TCH)

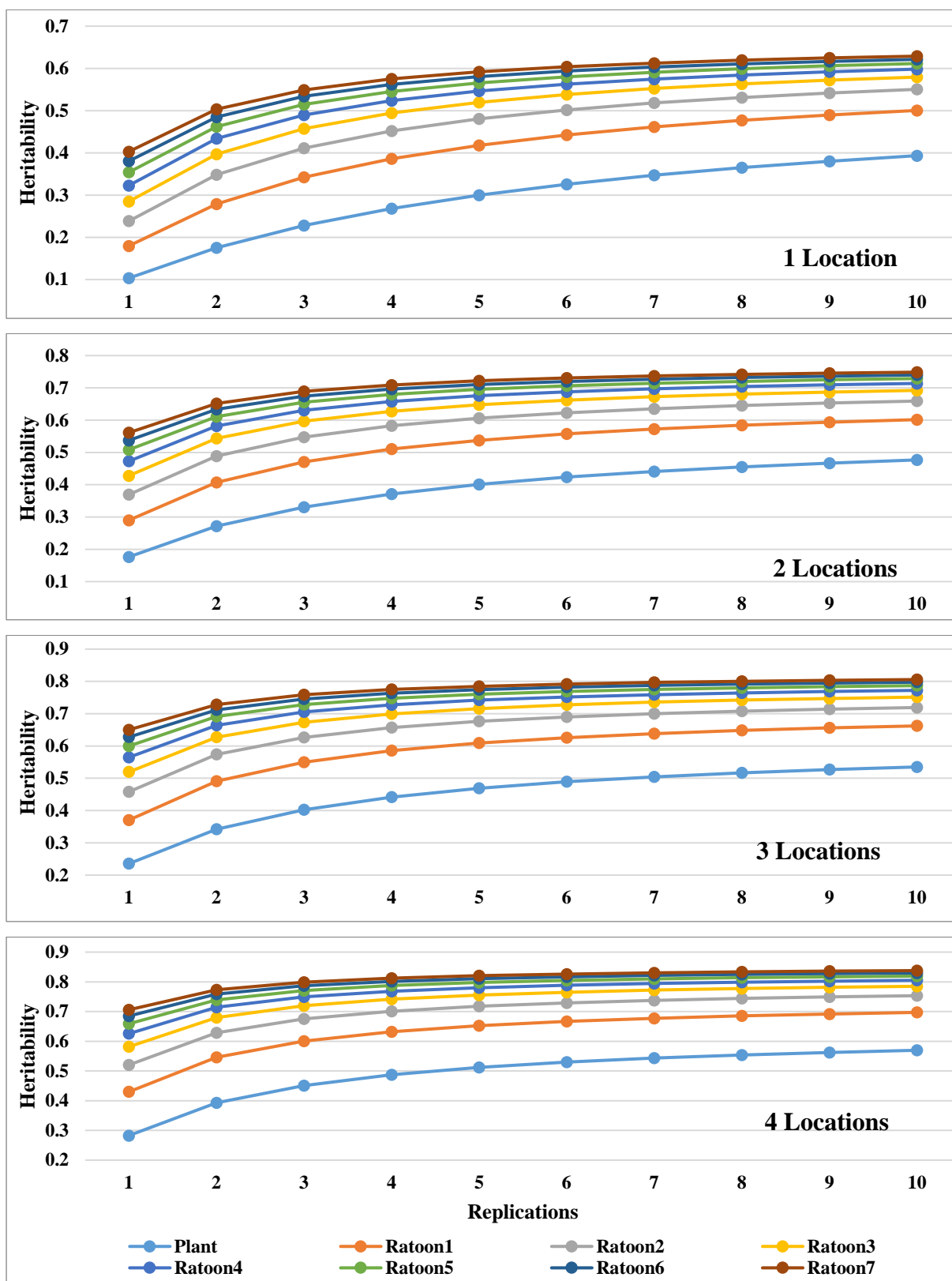


Figure 4.10: Crop-year effect on the broad sense heritability values for tons sucrose per hectare (TSH)

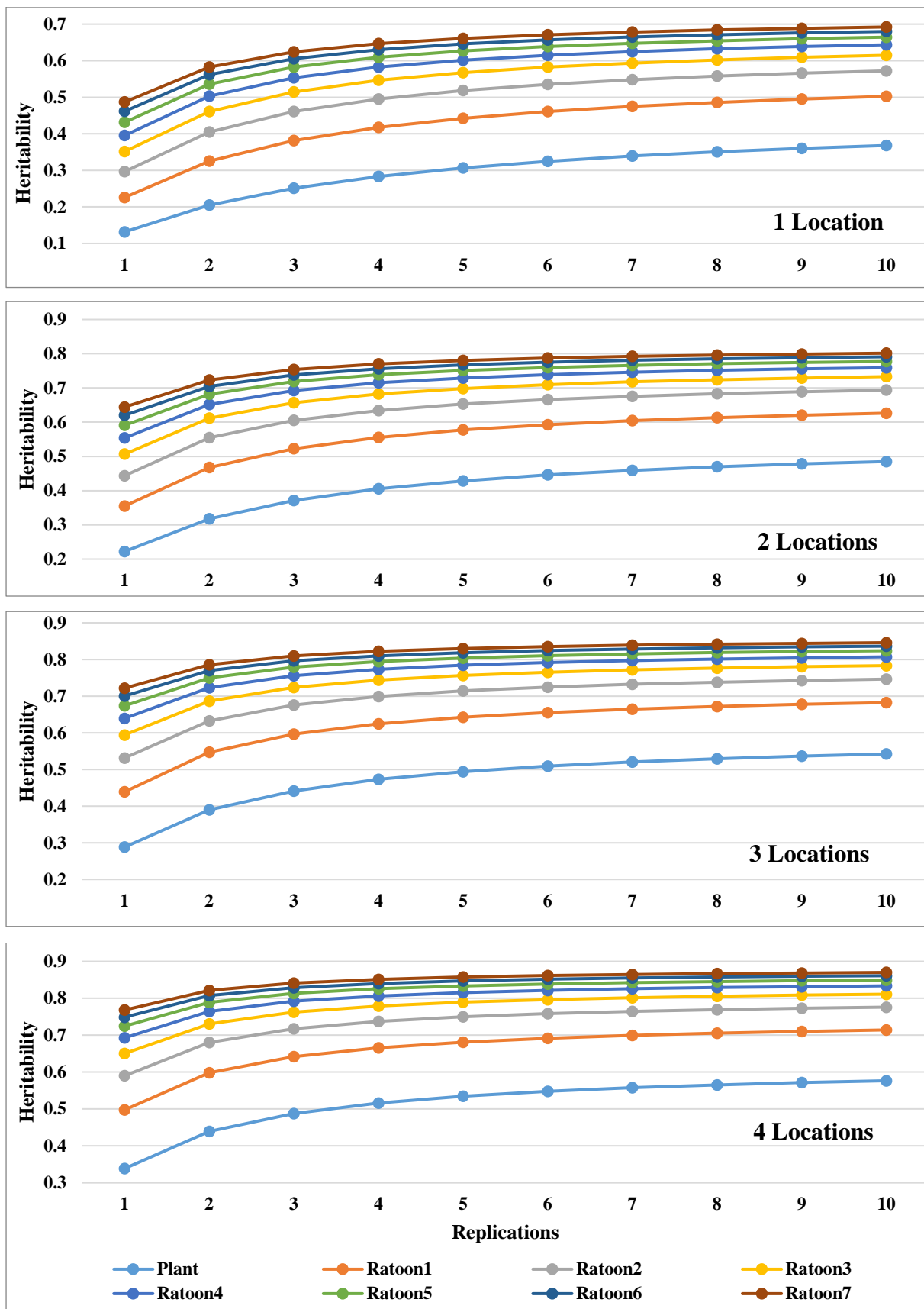


Figure 4.11: Crop-year effect on the broad sense heritability values for Suc% cane

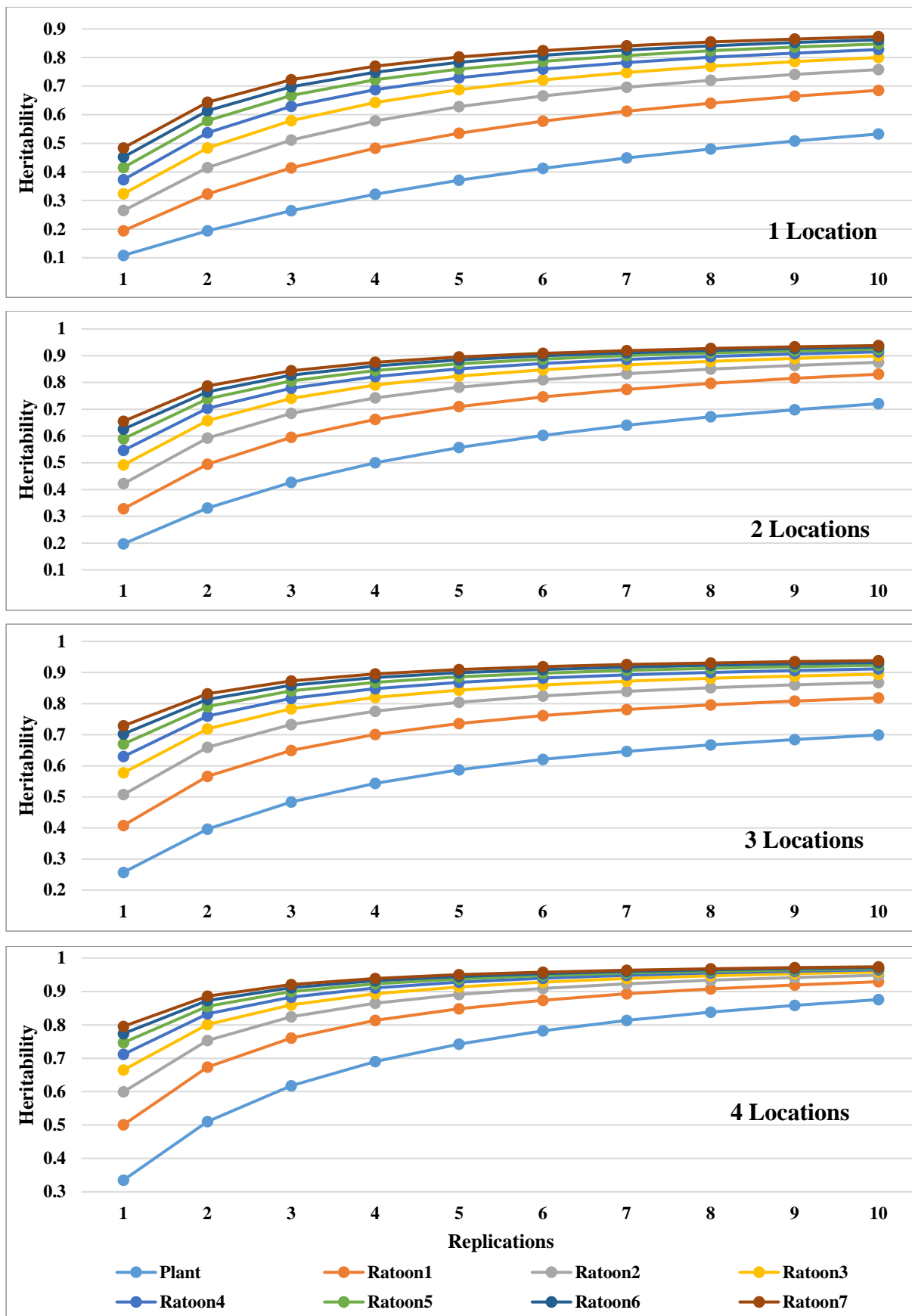


Figure 4.12: Crop-year effect on the broad sense heritability values for Fibre% cane

4.5 Discussion

The genotype (G) effect was the most important for all the traits, indicating that the genetic variance in this population of varieties was significant, hence varieties that perform well in traits of interest could be identified under these experimental conditions. This was evident from the very high broad sense heritability values demonstrated for all the traits (Table 4.1). Similar results were documented by Kimbeng *et al.* (2009) for the Rio Grande Valley Region of Texas. Zhou *et al.* (2011) also reported that G effect was dominant for the South African Sugarcane Research Institute (SASRI) irrigated breeding programme, suggesting higher stability among the variety population. Such results improve progress during the selection of high performing varieties for the different growing areas.

The variance components of GxL and GxC were highly significant ($p < 0.01$) for the yield traits (TCH and TSH), while for Suc% cane all the GEI components (GxL, GxC and GxLxC) were highly significant ($p < 0.01$) justifying their importance in the variety trials. For Fibre% cane, all the components of GEI were not significant ($p = 0.05$) indicating that fibre in these trials was largely dependent on the varieties (genetic control) as opposed to environmental factors, and the higher BSH supports this finding.

For the primary sugar yield components (TCH and Suc% cane), GxC was larger than GxL, implying that testing across multiple crop-years was more important than testing across locations. These findings emphasized the importance of testing for ratooning ability and suggested that substantial gains could be achieved from selection by increasing the number of crop-years over which varieties are tested. Similar results were reported by Rattey and Kimbeng (2001) for the Burdekin region of Queensland in Australia and Kimbeng *et al.* (2009) for the Rio Grande Valley Region of Texas. However, Jackson and Hogarth (1992) reported that testing across locations was of greater importance than across crop-years in Australia. Kimbeng *et al.* (2009) clarified that the Burdekin and Texas trials were grown under irrigation hence drought stress was not likely to be a limiting factor while the other Australian trials were rainfed. Contrary to this explanation, Zhou *et al.* (2011) reported that testing across locations was more important than testing for ratooning ability for the irrigated region of South Africa. This can probably be attributed to the differences in soil type, geographical locations and climatic conditions in the testing sites of the irrigated region of South Africa. Even then, this

clearly indicates the complexity associated with GEI, indicating that no one factor explains all GEI.

In this study, GxLxC was the largest source of variance compared to the other GEI components (GxL and GxC) for the Suc% cane indicating that the ratooning ability for this trait was influenced by the location. The higher GEI:G ratio for Suc% cane compared to the other traits confirmed that GxLxC was more influential on this trait. This is attributed to the fact that location in this study was defined by the combination of soil type and harvesting season (early and late seasons). Sucrose content in the sugarcane crop is heavily influenced by the season of harvest, hence varieties are classified as early or late maturing. Zhou (2015a) reported distinct differences between the performance of genotypes across seasons, low sucrose content in the early season and high sucrose content in the late season. This provides an opportunity to characterise the test varieties according to their time of maturity (season) thus enabling proper siting to maximise benefit from genetic gains. The three way interaction (GxLxC) is complex and suggests that variety testing needs to identify varieties that would significantly achieve yield gains in each location for the differing ratooning abilities (Zhou *et al.*, 2012). According to Kimbeng *et al.* (2009) these interactions are by nature difficult to interpret and model and that presents challenges if an attempt is made to select varieties for use as parents based on their performance for a primary trait such as TCH and Suc% cane. On the other hand, Milligan *et al.* (1990) argued that though the high order three-way interaction can be significant, it would generally contribute very little to the overall GEI variance. Zhou *et al.* (2012) emphasized that a better understanding of this interaction could assist identify varieties for specific seasons.

To identify the optimum number of resources (replications, locations and crop-years) required the broad sense heritability was calculated. Broad sense heritability (BSH) refers to the extent or degree at which the phenotype is controlled by the genetic makeup of the variety, hence it is also known as degree of genetic determination (Falconer, 1989). In this study, BSH measured the repeatability or consistency at which variety means could be differentiated based on the proportion of the variation among these means that was due to variety effects (Kimbeng *et al.*, 2009). In this study, the data were obtained from trials evaluating nine varieties at post-release stage. It is suggested that future studies should consider higher numbers of genotypes equivalent to that of advanced variety trials. Multi-location varietal evaluation trials in a systematic sugarcane breeding programme should involve planting about 20 varieties in replicated design (Shanthi *et al.* 2011).

Replication of treatments in a test is critical to statistical analysis, providing a means of estimating statistical error (Acquaah, 2007). The results indicated that the optimum number of replications required for these trials to adequately discriminate between variety means, was four. The minimal gains in BSH beyond replicate four showed that resources could be saved by reducing replication number from the current eight to four without jeopardising the accuracy at which differences between variety means are estimated. Similar results were reported by Zhou *et al.* (2012) at Dwangwa sugar estate in Malawi, and Brown and Glaz (2001) in Florida. These workers indicated that planting eight replications resulted in coverage of very large areas which could result in greater spatial variability, and reduce the precision of the trials in addition to the misuse of economically important resources. Kimbeng *et al.* (2009) found that reducing replications from four to two did not affect the advanced stage selection trials for the Texas Sugarcane Improvement Programme (TSIP). Rattey and Kimbeng (2001) reported that maintaining the replications at two was ideal for the Burdekin Sugar Experiment Station's breeding programme in Queensland, Australia. According to Acquaah (2007) the number of replications used usually varies between two and four; fewer replications may be used in early evaluation of genotypes while advanced yield tests usually have four replications.

Studies elsewhere indicated that the optimum number of replications depends on plot sizes. Studies by Polson (1964) and Taye *et al.* (2000) working on safflower and wheat, respectively, concluded that increasing plot sizes decreased the number of replications required to detect variances between genotype means. According to Klomsa-ard *et al.* (2013), for sugarcane trials, plot sizes are generally large due to wider row spacing to minimize inter-row plant competition. However, with the increasing area under mechanization, especially mechanical harvesting in both developed and developing economies, inter-row spacing has been increased to accommodate machinery with wider wheel axles without damaging the sugarcane crop. Hence, future studies aimed at identifying the optimum number of replications in sugarcane trials should consider changes in inter-row spacing.

With regard to crop-years, the results of this study showed that the plant and three ratoon crops (four crops) were sufficient to establish the ratooning ability of the test varieties. The current practice at the SSAVEP is to harvest trials at not less than five crops (plant and four crops) to inform the industry's variety recommendations programme. The finding of the study suggests that growers can benefit from genetic advancement by accessing newly released sugarcane varieties at least a year earlier. Kimbeng *et al.* (2009) and Zhou *et al.* (2012) also found that

four crops were optimum for the Dwangwa estate's VEP and TSIP, respectively. SASRI's plant breeding programme also uses four crops at the advanced variety trials (Zhou, 2013). Brown and Glaz (2001) reported that three crops were appropriate for testing variety ratooning ability in Florida. On the other hand, Rattey and Kimbeng (2001) concluded that increasing the number of years beyond four appeared to be beneficial as a result of the relatively large magnitude of GxC effect. Testing for ratooning ability is essential for the productivity and profitability of sugarcane growers because of the costs associated with establishing a new crop. It is suggested that future analysis should consider the longer ratoon performance (> 5 ratoon crops) as well to establish how the older ratoon crops correlate for genetic performance with the plant crop and early ratoon crops.

The analysis demonstrated that four locations were adequate to assess the interactions of test varieties with locations. Similarly, Rattey and Kimbeng (2001) and Kimbeng *et al.* (2009) found that four locations were adequate for the Burdekin breeding programme and TSIP. For this study, four trials were established at two different sites harvested at two different seasons, thus locations were constituted by a combination of soil type and harvest season. Hence, it is evident that the performance of varieties was dependent on the combined effect of soil type and harvest season. This again provides an opportunity to identify varieties for different soil types and harvest seasons where, if they are correctly sited, their genetic potential can be fully exploited. A GEI analysis conducted for the same sites and seasons by Dlamini and Ramburan (2016), utilising GGE biplots indicated that these four locations (two sites x two harvest seasons) were heterogenous, and a decision was made that variety testing across the two sites and two seasons should continue. Therefore, in considering these results, it is important to emphasize that the location/soil type factor was confounded by time of harvesting (seasons) in this study, hence these results should be used with that knowledge. In the study by Zhou *et al.*, (2012), the authors established that seasonal effects significantly influenced the yield of sugarcane varieties. These authors considered three seasons (early, mid- and late seasons) as opposed to the two seasons (early and late) in this study. In another study, Zhou (2015a) found that seasonal effects were larger than location effects, while genotype x season was larger than genotype x location, indicating the importance of seasons when breeding for irrigated sugarcane regions.

The advantage of decreasing the number of replications and crop-years is that resources saved could be reallocated to additional trials (Zhou *et al.*, 2012). Sugarcane growers are concerned

when the turnaround time for releasing newly improved varieties from breeding and selection programmes is prolonged especially when released varieties need further performance evaluation to refine recommendation. In most developing economies where sugarcane varieties are imported from other countries post-release, the VEPs take not less than five years before superior varieties are released to growers. To address this concern, this industry can investigate possibilities of accessing more sugarcane varieties from breeding and selection programmes at the pre-release stage. This strategy will not only give growers early access to improved varieties by reducing testing time, but will also enable SSA to test more varieties, increasing chances of identifying a larger pool of high performers. Hence the resources saved by reducing replication numbers and crop-years can be used to accommodate this strategy. Future studies with balanced GEI data will need to consider modelling these savings and express them in monetary terms.

4.6 Conclusions

The study has demonstrated that GEI are present within the variety evaluation programme of SSA, and the effect thereof are shown on sucrose yield and its components (cane yield and sucrose content). This emphasized the need to characterize varieties according to their suited seasons and locations to fully utilize their genetic potential and GEI effects. For the varieties studied, fibre content was not significantly influenced by environmental factors. Testing varieties across crop-years was more important than testing across locations in this study, indicating great opportunity of exploiting ratooning ability potential of test varieties. All the traits evaluated proved to be highly dependent on varieties' genetic component due to higher broad sense heritability values. Four replications, four locations and four crop-years appear to be an efficient resource combination to provide an adequate level of discrimination between sugarcane varieties in the VEP of SSA without compromising the level of experimental precision currently being attained with eight replications, four locations and five crop-years. The advantage associated with the reduced time of release is that growers will enjoy genetic improvements on newer varieties a year earlier than current practice while significantly reducing the testing costs per variety.

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CHAPTER 5

GENOTYPE BY ENVIRONMENT INTERACTION IN THE IRRIGATED SUGARCANE VARIETY TRIALS OF SWAZILAND AND SOUTH AFRICA

5.1 Abstract

The sugar industries of South Africa (SA) and Swaziland (SD), through their respective research institutes, the South African Sugarcane Research Institute (SASRI) and Swaziland Sugar Association (SSA), independently conduct evaluations of SASRI released irrigated sugarcane varieties that are common to both industries. These evaluations are conducted in replicated trials established at various sites representing the different sugarcane growing areas of the two industries so that variety recommendations can be provided to growers accordingly. However, there are strong sentiments among stakeholders that due to proximity of the irrigated region of SA and SD, there should be similarities between some of the testing sites across the borders. If this is proven true, redundant sites can be identified and eliminated from the testing network, and variety evaluations can be made on a joint basis.

To test this option, a combined data analysis of cane yield (TCH), sucrose content (Suc% cane) and sucrose yield (TSH) obtained from nine test environments (four from SASRI and five from SSA), collected over four years (2007-2010) was conducted. The analysis was restricted to seven varieties that were common in all environments. Environments were constituted by the combination of trial site and harvesting season. An AMMI ANOVA and a GGE biplot analysis were conducted using GenStat[®] 17th Edition statistical software. The analyses indicated that information from Komati-Early trials can be utilised to formulate variety recommendations for Mhlume-Early, Mhlume-Late, Pongola-Early, Malkerns-Early and Simunye-Early conditions. Hence, these environments can be dropped from the testing network to reduce costs. Alternatively, SSA and SASRI can combine research resources and widen the spectrum of variety testing across similar sites to represent a single agro-climatic region. However, for environments Ubombo-Late, Pongola-Late and Komati-Late, variety evaluation will have to continue independently, since they were not correlated to any other environments. Future studies will have to investigate genetic and environmental covariates characterising the genotype by environment interaction in this region to inform future variety recommendation strategies.

5.2 Introduction

The Swaziland Sugar Association (SSA) and South African Sugarcane Research Institute (SASRI) mutually entered into a research and development cooperation agreement (RADCA) more than two decades ago. The purpose of the RADCA was to enable SSA to access crop production research and development services from SASRI. These services include the provision of sugarcane varieties and technical services such as pest and disease identification and control, fertilizer advisory services, training and consultancy, and access to the SASRI library facilities and publications. The RADCA gives both parties the leverage to optimise resource usage while providing means for sharing technical expertise, research results, ideas and information in a protected environment. However, there is doubt if both SSA and SASRI are fully maximizing on the benefit derivable from the RADCA.

Both industries (SSA and SASRI), conduct variety evaluation programmes (VEP) separately to evaluate the performance of released varieties at various strategic sites chosen to represent different soil types and agro-climatic conditions prevalent within the industries. These multi-environment trials (METs) are essential owing to differential responses of varieties to different environments, a phenomenon known as genotype by environment interaction (GEI). GEI causes a change in performance rankings of varieties across environments. According to Voltas *et al.* (2002), GEI weakens the association between phenotype and genotype, hence reducing genetic progress in breeding programmes. The most important type of GEI is the crossover interaction, as it implies that the choice of the best genotype is determined by the environment (Ferreira *et al.*, 2006; Malosetti *et al.*, 2013). GEI is widely reported in sugarcane METs (Milligan *et al.*, 1990a; Parfitt, 2000; Brown and Glaz, 2001; Kimbeng *et al.*, 2009; Kumar *et al.*, 2011; Ramburan and Zhou, 2011; Zhou *et al.*, 2011; Khan *et al.*, 2013).

In the case of SSA and SASRI, Redshaw *et al.* (2005) indicated that despite their geographical proximity, these research institutes have independently evaluated sugarcane varieties that are common to both countries (Swaziland and South Africa) since the early 1980s. There is a very strong perception that varietal responses in the irrigated region of the South African industry are similar to those in Swaziland. As a result, it is alleged that there is some duplication of research across these industries. If this claim could be validated, some trial sites could be dropped from the network, and as such it could be possible to make more effective use of resources, since variety evaluation and recommendations could be made on a regional basis.

Under such arrangement, Swaziland can utilise the data and recommendations from SASRI to make variety recommendations for similar environments when a new variety is released. This can significantly reduce the cost of testing varieties in the Swaziland sugar industry. Alternatively, resources from both industries could be pooled to evaluate a broader spectrum of varieties across similar sites to represent a single agro-climatic region (Redshaw *et al.*, 2005).

On the other hand, Swaziland growers are concerned about the delay in benefiting from genetic gains attainable from released SASRI varieties. This is exacerbated by the further performance evaluation of SASRI released varieties under local conditions (for not less than five years) before release for commercial cultivation. The objective of these evaluations is to ensure that varieties released to growers for commercial growing are high yielding, sustainable (good ratooning ability) and resistant or tolerant to pests and diseases. Therefore, identifying testing sites that interact similarly with varieties between the two industries can accelerate the adoption of SASRI released varieties by Swaziland growers since SSA will utilize information from SASRI trials for variety recommendations. This will ensure that SSA extracts maximum value from the RADCA for the benefit of the Swaziland sugar industry.

The GGE (genotype + genotype x environment) biplot analytical technique proposed by Yan *et al.* (2000) has gained popularity as a tool to investigate various aspects of GEI (Ramburan and Zhou, 2011) including its use to group test environments according to their similarities. Once test environments are grouped according to their similarities, Yan and Tinker (2006) proposed that within a single mega-environment high yielding and highly stable varieties, and discriminating and representative test environments should be identified. Sandhu *et al.* (2012), Klomsa-ard *et al.* (2013) and Luo *et al.* (2014; 2015) used the GGE biplot technique to evaluate sugarcane varieties and to identify efficient test sites for METs in India, Thailand and China, respectively. Ramburan (2012) used the technique to investigate GEI in the Midlands region of South Africa. Rakshit *et al.* (2012) utilised the tool to demonstrate the usability of the biplot graphical approach in analysing and interpreting the complex GEI in MET data.

GGE biplots have also been used to interpret MET data in other crops such as maize (*Zea mays* L.) [Muungani *et al.*, 2007; Setimela *et al.*, 2010], wheat (*Triticum aestivum* L.) [Yan and Hunt, 2001; Kaya *et al.*, 2006; Hagos and Abay, 2013], soybeans (*Glycine max* L.) [Asfaw *et al.*, 2009], sorghum (*Sorghum bicolor* L.) [Rakshit *et al.*, 2012], sunflower (*Helianthus annuus* L.)

[Mostafavi *et al.*, 2012] and chickpea (*Cicer arietinum* L.) [Farshadfar *et al.*, 2013]. Notwithstanding its popularity, the GGE biplot method has not been tried in MET data analyses in the VEP of SSA. The objective of this study was to conduct a simultaneous analysis of irrigated sugarcane trials for SSA and SASRI to establish existence of relationships and similarities between trials using the GGE biplot analytical technique.

5.3 Materials and methods

5.3.1 Trial treatments

Nine irrigated variety trials (SASRI: four; SSA: five) established between 2003 and 2014, consisting of seven to ten commercial varieties and all harvested at 12 months were used in this study (Table 5.1). The SASRI trials were planted at Pongola and Komati, both early and late season at each site. The SSA trials were planted at Mhlume (both early and late), Simunye (early), Ubombo (late) and Malkerns (early). The trials were coded using the first letter of the site and the harvest season, except for Malkerns where an additional letter from the site name had to be used to differentiate it from Mhlume-early trial. For example, code – PE, represents a trial established at Pongola for harvesting early season. Early trials were harvested within April and May while late trials were harvested within October and November. In this study each trial was regarded as an environment. To ensure a balanced set of data, first, only seven varieties that were common to all trials were included in the analysis. Second, since the trials were not all established in the same year, only yield data collected over four common years, that is between 2007 and 2010, were considered for analysis (Table 5.2). In this analysis, environments were considered as trial x year combinations and the variation in actual ratoon number between trials within a year was considered negligible.

Table 5.1: Details of the nine trials used for the analysis in this study

Trial code	Country	Location	Harvest season	Irrigation system	Varieties
PE	South Africa	Pongola	Early	Drip	N19, N25,
PL	South Africa	Pongola	Late	Drip	N26, N32,
SE	Swaziland	Simunye	Early	Sprinkler	N36, N40 &
UL	Swaziland	Big Bend	Late	Sprinkler	N41
ME	Swaziland	Mhlume	Early	Sprinkler	
ML	Swaziland	Mhlume	Late	Sprinkler	
KE	South Africa	Komati	Early	Drip	
KL	South Africa	Komati	Late	Drip	
MKE	Swaziland	Malkerns	Early	Sprinkler	

Table 5.2. Details of the crop-years of the nine trials used for the analysis in this study

Trial codes	Crop Years											
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
KE	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
PE	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
ME	-	R0	R1	R2	R3	R4	R5	R6	R7	-	-	-
SE	-	R0	R1	R2	R3	R4	R5	R6	R7	R8		
ML	-	R0	R1	R2	R3	R4	R5	R6	-	-	-	-
UL	-	R0	R1	R2	R3	R4	R5	R6	-	-	-	-
PE	-	-	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9
MKE	-	-	R0	R1	R2	R3	R4	R5	R6	-	-	-
KL	-	-	-	R0	R1	R2	R3	R4	R5	R6	R7	R8

R0 = plant crop, R1 = first ratoon crop ... R11 = eleventh ratoon crop

5.3.2 Experimental design

The trials were established in a randomised complete block design (RCBD) with six to ten replicates. Trial plots consisted of five or six rows that were 8.0 to 10.0 m long and spaced 1.4 to 1.5 m apart. In all trials, plot spacing ranged from 1.0 to 2.0 m and a 1.0 m end of row effect was provided for in all plots. The outer rows on each side of each plot were considered as buffer rows, hence they were not regarded in the analysis.

5.3.3 Site selection and description

The primary objective of these trials was to identify varieties suited to the different environmental conditions for the purpose of refining variety recommendations. Table 5.3 shows the soils, geographical and climatic information for the trial sites, and Figure 5.1 shows the geographical position of the different testing sites.

Table 5.3: Soil types, geographical locations and climatic information for the four sites

Sites	Soil forms (SA) [#]	Soil set (SD) [*]	Latitude (°S)	Longitude (°E)	Altitude (m)	Annual rainfall (mm)	Annual solar radiation (MJ/m ²)	Annual heat units (T _b = 10°C)
Ubombo	Glenrosa	S	26.77	31.93	106	632	6772	4526
Simunye	Shortlands	R	26.20	31.90	233	711	7094	4414
Malkerns	Hutton	M	26.50	31.20	740	931	6564	3481
Mhlume	Katspruit, Bonheim	H C	26.03	31.80	280	798	7233	4348
Pongola	Hutton	R	27.40	31.58	308	683	6947	4071
Komati	Shortlands	R	25.33	31.87	179	629	7196	4524

[#]South African classification ^{*}Swaziland classification
Sourced from Redshaw *et al.* (2005)

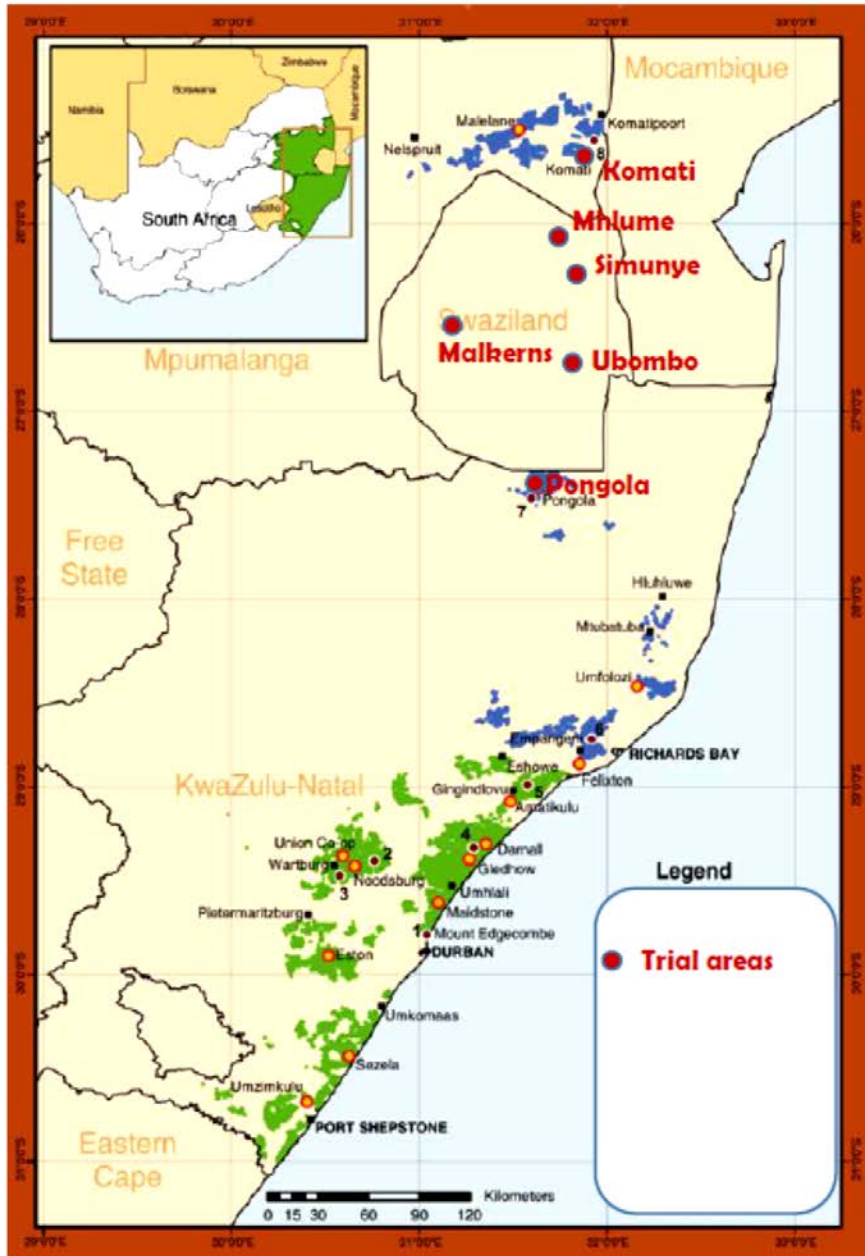


Figure 5.1: The six variety evaluation locations of SSA (Ubombo, Simunye, Malkerns and Mhlume) and SASRI (Pongola and Komati) considered in this study.

5.3.4 Crop maintenance

Although trials were established under different irrigation systems (Table 5.1), the general crop management practices such as irrigation, fertiliser application and weed control were conducted as per commercial practices in all the estates at which they were established.

5.3.5 Data collection

In all trials, yield data were collected at time of harvesting (12 months cycle) on a per plot basis. To remove the extraneous matter, the cane was burnt a day prior to harvesting. On the day of harvesting, the end-rows were cut and removed from the net plots and placed in a windrow together with cane from the buffer rows. The cut cane was topped according to estate practice which is usually below the natural breaking point. Cane from the net rows (buffer rows discarded) was also cut and weighed using a scale mounted on a tractor-operated hydraulic boom to determine cane yield in tons cane per hectare (TCH). After the weighing, a total of 16 stalks of cane per plot were sampled at random from the net rows and sent to the laboratory to determine sucrose content (Suc% cane) (Shonnees-Muir *et al.*, 2009). To calculate sucrose yield per hectare (TSH), TCH was multiplied by the Suc% cane.

5.3.6 Data analysis

To explain main effects of varieties and environments, and their interaction effects (GEI), the TSH, TCH and Suc% cane data were subjected to an AMMI ANOVA. The following AMMI model equation was used:

$$Y_{ij} = \mu + G_i + E_j + \sum \lambda_k \alpha_{ik} \delta_{jk} + R_{ij} + \varepsilon \quad (1)$$

Where, Y_{ij} is the value of the i^{th} variety in the j^{th} environment; μ is the grand mean; G_i is the deviation of the i^{th} variety from the grand mean; E_j is the deviation of the j^{th} environment from the grand mean; λ_k is the singular value for principal component (PC) axis k ; α_{ik} and δ_{jk} are the PC scores for axis k of the i^{th} variety and j^{th} environment, respectively; R_{ij} is the residual and ε is the error term (Gauch, 1992).

To establish the relationship within and between locations and varieties in graphical form, a first set of GGE biplots (Yan *et al.*, 2000) were drawn using GenStat[®] 17th Edition statistical software (VSN International, 2015). These biplots allow the graphical display of the two way data and visualisation of the interrelationship among environments, varieties and interactions between varieties and environments (Dehghani *et al.*, 2006). The GGE biplots were constructed by plotting the first principal component (PC1) scores of the varieties and the environments against their respective scores for the second principal component (PC2) that result from singular value decomposition (SVD) of environment-centered GEI data (Yan *et al.*, 2007).

To identify varieties with high performance and high stability, and to identify environments that are both discriminating and representative, a second set of GGE biplots were drawn based on the environment-centered GEI two-way table, using the comparison plot. The difference between environment and variety evaluation biplots is that environment evaluation biplots are environment-metric preserving (singular value partitioning, $SVP = 2$) while variety evaluation biplots are variety-metric preserving ($SVP = 1$). A third set of GGE biplots were drawn to show distinct mega-environments, that is, environments that segregate varieties similarly grouped together. This was meant to assist in identifying environments that have similar effects on varieties between the two industries so that redundant ones may be removed from the network. More details of the GGE biplot analysis were given in Chapters 2 and 3.

5.4 Results

5.4.1 AMMI analysis of variance

Tables 5.4, 5.5 and 5.6 show the effects of varieties, environments and their interactions on sucrose yield, cane yield and sucrose content, respectively. In this analysis, years were considered as blocks. The highly significant ($p < 0.001$) block effects for all three traits suggested that variety (genotype) performance was not similar across years. Both varieties and environments, together with their interactions were highly significant ($p < 0.001$) for all the studied traits. The first two interaction principal components axes (IPCAs) were highly significant ($p < 0.001$) for sucrose yield. For cane yield, IPCA 1 was significant at $p < 0.001$ while IPCA 2 was significant at $p < 0.01$. For sucrose content, IPCA 1 was significant at $p < 0.001$ while IPCA 2 was significant at $p < 0.05$. Environments were the largest source of variation accounting for 79.98%, 85.72% and 88.15% of the treatments SS (SS) for sucrose yield, cane yield and sucrose content, respectively. Varietal effects were larger than the GEI effects for all three traits. The first two IPCAs captured a very high portion of the GEI, together explaining 79.06%, 80.70% and 82.45% of the total GEI for sucrose yield, cane yield and sucrose content, respectively.

Table 5.4: AMMI analysis of variance for sucrose yield (TSH) of seven varieties tested over nine environments (df: degrees of freedom; SS: sum of squares; MS: mean of squares)

Source of variation	df	SS	%SS	Explained % of interaction	MS	P-value
Total	251	2882.3			11.48	
Treatments	62	1593.8			25.71	<0.001
Genotypes	6	217.4	13.64%		36.23	<0.001
Environments	8	1274.7	79.98%		159.34	<0.001
Block	27	1184.3			43.86	<0.001
Interactions	48	101.7	6.38%		2.12	<0.001
IPCA 1	13	57.7	3.62%	56.74%	4.44	<0.001
IPCA 2	11	22.7	1.42%	22.32%	2.07	<0.001
Residuals	24	21.3			0.89	0.1231
Error	162	104.1			0.64	

Table 5.5: AMMI analysis of variance for cane yield (TCH) of seven varieties tested over nine environments (df: degrees of freedom; SS: sum of squares; mean of squares)

Source of variation	df	SS	%SS	Explained % of interaction	MS	P-value
Total	251	183481			731	
Treatments	62	128772			2077	<0.001
Genotypes	6	14605	11.34%		2434	<0.001
Environments	8	110380	85.72%		13798	<0.001
Block	27	51311			1900	<0.001
Interactions	48	3787	2.94%		79	<0.001
IPCA 1	13	2371	1.84%	62.61%	182	<0.001
IPCA 2	11	685	0.53%	18.09%	62	0.0013
Residuals	24	731			30	0.0913
Error	162	3399			21	

Table 5.6: AMMI analysis of variance for sucrose content (Suc% cane) of seven varieties tested over nine environments (df: degrees of freedom; SS: sum of squares; MS: mean of squares)

Source of variation	df	SS	%SS	Explained % of interaction	MS	P-value
Total	251	1158.3			4.61	
Treatments	62	1034.2			16.68	<0.001
Genotypes	6	103.8	10.04%		17.3	<0.001
Environments	8	911.6	88.15%		113.95	<0.001
Block	27	103.7			3.84	<0.001
Interactions	48	18.8	1.82%		0.39	<0.001
IPCA 1	13	12.4	1.20%	65.96%	0.95	<0.001
IPCA 2	11	3.1	0.30%	16.49%	0.29	0.0134
Residuals	24	3.3			0.14	0.3502
Error	162	20.4			0.13	

5.4.2 GGE biplot analysis

5.4.2.1 Correlations between test environments

Environments in this analysis were constituted by the combination of test site/location and season. For example, a trial in Pongola (P) that was harvested early season (E) forms the PE environment, while a trial at Mhlume (M) harvested late season (L) forms the ML environment. This was done to account for seasonal effect as previous studies report that seasons significantly affect the performance of sugarcane varieties (Redshaw and Nuss, 2001; Gilbert *et al.*, 2006; Di Bella *et al.*, 2008; Ramburan *et al.*, 2010; Zhou, 2015a). It is worth noting that not all test sites had trials established at both harvesting seasons (early and late). For example, Malkerns and Simunye trials were only planted early season, while at Ubombo, only the late trial had usable data.

In GGE biplots, the lines that connect the test environment markers to the biplot origin are called environment vectors. The vector views of the GGE biplot provide concise summaries of the interrelationships between test environments in relation to the trait of interest (Yan, 2002). These vector views; first, are based on environment-centered variety by environment values without scaling; second, they are environment-metric preserving; and third, the axes are drawn on scale (Yan and Tinker, 2006). By interpretation, the cosine of the angle between the vectors of two environments approximates the correlation coefficient between them (Farshadfar *et al.*, 2012). An obtuse angle ($> 90^{\circ}$) indicates a negative correlation, an acute angle ($< 90^{\circ}$) indicates a positive correlation, and a right angle ($= 90^{\circ}$) implies no correlation. Another useful property of the vector view of the biplot is that the length of the environment vectors approximates the standard deviation within each environment, which is a measure of their discriminating ability (Dehghani *et al.*, 2006).

For TSH, the GGE biplots explained 78.37 to 88.55% of the GEI (Figure 5.2). Environment KL showed weak to negative correlation with most environments in Years 2 and 4 as demonstrated by the obtuse angles. In Year 1, this environment was negatively associated with UL and PL while in Year 3, it was negatively associated with UL, PL, MKE and ML. This suggests that data from this environment (KL) cannot accurately predict variety performance in environments UL, PL, MKE and ML due to crossover GEI. The acute angles between the other eight environments across all test years indicate that these environments are positively correlated since there are no crossover interactions between them. This indicates that variety

performances can be predicted in any of these environments based on performance in any of the environments as there is no rank change from one environment to the other. A general observation in this analysis (Figures 5.2) was that early harvested trials were more discriminating compared to late harvested trials as demonstrated by their often longer vectors. An exception was KL in the fourth year.

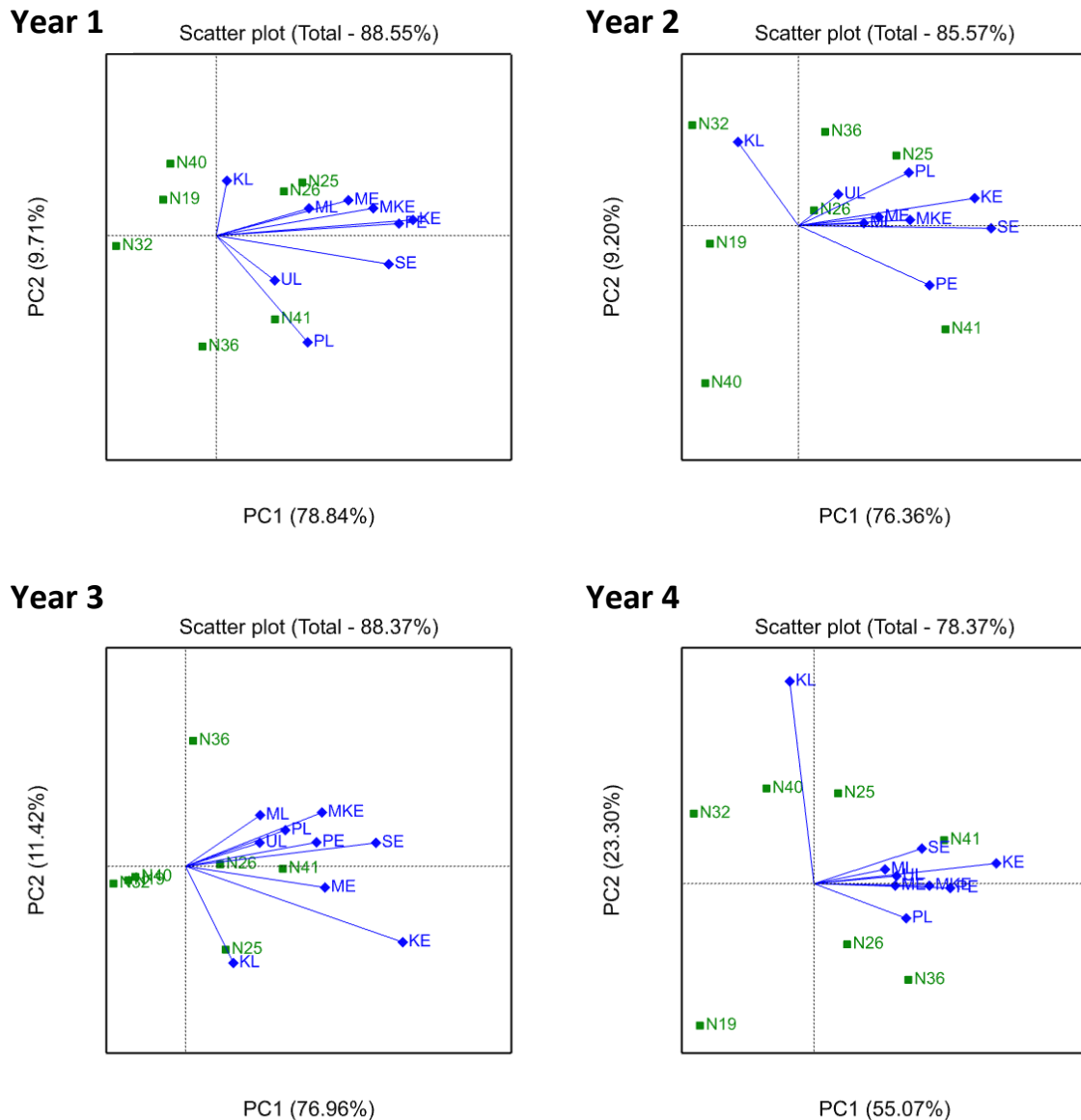


Figure 5.2: GGE biplot showing the correlations among seven varieties tested over nine test environments across four consecutive years (Year 1, Year 2, Year 3 and Year 4) on sucrose yield (TSH). Varieties are shown by squares while environments are shown by diamond shapes. The percentage variance accounted for by each principal component (PC axis) is indicated in parentheses

For TCH, the GGE biplots accounted for 89.25% to 95.40% of the total GEI (Figure 5.3). While environments were closely correlated across years, there were exceptions in Years 1 and 4. In Year 1, environments PL and KL were negatively correlated as shown by the obtuse angle between vectors. In Year 4, the right angle between the vectors of the same environments (PL and KL) demonstrated that there is no relationship between them. The relatively longer vectors in Years 1 and 4 suggests that the standard deviation within the environments was higher compared to Years 2 and 3. This was further explained by the relatively wider angles between environment vectors during these two years. The discriminatory effect of early harvested trials observed on TSH (Figure 5.2) was shown for TCH (Figure 5.3), except for KL in Year 4.

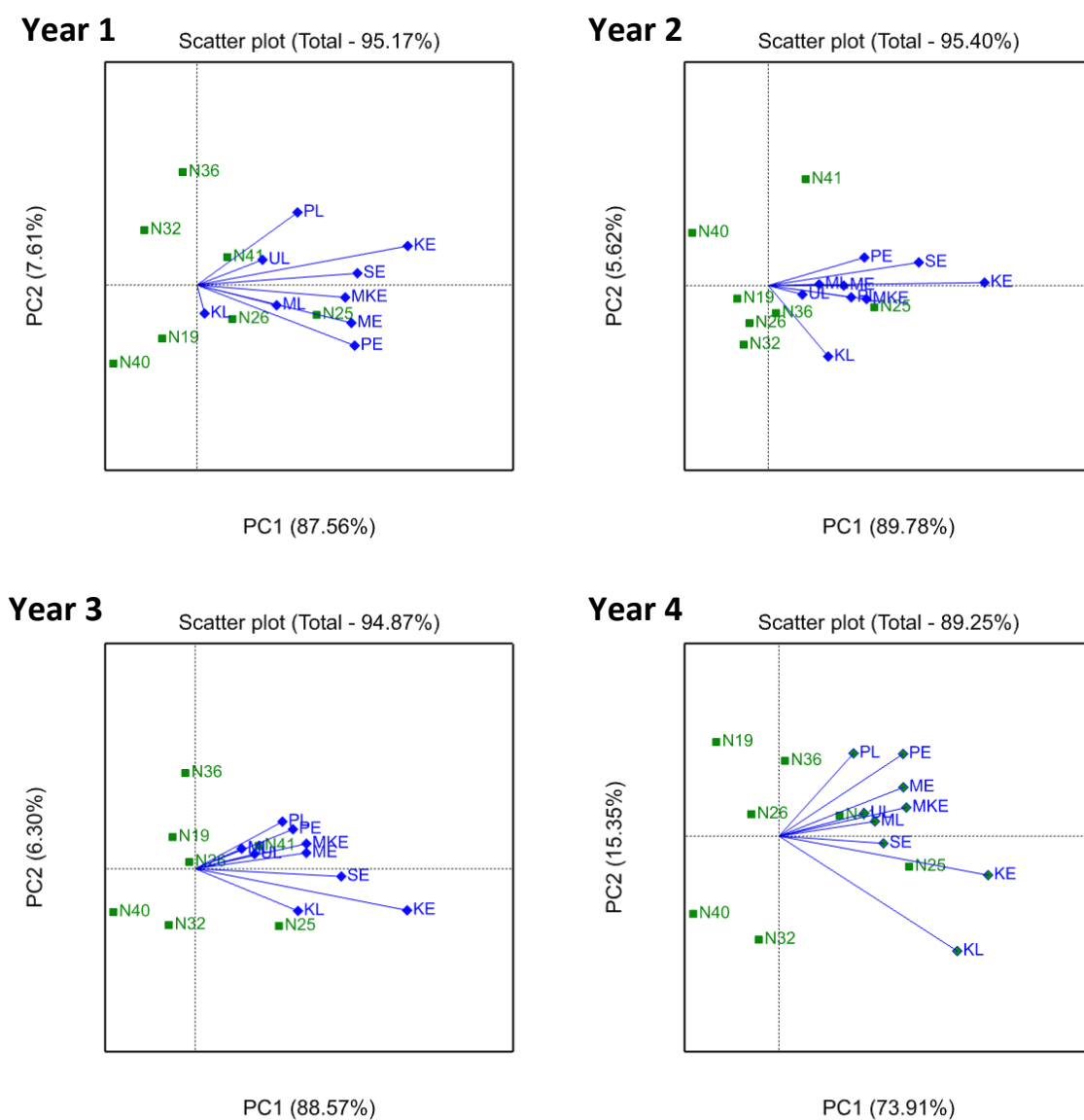


Figure 5.3: GGE biplot showing the correlations among seven varieties tested over nine environments across four consecutive years (Year 1, Year 2, Year 3 and Year 4) on cane yield

(TCH). Varieties are shown by squares while environments are shown by diamond shapes. The percentage variance accounted for by each principal component (PC axis) is indicated in parentheses

Figure 5.4 presents Suc% cane results for all the four years studied. For Suc% cane, the GGE biplots accounted for 90.19% to 93.61% of the total GEI (Figure 5.4). While there were two or three environments that had larger acute angles, generally, there were very close positive correlations between environments for this trait within years. Compared to TSH and TCH, there were no negative or zero correlations for Suc% cane. This implies that varieties were more stable across the environments on sucrose content compared to the other two quantitative traits. The frequent weak positive correlations (large acute angles) between KL and some environments, i.e. PE, suggests that extrapolation of variety performances (Suc% cane) between KL and these environments may not be accurate.

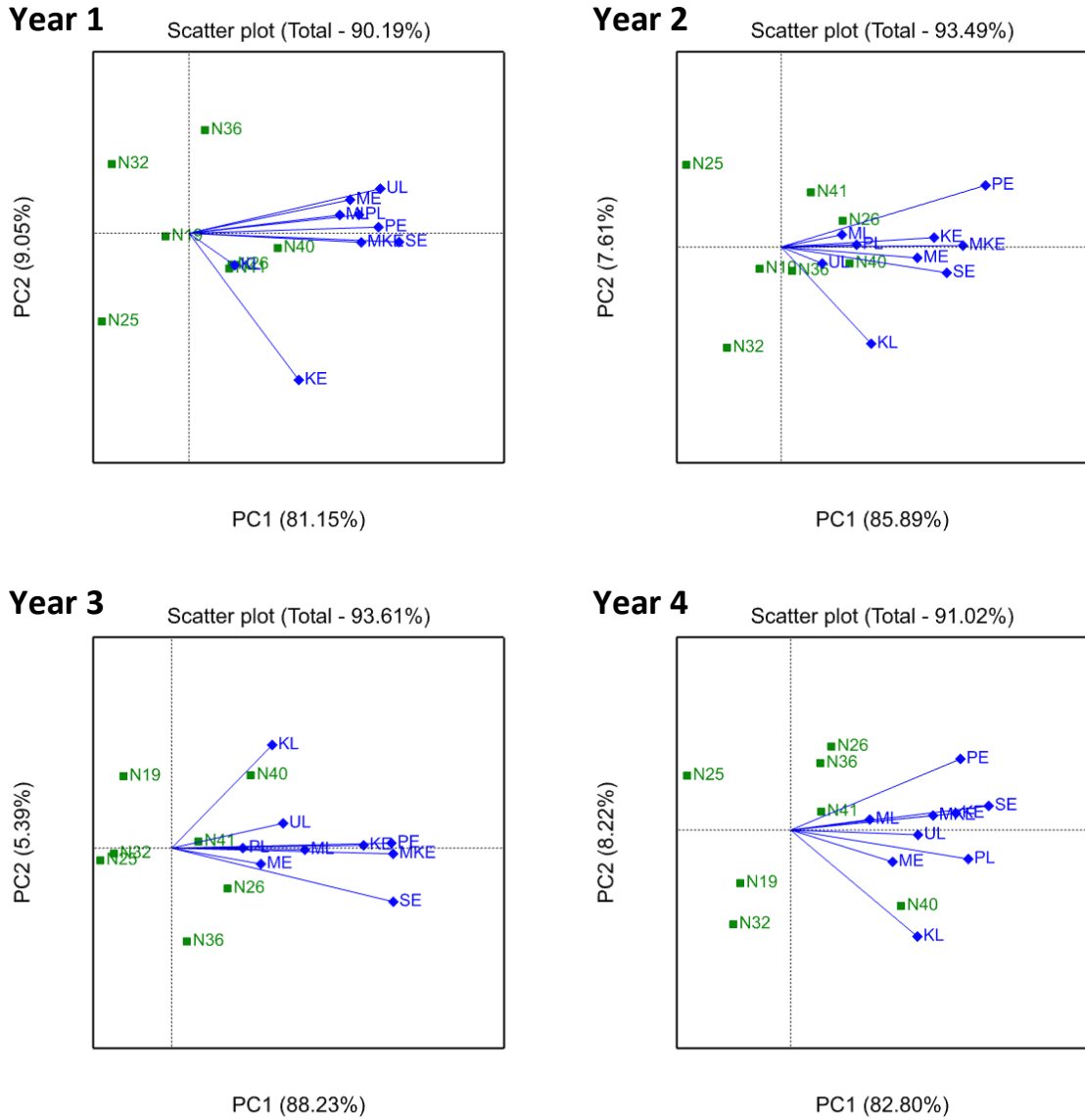


Figure 5.4: GGE biplot showing the correlations among seven varieties tested in nine environments across four consecutive years (Year 1, Year 2, Year 3 and Year 4) on sucrose content (Suc% cane). Varieties are shown by squares while environments are shown by diamond shapes. The percentage variance accounted for by each principal component (PC axis) is indicated in parentheses

5.4.2.2 Mega-environment analysis

Another important function of the GGE biplot analysis is the ability to divide the target environments into meaningful mega-environments (MGEs) (Yan and Tinker, 2006). Dividing the target environment into different MGEs and deploying different varieties in different MGEs is the ideal way to exploit GEI (Mostafavi *et al.*, 2012). To achieve this, a polygon was first drawn on varieties that are furthest (vertex varieties) from the biplot origin so that all varieties were contained within the polygon. The vertex varieties were the best or poorest in some or all of the environments. Thereafter, perpendicular lines were drawn on each side of the polygon starting from the origin such that sectors representing different MGEs were formed. By definition, a MGE is a group of locations that consistently share the best set of varieties across years (Yan and Rajcan, 2002). According to Ramburan and Zhou (2011) environments within the same MGE sector have the same effects on variety performance, hence should be considered as a homogenous group. Thus, the purpose of this analysis was to identify test environments that are repeatedly located within the same MGE so that redundant ones are eliminated. Repeatable grouping of the environments across years, although not sufficient, is necessary for declaring different MGEs (Yan *et al.*, 2007).

For TSH, environments ML, ME, MKE, PE, KE and SE were consistently located in one MGE across the four year testing period (Figure 5.5). Variety N41 was the dominant variety in three of four years in this MGE. UL and PL grouped together in the N41 and N25 sectors in Years 1 and 2, respectively. KL was repeatedly located in a different sector in Years 2, 3 and 4. Varieties N32 and N25 were associated with this environment (KL) in Years 2 and 3, respectively, while there were no associations in Year 4. PL was isolated in variety N36 sector in Year 4.

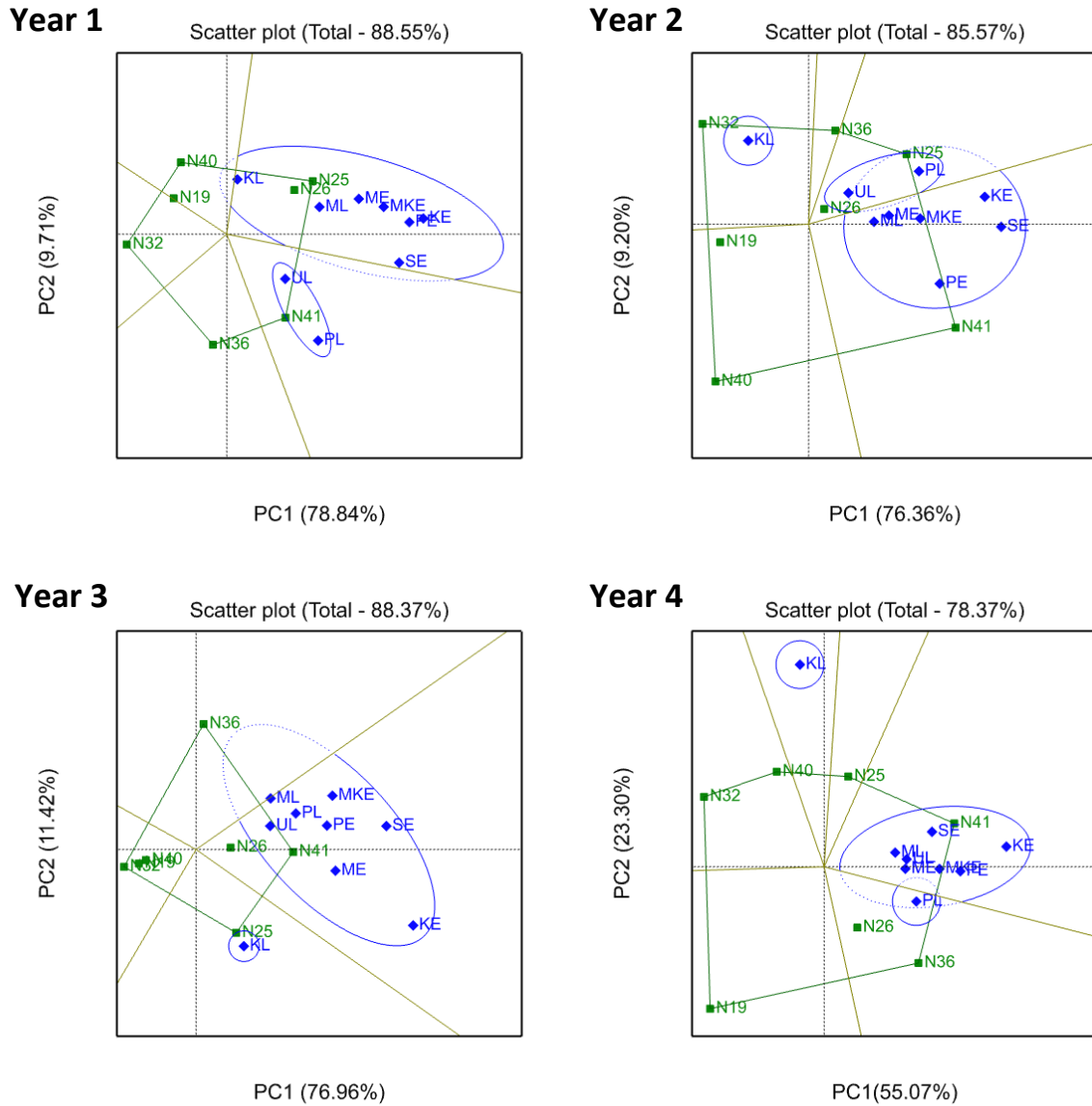


Figure 5.5: GGE biplots showing mega-environments for nine test environments planted with seven varieties harvested across four consecutive years (Year 1, Year 2, Year 3 and Year 4) evaluated for sucrose yield (TSH). Varieties are shown by squares while environments are shown by diamond shapes. The percentage variance accounted for by each principal component (PC axis) is indicated in parentheses

For TCH, the environments were consistently located in one MGE in Years 1, 2 and 4, the dominant variety being N25 across all three years (Figure 5.6). Generally, varieties were more stable for TCH compared to TSH as observed from the minimum crossover interactions. In Year 3, environments PE and ML grouped together in the N41 sector, while KE, SE, KL, MKE and ME grouped together in the N25 sector. Environment PL was located between the N41 and

N25 sectors, indicating that both varieties interacted similarly with this environment on TCH in Year 3.

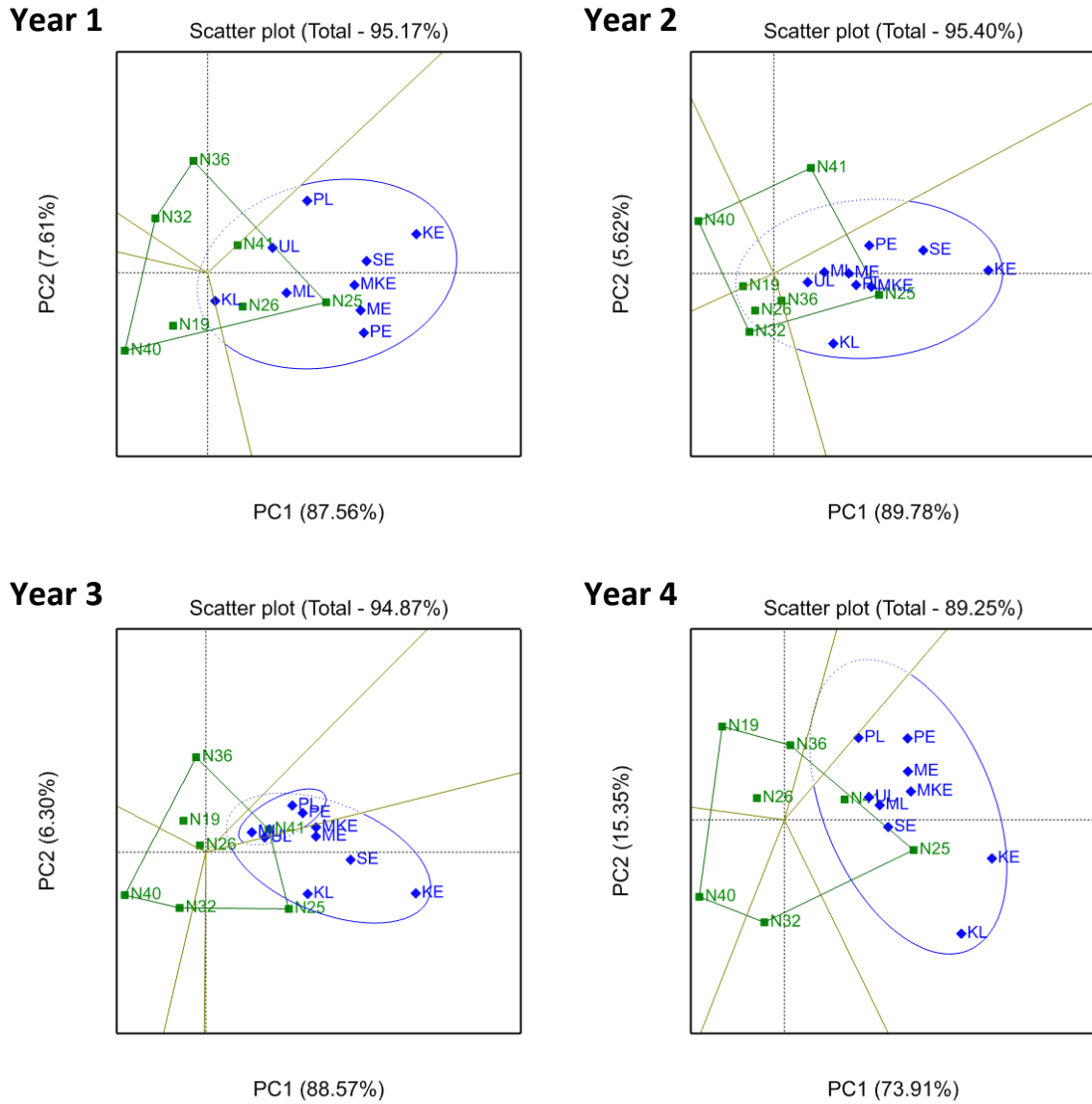


Figure 5.6: GGE biplots showing mega-environments for nine test environments planted with seven varieties harvested across four consecutive years (Year 1, Year 2, Year 3 and Year 4) evaluated for cane yield (TCH). Varieties are shown by squares while environments are shown by diamond shapes. The percentage variance accounted for by each principal component (PC axis) is indicated in parentheses

On Suc% cane, all the environments grouped in one MGE, and N40 was the dominant variety in Years 1 and 4 (Figure 5.7). In Year 2, environments PE and ML fell in the N26 sector, while the rest of the environments were clustered together in the N40 sector. In Year 3, SE was isolated in the N26 sector.

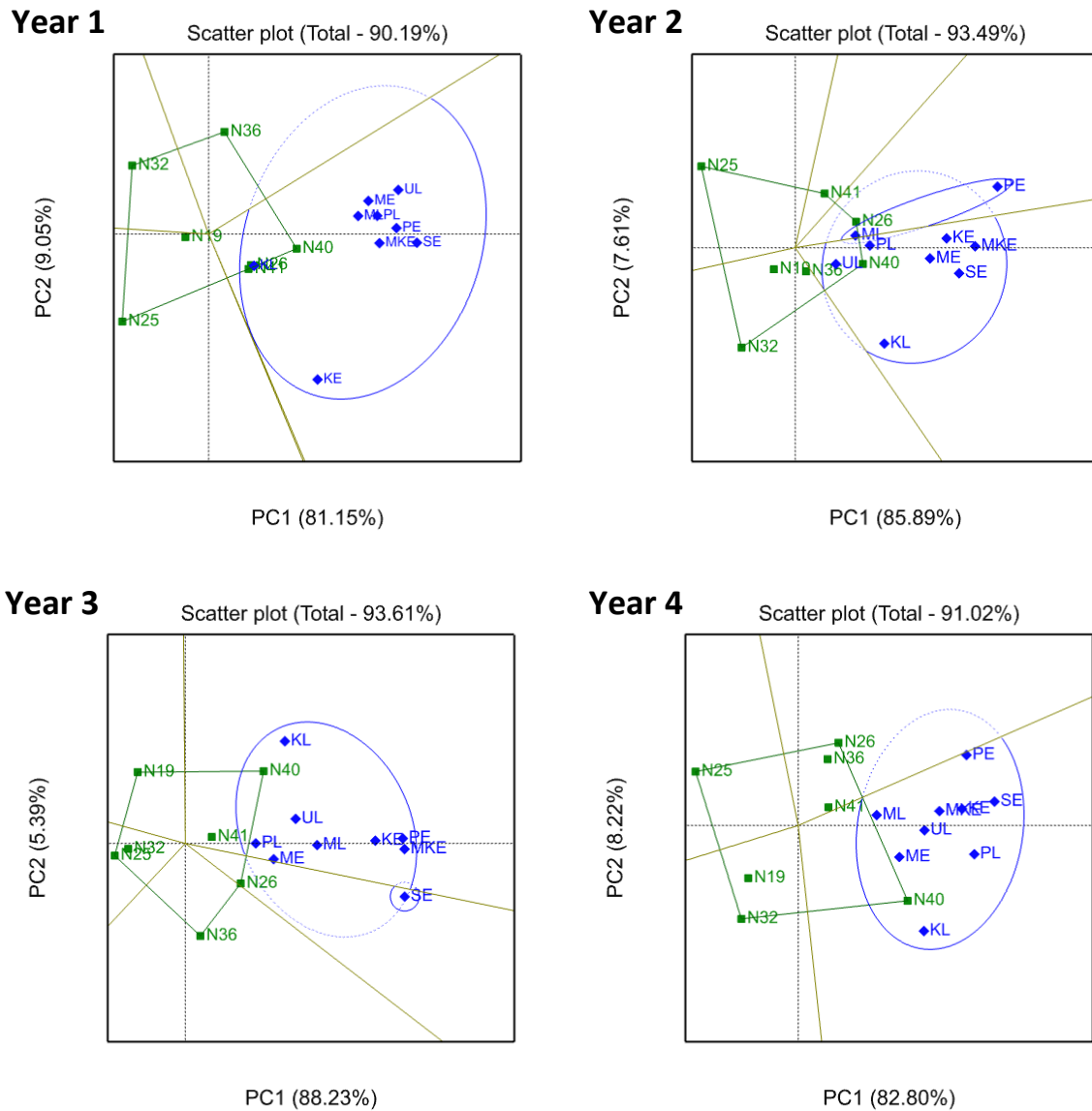


Figure 5.7: GGE biplots showing mega-environments for nine test environments planted with seven varieties harvested across four consecutive years (Year 1, Year 2, Year 3 and Year 4) evaluated for sucrose content (Suc% cane). Varieties are shown by squares while environments are shown by diamond shapes. The percentage variance accounted for by each principal component (PC axis) is indicated in parentheses

5.4.2.3 Discriminating ability and representativeness of test environments

Once the MGEs issue is addressed, test environment and variety evaluations become meaningful (Yan *et al.*, 2007). Within a single MGE, test environments should be evaluated for being, or not being, representative of the target MGE and for their ability to discriminate among varieties; and varieties should be evaluated for mean performance and stability across environments.

Figure 5.8 shows the discriminating ability and representativeness of the nine test environments for the three traits (TSH, TCH and Suc% cane) of interest. These analyses are based on the combined four year data for the different traits. Kaya *et al.* (2006) classify test environments into three: ideal, favourable and unfavourable. An ideal test environment is one that is most discriminating and most representative of the target environment. In the biplot, it is located on the average environment axis (AEA) in the positive direction with a distance to the biplot origin equal to the longest vector of all environments. The average environment axis (AEA) is the single-arrowed line that passes through the biplot origin and the average environment (Yan *et al.*, 2007). This average environment has the average coordinates of all the test environments (Yan and Tinker, 2006), and it is represented by the small circle behind the AEA arrow. A favourable environment is located between the average environment and the ideal environment. Environments located outside the concentric circle of the average environment are classified as unfavourable.

The ideal test environment (Figure 5.8) in biplots is at the center of the concentric circles (Yan and Tinker, 2006). The concentric circles were drawn to assist in visualizing the distance between each environment and the ideal environment (Yan and Rajcan, 2002). While such an ideal environment may not exist in reality, it can be used as a reference for variety evaluation in METs (Kaya *et al.*, 2006). An environment is more desirable (discriminating and representative) if it is located closer to the ideal environment. Non-discriminating environments have shorter vectors (closer to point of origin) while non-representative environments have wider angles with the AEA.

For sucrose yield (TSH), SE and KE were the most discriminating and most representative environments because they were closest to the 'ideal environment'. PE, MKE and ME were also favourable environments since they had relatively longer vectors that had smaller angles

with the AEA. ME had sucrose yield equal to the average environment. ML and UL were representative (smaller angles with the AEA) but were not discriminating due to shorter vectors. PL and KL were neither discriminatory (shorter vectors) nor representative (wider angles with the AEA). Generally, the early harvested environments were more discriminatory and more representative compared to the late harvested environments.

For cane yield (TCH), KE was the most discriminating and most representative environment, followed by SE. These analyses further confirm that early harvests were more discriminatory compared to late harvests (similar to TSH). MKE and ME were also favourable environments since they had smaller angles with the AEA, and they had marginally higher mean cane yields than the average environment. ML and UL were representative but due to their short vectors they are characterised as non-discriminating. Compared to the other environments, PL and KL can be described as unfavourable since they had cane yield lower than the average environment, and relatively shorter vectors.

On Suc% cane, environment SE was the most discriminating and most representative followed by MKE. This is because of their closeness to the center of the concentric circles. PE was highly discriminating (longer vector) but not representative as shown by the wider angle with the AEA. KE was a favourable environment because of its relatively longer vector and being the same distance from the ideal environment as the average environment. While ML was most representative but the shorter vector made it an unfavourable environment. KL was neither discriminating nor representative hence unfavourable. UL, PL and ME were also unfavourable environments due to shorter vectors.

The biplots explained 92.15%, 95.53% and 95.79% of the total GEI for TSH, TCH and Suc% cane, respectively. These biplots captured more GEI for their respective traits compared to those explained by the AMMI analysis of variance (TSH, Table 5.4: 79.06%; TCH, Table 5.5: 80.70%, and Suc% cane, Table 5.6: 82.45%).

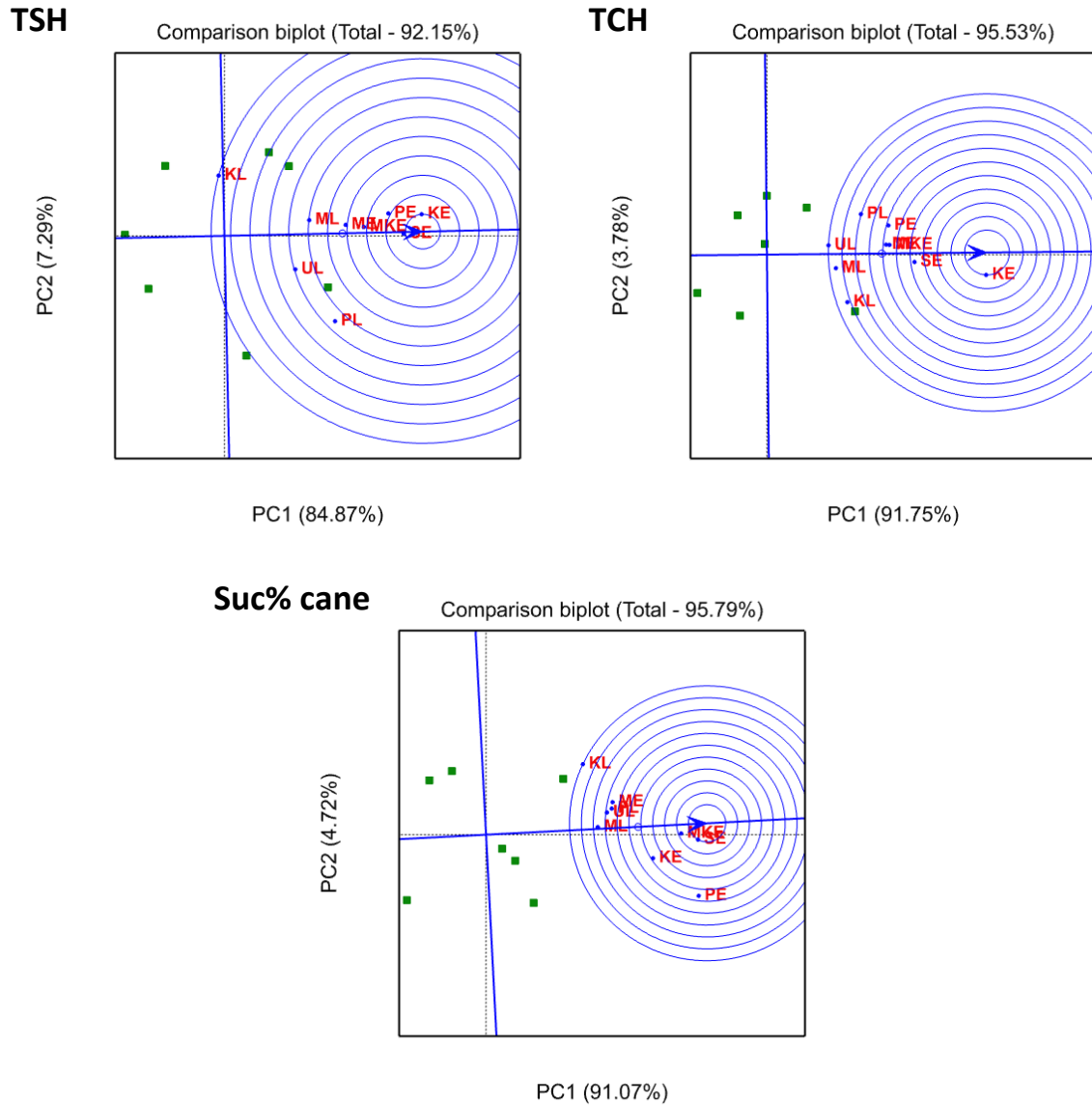


Figure 5.8: GGE biplot showing discriminating ability and representativeness of nine test environments planted with seven varieties averaged over four years for sucrose yield (TSH), cane yield (TCH) and sucrose content (Suc% cane). Varieties are shown by square shapes while environments are shown by small circles and their codes as explained in Table 5.1. The percentage variance accounted for by each principal component (PC axis) is indicated in parentheses

5.4.2.4 Mean performance and stability of varieties

Within a single MGE, an ideal variety is evaluated on both mean performance and stability across environments (Yan and Tinker, 2006; Yan *et al.*, 2007). According to Mostafavi *et al.* (2012) an ideal variety should have the highest mean performance and be absolutely stable.

Such an ideal variety is defined by having the greatest vector length of the high-yielding varieties and with zero GEI (Yan, 2001; Yan and Rajcan, 2002). While such a variety may not exist in reality, it can be used as a reference for variety evaluations (Kaya *et al.*, 2006). A variety is more desirable if it is located closer to the ideal variety. In Figure 5.9, the ideal variety is at the center of the concentric circles of TSH, TCH and Suc% cane biplots. These biplots explain the same proportion of the total GEI as their equivalents in Figure 5.8 (environment evaluation), the difference being that they are genotypic-metric preserving ($SVP = 1$) while those in Figure 5.8 are environment-metric preserving ($SVP = 2$). The line that passes through the point of origin and is perpendicular to the AEA is called the average environment coordination (AEC) ordinate (Kaya *et al.*, 2006). Moving along the AEC ordinate either direction away from the biplot origin indicates greater GEI effect and reduced stability. Furthermore, the AEC ordinate separates varieties with below-average means from those with above average means. Varieties on the positive side have above average mean yield while those on the negative side have mean yield below the average.

Variety N41 was high-yielding and most stable on TSH compared to the other varieties, since it was closest to the center of the concentric circles (Figure 5.9). N32 was very close to the AEA implying that its performance was consistent (stable) across environments, however, its location on the furthest negative direction indicates that it was consistently the poorest. Varieties N25, N26 and N36 had above average TSH and they were adapted to specific environments (unstable). N40 and N19 were unstable and had mean yield below average TSH.

Variety N25 was the most high-yielding and most stable on TCH. N41 was not stable but had mean yield that was above the overall average TCH across environments. N26 had relatively stable performance on TCH as shown by its proximity to the point of origin. However, its mean yield was similar to the overall average TCH as demonstrated by its position on the AEC ordinate. Similarly, N36 had mean TCH that was equal to the overall average yield. N19, N32 and N40 had mean cane yields that were below the overall average. Varieties were not widely dispersed from the point of origin on TCH when contrasted to TSH. This confirms that the tested varieties were more stable on TCH than TSH.

Variety N40 was the highest performing and most stable on Suc% cane as it was closer to the ideal variety. N36 and N41 were relatively stable and they had Suc% cane above the overall average across environments. While N26 performed above the overall average on this trait, its

performance was not consistent over the environments. Varieties N19, N32 and N25 were unstable and had mean Suc% cane below the overall average.

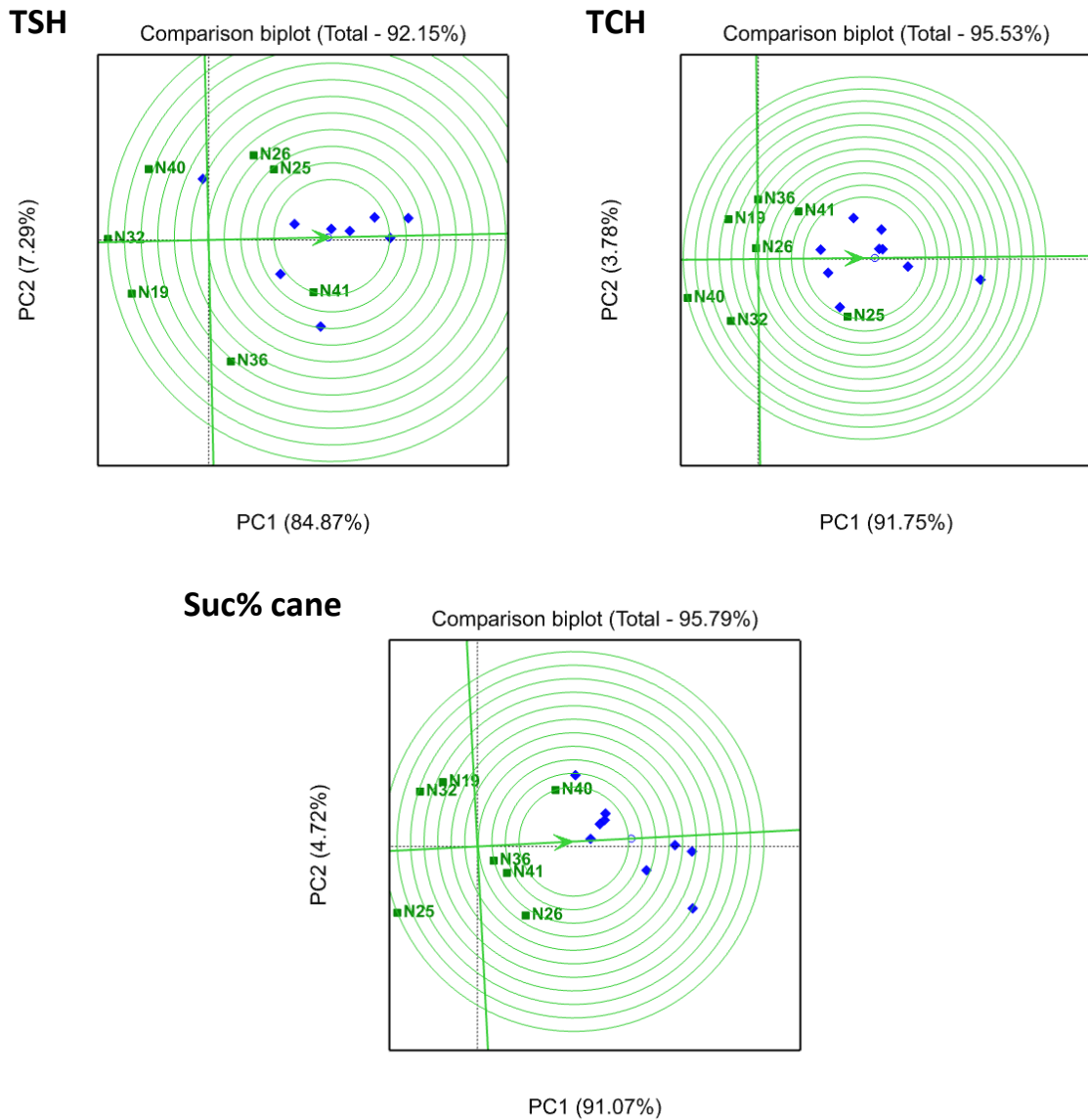


Figure 5.9: GGE biplot showing yield performance and stability of seven varieties tested over eight environments for sucrose yield (TSH), cane yield (TCH) and sucrose content (Suc% cane) averaged over four consecutive years. Environments are shown by square shapes and their names while environments are shown by diamond shapes. The percentage variance accounted for by each PC axis is indicated in parentheses

5.5 Discussion

The results of this study provided valuable information and insight on the GEI characterising the northern irrigated region of South Africa and Swaziland. The purpose of the study was to establish if there exist similarities between testing sites of the two sugar industries so that shared information from variety evaluation trials is utilised appropriately. SASRI generates information for released varieties earlier than SSA, hence there was a need to establish if SSA can utilize this information to recommend new varieties for local growers. The benefits accruable from such an exercise could include the cost effective use of research resources, and accelerated adoption of SASRI varieties by Swaziland growers. To investigate this, a combined analysis of data collected from post-release variety trials with varieties common to both industries was conducted.

The order of effects on phenotypic stability for all three traits (TSH, TCH and Suc% cane) was environment > variety > GEI. The very large effect of environments indicated that the test environments were highly variable, as a result their potential for sugarcane production differed significantly. Diversity in environmental conditions results in great variation in crop productivity between test environments (Klomsa-ard *et al.*, 2013). The incidence of pests and disease, annual rainfall, drought, soil fertility, soil waterlogging, soil depth and photoperiod are some of the factors responsible for environmental differences (Ferreira *et al.*, 2006). Allard and Bradshaw (1964) classified variations due to environments into two groups: predictable (climate, soil type, day length, some agronomic practices, and many others) and unpredictable (rainfall, radiation, temperature and many others). According to these authors, most of the factors responsible for environmental variations are unpredictable. In this study, seasonal effect might have also contributed to this larger environmental effect, since time of harvest was confounded with environmental effects, yet seasons are known to affect sugarcane performance (Redshaw and Nuss, 2001; Gilbert *et al.*, 2006; Di Bella *et al.*, 2008; Ramburan *et al.*, 2010; Zhou, 2015a).

Environments accounted for 79.98% (TSH), 85.72% (TCH) and 88.15% (Suc% cane) of the total variation in variety performances. Generally, environment main effect accounts for 80% or more of the total yield variation (Yan, 2002). Ramburan (2011) reported 63.90%, 70.70% and 61.40% for TSH, TCH and Suc% cane, respectively. Rodriguez *et al.* (2010) and Da Silveira *et al.* (2013) reported 65.20% and 73.36% for TSH, respectively. Similar results were

reported for other crops: Voltas *et al.* (2002), Ahmadi *et al.* (2012) and Akbarpour *et al.* (2014) reported 70.4%, 87.6% and 79.0% for barley, respectively; Muungani *et al.* (2007) and Malosetti *et al.* (2013) reported 88.6% and 79.9% for maize, respectively; Rakshit *et al.* (2012) reported 76.30% for sorghum; Farshadfar *et al.* (2013) reported 86% for wheat; and Nowosad *et al.* (2016) reported 69.82% for rapeseed. An exception was reported by Kumar *et al.* (2011) who found that only 22.34% of the total SS was attributable to environment for TCH. This suggests that results from GEI studies are not universal, such that the implications and potential selection strategies that develop from them may differ among crop improvement programmes (Kimbeng *et al.*, 2009).

The highly significant variety main effect for all characters (TSH, TCH and Suc% cane) indicated that there was a considerable amount of phenotypic variability within the varieties. This is traced back to the heterozygous and polyploid nature of the sugarcane crop which has resulted in generation of greater genetic variability (Chaudhary, 2001). Since sugarcane is vegetatively propagated, variability between varieties is created by hybridisation, a common practice in all sugarcane breeding programmes. The parents used as breeding material are heterozygous, as a result the progeny of the crosses possess a large amount of genetic variability. Importantly, this genetic variability is ideal for making progress during selection of superior varieties (Zhou *et al.*, 2011). It is well documented that the success of any variety improvement programme depends largely on the amount of genetic variability present within the population (Jamoza *et al.*, 2014). So, the diversity in performance of the experimental varieties illustrates the progress made within the SASRI breeding programme to widen their genetic base.

The highly significant GEI effect suggests that the performance of the varieties for TSH, TCH and Suc% cane performance was dependent on the test environments. This validated the necessity of conducting variety evaluation trials at more than one testing site. Significant GEI effects restrict breeders' efforts to release high yielding varieties over target area. Once significant GEI is detected, the next step is exploring the potential causes of GEI. To achieve this, data of genetic and environmental covariates are required (Yan and Tinker, 2006); Yan *et al.*, 2007). Ramburan (2011) and Ramburan (2012) used the AMMI biplot analysis to investigate and interpret environmental covariates in GEI studies. Yan and Tinker (2006) exploited the GGE biplot analysis to interpret GEI of oat yield using genetic values of explanatory traits. Voltas *et al.* (2002) used factorial regression models and the AMMI analysis

to investigate associations between genetic and morpho-physiological covariates in barley. Such studies have the potential to identify salient opportunities for using multivariate techniques to interpret GEI, and the results thereof are expected to significantly improve variety recommendation strategies (Ramburan and Zhou, 2011).

This significant GEI was also shown by the GGE biplots analysis, and it was distinct on TSH (Figure 5.2) and TCH (Figures 5.3). For TSH, crossover GEI was observed across all four test years while for TCH it was observed in two of four years. For Suc% cane, there was no crossover GEI over the four years (Figure 5.4). This confirms the understanding that cane yield has more complex GEI compared to sucrose content (Jackson and McRae, 2001). This is mainly caused by the large number of genes with small individual additive effects that control this trait (Zhou *et al.*, 2011; Zhou, 2015a). Sucrose content, on the other hand, is more strongly influenced by genetic composition than GEI (Nayamuth *et al.*, 1999; Ramburan and Zhou, 2011). Several GEI studies have reported that sucrose content is more stable than cane yield (Milligan *et al.*, 1990b; Kang and Miller, 1984; Rosa *et al.*, 2010; Sandhu *et al.*, 2014; Masri and Amein, 2015). It is on this premise that more experimental resources are required to detect differences among varieties on cane yield compared to sucrose content (Kimbeng *et al.*, 2009).

TSH had more pronounced crossover interactions compared to its components (TCH and Suc% cane), yet it represents the product growers are paid for in their crops (Zhou, 2013). TSH is a product of the primary traits: cane yield and sucrose content (Kang *et al.*, 1991; Jackson, 2005; Zhou, 2015b), hence it described as a secondary trait. The additive effects of the variability for the primary traits result in the higher variability on this secondary trait (Zhou, 2015b). Each primary trait is controlled by different set of genes, and the influence of environments on the cumulative expression of different set of genes vary considerably (Rakshit *et al.*, 2012). In addition, the un-relatedness and sometimes negative association between TCH and Suc% cane magnify the variability in TSH (Klomsa-ard *et al.*, 2013). This, therefore, demands greater precaution when selecting for sucrose yield (Zhou, 2015b). Milligan *et al.* (1990a) and Jackson (2005) reported that the contribution of cane yield to sucrose yield exceeds that of sucrose content for released varieties. However, there is a need for future studies to investigate the degree of correlation between these traits. It is envisaged that recent developments in biotechnology will assist breeders to incorporate useful genes from promising sources to advanced sugarcane varieties, and possibly overcome the incompatibility between cane yield and sucrose content.

In this analysis, the variety main effects were larger than GEI effect for all three traits. This suggests that TSH, TCH and Suc% cane were influenced to a larger extent by genetic effects as opposed to GEI effects (Ramburan and Zhou, 2011). Similar findings were reported by Ramburan (2011) for TCH and Suc% cane, and Nowosad *et al.* (2016) on rapeseed. However, Kumar *et al.* (2011) reported a GEI effect that was 3.8 times higher than variety effect on TCH. Similar GEI studies including those of Rodriguez *et al.* (2010); Da Silveira *et al.* (2013); Luo *et al.* (2014) - (sugarcane); Muungani *et al.* (2007); Kamutando *et al.* (2013); Malosetti *et al.* (2013) - (maize); Voltas *et al.* (2002); Ahmadi *et al.* (2012); Akbarpour *et al.* (2014) - (barley) and Farshadfar *et al.* (2013) - (wheat) reported higher GEI than variety effects. In such cases, breeding and selection for better varieties is difficult, since varietal performance is largely dependent on the environment.

To establish the relationships between environments in this study the GGE biplot analysis was utilised. This analysis provides one of the most advanced statistical tools used to identify relationships between test environments. Large variation due to environment justifies the selection of a GGE biplot as the appropriate method for analysing MET data (Dehghani *et al.*, 2006). The GGE biplots were constructed from the first two principal components (PC1 and PC2) derived from PC analysis of the environment-centered data. GGE biplots are useful in a number of aspects. In this study they were utilised to: identify correlations between environments, construct mega-environments (MGE), identify ideal test-environments, and identify high yielding and stable varieties for the MGEs. In this analysis, the first two principal components (PCs) of the biplots explained more than 78% of the total GEI, and the combined G + GEI effects accounted for more than 11% of the total variability. According to Rakshit *et al.* (2012), if the first two PCs explain more than 60% of the GEI in the data, and the combined (G + GEI) effect account for more than 10% of the total variation, then the biplot adequately approximates the variability in the GEI data. This, therefore, suggests that the biplots in this study were effective graphical representation of the variability present in the data and, as such meaningful.

The first two PCs of the GGE biplots (Figures 5.8 and 5.9) captured more GEI than the first two PCs of the AMMI analysis (Tables 5.3, 5.4 and 5.5) in all three traits. This confirms Yan *et al.* (2007)'s statement that the GGE biplot explains more G+GEI than the AMMI method and is, therefore, a more accurate presentation of the GGE of the data. According to Malosetti *et al.* (2013) GGE biplots approximate overall G+GEI while AMMI analysis approximate only

the GEI part of the phenotype. Yet G and GEI must be considered simultaneously because they are the two sources of variation in variety evaluation (Yan and Hunt, 2001). GGE is more logical and biological compared to AMMI in explaining PC1 score, which represents genotypic effect rather than additive main effect (Yan, 2002). Statistical and biological reasons for preferring GGE over AMMI in assessing complex GEI are explained by Crossa *et al.* (2010). Rodriguez *et al.* (2010) used both multivariate analyses (AMMI and GGE), and found that they were similar in showing variety performance across environments, and the difference being that the GGE captured more GEI than the AMMI.

There was a general observation that early harvested trials were more discriminatory on varieties for TSH (Figure 5.2) and TCH (Figure 5.3) compared to late harvested trials in this study. This is attributed to greater exploitation of more favourable conditions (radiation, temperature and rainfall) in the early season which coincides with period of stalk elongation, while late season - the phase of stalk elongation correspond to periods of lower levels of radiation, rainfall and temperature (Ramburan, 2011). It is general knowledge that early harvested cane yields higher TCH than late harvested cane. For variety improvement programmes, these macro-environments produced by the early and late seasons create a great challenge (Zhou, 2015a). As a result, Zhou (2015a) recommended that the SASRI breeding programme for the irrigated region be split into two, one for early season and the other for late season.

While most environments were positively related for TSH across years, KL was often negatively related to some environments (Figure 5.2). This implies that KL interacted differently with some varieties compared to the other environments. Six environments (ML, ME, MKE, PE, KE and SE) repeatedly grouped in the same MGE across all four years (Figure 5.5). In this MGE, N41 was the winning variety in the final three years while N25 was the winning variety only in Year 1. This suggests that some environments may be dropped from the testing network without much loss of information about the varieties (Yan and Tinker, 2006; Yan *et al.*, 2007; Muungani *et al.*, 2007). Environments UL and PL were located in the same sector in Years 1 and 2, however, with different winning varieties per sector. According to Yan and Rajcan (2002) and Yan and Kang (2003), the requirement for MGE division is a repeatable “which-won-where” pattern rather than merely a repeatable environment grouping pattern. Hence, the UL and PL sector may not be classified as a MGE.

For TCH, while there were some negative correlations, generally the test environments were positively correlated across years (Figure 5.3). The repeatable grouping of environments in one MGE across years (Figure 5.6) suggests that testing can be done in fewer sites than current practice without prejudicing test results. Variety N25 was consistently the winning variety in this MGE. An exception was in Year 3, where PE, PL and ML were located in the N41 sector, however, even then the data showed that N25 had higher TCH and N41 was second in rank. For Suc% cane, the biplots showed positive associations between the environments (Figure 5.4). This was further confirmed by the tendency of environments to group in one MGE across years, and the winning variety was N40 (Figure 5.7). Exceptions were PE and ML (Year 2) and SE (Year 3) which fell in the N26 sector.

The presence of a repeatable MGE suggests that there is no need to initiate separate testing sites within that MGE. In this study, a majority of the test environments were invariably located in one major MGE across the four years studied for all traits. Kamutando *et al.* (2013) also reported the existence of one MGE for maize GEI studies in Zimbabwe. Conducting METs for sugarcane is very costly owing to the resources required in the form of land, time, equipment and labour. The cost of testing can be reduced and testing efficiencies improved by using a smaller number of test environments. Klomsa-ard *et al.* (2013) identified four of nine test locations that were redundant, and they were omitted from the trial networks of sugarcane varieties in Thailand. Ramburan and Zhou (2011) using GGE biplots discovered that some trial sites that had potential to be removed for post-release evaluation purposes under the rainfed conditions of South Africa. Dehghani *et al.* (2006) identified two locations that provided similar information on barley varieties, and suggested that one be dropped to reduce testing costs. Ramburan (2012) identified two redundant test sites of six sites in the Midlands, and suggested that these be eliminated because they provided no unique information about the released varieties.

The next step after identifying MGEs is locating better testing environments. A better testing environment should be both discriminating of the varieties and representative of the MGE (Yan *et al.*, 2007). In the GGE biplots (Figure 5.8), the ideal testing environments were located closer to the theoretically ideal environment, which is at the centre of the concentric circles. Environments SE and KE were identified as the most discriminating and most representative on TSH, hence they are ideal for selecting widely adapted varieties. Both environments, apart from being early harvests (highly discriminating), are characterised by soils (Shortlands form)

with an A horizon that is red to reddish brown well-structured clay loam, and a B horizon that is dark red to reddish brown clay loam with a moderate structure. The B horizon has an effective rooting depth above 900 mm, hence these soils are classified as high potential soils in the sugar industry (Nixon, 2006). For TCH, KE was also identified as the most discriminating and most representative test environment, thus ideal for selecting widely adapted varieties on this trait. For Suc% cane, SE was identified as the most discriminating and most representative test environment. As a result this environment is appropriate for identifying generally adapted varieties on Suc% cane.

Another objective of this analysis was to identify superior varieties for the target region. To achieve this, biplots were constructed as discussed in sub-sub section 5.4.2.4. An ideal variety is one that has the highest mean performance and is absolutely stable across test environments. The interpretation follows that of environments, the superior variety is one that is located closer to the “ideal variety” at the centre of the concentric circles (Figure 5.9). Ramburan and Zhou (2011) and Mostafavi *et al.* (2012) stated that a variety nearest to the “ideal variety” in GGE biplots can be used as a reference in variety selection programmes. In this analysis varieties N41, N25 and N40 were identified as superior varieties for TSH, TCH and Suc% cane, respectively. As a result, these varieties are recommended as commercial checks in variety improvement trials for the different traits.

Variety N25 is a widely grown variety in the irrigated region covering more than 30% and 40% of the sugarcane area in South Africa (irrigated area) and Swaziland, respectively, due to its relatively higher cane yield. However, it is characterised by a relatively low inherent sucrose content, thus, it is recommended for planting mid to late season across all soil types. N40 has the highest sucrose content relative to other varieties in the irrigated region, however, its very low cane yield has hindered its adoption by growers. Due to its high sucrose content and low cane yield, it is recommended for growers that are a long distance from mills. N41 is relatively new when compared to N25 and N40, and its adoption by growers is increasing at an appreciable rate. This variety combines a higher cane yield (although generally lower than N25) and a relatively high sucrose content to produce higher sucrose yield. While it performs very well early season, its sucrose yield is stable across all seasons and soil types. Hence, the results of the study reflect actual on-farm performances.

5.6 Conclusions

This study revealed that environments had a greater influence on variety performances than variety and GEI effects, and variety effect was larger than GEI effect for sucrose yield, cane yield and sucrose content. Generally, early harvested trials were more discriminating compared to late harvested trials. The tested varieties were more stable for sucrose content than cane yield. Sucrose yield was more unstable compared to its primary components. Variety N41 was both high yielding and most stable for sucrose yield across environments, indicating that its performance was not dependent on environments when compared to other varieties. Varieties N25 and N40 were higher yielding and stable for cane yield and sucrose content, respectively.

Importantly, the study showed a great opportunity for reducing testing environments, and consequently reduce testing resources. For sucrose yield, the product of value to growers, testing sites ML, ME, MKE and PE can be dropped from the testing network, and data from either KE or SE can be used to formulate recommendations for these areas. SE and KE were actually the most discriminating and most representative test environments in the MGE constituting the environments ML, ME, MKE, PE, SE and KE. In essence, SSA can utilise information accumulated from SASRI environment KE to formulate variety recommendations for ML, ME, MKE and SE. By so doing, Swaziland would move in the right direction towards fully maximizing the benefits accruable from the research and development cooperation agreement between the two sugar industries of Swaziland and South Africa. Alternatively, resources from both industries could be combined to evaluate a wider range of varieties across similar sites to represent a single agro-climatic region.

Due to inconsistency in the results of UL and PL, it is recommended that testing in these environments continue independently. KL was consistently disassociated from the rest of the environments, hence testing in this environment should continue. Future studies will have to investigate and interrogate the factors behind these differential responses to direct regional variety recommendation strategies.

5.7 References

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The Swaziland sugar industry from the early 1980s has been importing sugarcane varieties from the South African Sugarcane Research Institute (SASRI) for testing prior to release for commercial cultivation. The testing is conducted across seasons and soil types over multiple crop-years. The test sites were selected to represent the prevalent soil characteristics under which sugarcane is grown in the country. The drive was the fact that sugarcane varieties interact differently with different soil types. The findings from these trials determine the variety recommendation programme of the industry. Historically, not all the imported varieties were tested across all soil types and seasons, probably due to resource constraints (land and seed material), trial failure, and/or lack of knowledge. In such instances, variety recommendations for certain sites were inferred from results of other sites, ignoring the effect of GEI. Prior to this study, there was no evidence of any research initiated to gain understanding of GEI within the industry. While so much data were collected over the years, it was rarely utilized to its full capacity to investigate GEI. Yan and Tinker (2006) lamented that genotype by environment data is often limited to genotype evaluation which is based on genotype main effect while GEIs are treated as noise or a confounding factor.

Chapters 3 and 4 of the study showed significant GEI within the Swaziland sugar industry. GEI according to Crossa *et al.* (2010), is known to cause inconsistent responses of some varieties relative to others due to genotypic rank change, or as changes in the absolute differences between varieties without rank change. As a result, this study was able to characterise varieties as either stable (wider adaptation) across environments or specifically adapted to certain environments. GEI can be exploited by either selecting widely adapted and stable varieties across a wide range of environments or selecting superior varieties for each specific target environment (Ceccarelli, 1989). Consequently, this will assist to maximize benefit from imported varieties. One of the greatest challenges in most sugar industries (and other crops) is the indiscriminate planting of high yielding but specifically adapted varieties. This eventually leads to them becoming unpopular due to poor performance or to their demise due to spread of pests and diseases and/or poor management (Ramburan, 2015). It is suggested that systematic and simultaneous testing be carried out across locations/soil types, seasons and crop-years covering all the experimental sites of the industry for a comprehensive study of GEI.

The attention of plant breeders in times past was more centered on the economic yield, for example sucrose yield in sugarcane. In recent years, there has been a growing interest in understanding the developmental pathways by which the final yield is reached. Gaining an understanding of the biochemical, physiological, or morphological basis of the interplay between variety and environment is expected to improve the efficiency of breeding (Allard and Bradshaw, 1964). In sugarcane, breeders have also carried out studies on character associations (covariance analysis) (Milligan *et al.*, 1990; Ramburan, 2012; Bora *et al.*, 2014; Jamoza *et al.*, 2014; Tadesse and Dilnesaw, 2014). Voltas *et al.* (2002) lamented that most GEI studies are purely empirical, describing postdictively fixed varietal performances across a fixed sample of environments, yet a preferred analytical approach should characterize both varieties and environments in terms of a number of biotic and abiotic variables which directly affect performance. Ramburan (2012) demonstrated the possibility of establishing relationships between sugarcane phenology and environmental covariates. Identifying significant GEI is not sufficient, efforts have to be made to identify and explain the factors defining significant GEI. As a result, it is recommended that future studies should consider combined analyses of genetic correlations and environmental covariates for comprehensive interpretation of GEI. Such studies can also assist in crop modelling, and as such automatic weather stations should be provided in each test location (Ramburan, 2012).

Throughout the study variety main effect was significant. Chapters 3 and 4 dealt primarily with varieties imported from Mauritius (R570, M1176/77, M1186/86, M1246/84, M1400/86, M1551/80, M695/69 and M96/82), while Chapter 5 dealt with varieties imported from South Africa (N19, N25, N26, N32, N36, N40 and N41). According to D'Hont *et al.* (1996) modern sugarcane varieties are aneuploid, interspecific hybrids with chromosome numbers ranging from $2n = 100$ to 130, comprising 70 to 80% *Saccharum officinarum*, 10 to 20% *Saccharum spontaneum*, and 10% recombinant chromosomes between the two species. In sugarcane breeding, improving the genetic base of the modern sugarcane varieties is the major concern for sugarcane breeders worldwide (Govindaraj *et al.*, 2012). However, the highly heterozygous nature of the sugarcane crop combined with its higher polyploidy makes sugarcane breeding a complex exercise. While exploring the genetic structure of the sugarcane crop was beyond the scope of this study, the diversity in performance within the populations studied was evident. Chapters 4 and 5 showed opportunities at the industries' disposal to rationalize the use of testing resources. While testing across seasons in all locations was suggested, the analysis in Chapter 4 indicated that four replications and four crop-years are sufficient to discriminate

variety mean performances. This indicated a saving of up to four replications and a crop-year. Analysis in chapter 5 demonstrated opportunities for the Swaziland sugar industry to utilize information from SASRI's irrigated regions to recommend varieties to local growers. The benefits accruable from this is the adoption of new SASRI varieties early, eliminate unnecessary testing and reduce variety testing costs. It is suggested that planned simultaneous testing across SSA and SASRI irrigated trials involving 10 or more varieties be considered to validate these findings. The savings on resources may be utilised to import and test varieties from other sources where irrigated breeding programmes exist. These include Australia, USA, Réunion, Mauritius and Zimbabwe.

Another opportunity would be sourcing SASRI varieties at pre-release stage which ordinarily requires more land and finance. However, this additional costs will be covered by the benefit of being able to introduce new varieties earlier with a compounding effect on returns. In addition, this would have the advantage testing and identifying varieties for other needs that are specific to Swaziland i.e. mechanical/green cane harvesting and energy cane. This avenue needs to be carefully explored, as other sugarcane growing countries within the region such as Zimbabwe and Malawi import SASRI varieties at pre-release to test their adaptability to local conditions.

The utilization of broad sense heritability (BSH) estimated from variance components to simulate the optimum number of resources required for variety testing was also an intriguing part of the study. While several studies elsewhere used BSH for the same objectives (Swallow and Wehner, 1989; Brown and Glaz, 2001; Rattey and Kimbeng, 2001; Kimbeng *et al.*, 2009; Zhou *et al.*, 2012) no similar studies have been conducted in Swaziland. BSH predicts the phenotypic variability that is due to total genetic variance. The limitation of the analysis in this study was the use of only four locations which were constituted by the combination of test site and harvesting seasons, planted with only nine common varieties. As a result, it is strongly recommended that future studies consider expanding the number of locations and varieties to validate the findings of this study.

The GEI analysis indicated that seasons have a significant effect on the performance of varieties (GxS). Due to resource availability, variety testing at present is done in early and late seasons. Zhou *et al.* (2012) reported significant GxS interaction, where seasons included early, mid and late. Sugarcane varieties are classified into three distinct maturity groups (early, mid-

, and late) as per their patterns of sucrose accumulation (Nayamuth *et al.*, 2005). Variety testing in other industries such as South Africa (Ramburan, 2011), Malawi (Zhou *et al.*, 2012), Zimbabwe (Zhou, 2004) and Mauritius (Nayamuth *et al.*, 2005) is done consistently across all three seasons. Hence, resources saved from reducing the number of replications and crop-years as well as from collaboration with SASRI could be used to establish more trials equally split across three seasons. This would assist to identify varieties suited to the different seasons, thus maximizing benefit derivable from GEI effects. Matching varieties with locations/soil types and seasons is the ultimate goal of multi-environment trials.

Good analytical methods are a prerequisite for accurately predicting the performance of varieties in crops (Malosetti *et al.*, 2013). In this study, variance components and biplots were used to clarify GEI and summarise the patterns and relationships of varieties and environments. Variance component analyses were primarily targeted at evaluating the relative contributions of different sources of variation to GEI. In Chapters 3, 4 and 5, the variance components indicated the amount of yield variation attributed to each component. Worth noting is that, while genotype x location (GxL) and genotype x crop-year (GxC) interactions were significant for sucrose yield, variety x location x crop-year (GxLxC) was not significant (Chapter 3 and 4). This implied that ratooning ability of the tested varieties was not dependent on location, hence, the evaluation of ratooning ability at a particular location was sufficient to characterize the varieties on this attribute. However, this conclusion should be cautiously handled due to the limited number of locations used in this study. Ratooning ability is an essential criterion in assessment of a variety before release for commercial cultivation.

The AMMI and GGE biplots are the two multivariate analyses that were used in this study. While variance components analysis provides knowledge of the components of GEI, they do not provide detailed information on the performance of varieties across a series of environments. Biplots establish and display the pattern of genetic response across environments in graphical forms. Although both analytical methods appeared to converge at similar conclusions in Chapter 3, each had its own strengths and weaknesses. The AMMI 1 biplot was useful at displaying variety performances and stability. The AMMI 2 biplot was used to determine the correlations between varieties and test environments. The strongest function of the GGE biplot analysis was the stratification of test environments into homogenous agroecological zones (mega-environments) and the identification of winning varieties in each mega-environment. In Chapter 5, the GGE biplot was also used to identify the ideal variety and

environment. The choice of analytical method seems to depend largely on the type of data and analysis required. For example, while in this study the GGE biplot was the most preferred due to the ability to characterise environments into mega-environments, Ramburan (2012) preferred the AMMI biplot because of its ability to correlate environmental covariates and genotypic traits.

Moving forward, all future variety testing trials will be adjusted to comprise four replications and tested across four crop-years (plant plus three ratoon crops). This will require redesigning and modification of present testing panels at each test site. The issue of using information from SASRI derived information to recommend varieties to Swaziland growers will have to be discussed further for possible incorporation into the Research and Development Cooperation Agreement between the two industries. Based on the performance of Mauritian imported varieties across test environments, a motivation will be made through industry structures to have the two top varieties M1176/77 and M1400/86 approved for commercial cultivation in Swaziland. Once approved, the necessary procedures to have the varieties gazetted as indicated in the industry's Sugar Act of 1967 will be followed. Consequently, the variety recommendation information for the Swaziland sugar industry will be updated accordingly.

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SUMMARY

The primary aim of this study was to assess the genotype by environment interaction (GEI) in the sugarcane Variety Evaluation Programme (VEP) of the Swaziland Sugar Association (SSA). This was achieved by pursuing the specific objectives: to evaluate the adaptability and phenotypic stability of imported sugarcane genotypes in Swaziland; to determine the optimum combination of locations, replications and crop-years necessary to provide an adequate level of discrimination among genotypes within the SSA VEP; and to undertake a combined data analysis of irrigated sugarcane variety trials in Swaziland and South Africa. Data from replicated trials established across locations, seasons and crop-years were used, and the GenStat® 17th Edition software was used for the analyses.

The study indicated that the main effects of variety, location and crop-years were significant, showing genetic diversity amongst the tested material and the complex environments under which they are tested. Consequently, these resulted in significant genotype by environment interactions (GEI). For sucrose yield, variety x location and variety x crop-year interactions were significant, while the higher order interaction (variety x location x crop-year) was not significant. Consistently, environments accounted for a larger portion of yield variation. The ratio of variety:GEI was largely dependent on the source of varieties. Varieties imported from Mauritius had a larger GEI effect than variety effect, while South African varieties had larger variety than GEI effects. This is attributed to the fact that conditions at which South African varieties are tested are similar to those in Swaziland. The grouping of test environments according to season clearly indicated that seasonal effects are stronger than location effects, as a result more emphasis should be put on testing across seasons than locations/soil types. Variety M1176/77 proved to be high yielding, stable and widely adapted to the sugarcane growing conditions of Swaziland, while M1400/86 was high yielding but specifically adapted to good draining soil conditions.

To model the optimum number of locations, replications and crop-years necessary to provide adequate discriminating ability among varieties, the broad sense heritability was used. The results indicated that four locations, four replications per trials, and four crop-years were sufficient for variety testing within the industry. While the data used for the combined analysis of irrigated sugarcane variety trials of Swaziland and South Africa were

largely unbalanced, the analysis indicated potential for SSA to utilise results from SASRI post-release trials to inform the industry's variety recommendation. Both industries should consider pooling together testing resources and conduct variety evaluations at regional level. It is envisaged that making these adjustments will ensure that SSA derives maximum benefit from the resources used to test imported varieties.

Keywords: genotype by environment interaction, resources, sucrose, sugarcane, Swaziland, trials, varieties, yield

OPSOMMING

Die hoofdoel van hierdie studie was om genotipe by omgewing interaksie (GEI) in die suikerriet Cultivar Evaluasie Program (VEP) van die Swaziland Suiker Assosiasie (SSA) te bepaal. Dit is bereik deur spesifieke doelwitte: om die aanpasbaarheid en fenotipiese stabiliteit van ingevoerde suikerriet genotipes in Swaziland te bepaal; om die optimum kombinasies van lokaliteite, herhalings en gewas-jare te bepaal wat nodig is om 'n goeie vlak van diskriminasie tussen genotipes binne die SSA VEP te kry; en om gekombineerde data analise van besproeide suikerriet cultivar proewe in Swaziland en Suid Afrika te doen. Data van gerepliseerde proewe oor omgewings, seisoene en gewas-jare is gebruik, en GenStat® 17th uitgawe sagteware is gebruik vir analise.

Die studie het getoon dat hoofeffekte van cultivar, omgewing en gewasjare betekenisvol was, wat genetiese diversiteit in die getoetsde materiaal en die komplekse omgewings waar dit getoets is, getoon het. Dit het gevolglik gelei tot betekenisvolle genotipe by omgewings interaksies (GEI). Vir sukrose opbrengs, was cultivar x omgewing en cultivar x gewas-jaar interaksies betekenisvol, terwyl hoër vlak interaksie (cultivar x omgewing x gewas-jaar) nie betekenisvol was nie. Die omgewings het konstant vir 'n groot deel van die variasie gesorg. Die verhouding van genotipe:GEI was grootliks afhanklik van die bron van die cultivars. Cultivars ingevoer vanaf Mauritius het 'n groter GEI effek as cultivar effek getoon, terwyl Suid Afrikaanse cultivars groter cultivar as GEI effekte getoon het. Dit kan toegeskryf word aan die feit dat toestande waaronder Suid Afrikaanse cultivars getoets word, ooreenstem met toets-toestande in Swaziland. Die groepering van toetsomgewings volgens seisoen het duidelik getoon dat die seisoen effekte groter as omgewingseffekte was, daarom moet daar meer aandag gegee word aan toetsing oor seisoene as oor omgewings/grondtipes. Cultivar M1176/77 het hoë opbrengs gegee, was stabiel en wyd aangepas in die suikerriet groeitoestande van Swaziland, terwyl M1400/86 hoë opbrengs gegee het, maar spesifiek aangepas was vir grond met goeie dreinerings.

Om die optimum aantal omgewings, herhalings en gewas-jare te modelleer wat goeie diskriminasie tussen cultivars sal gee, is breë sin oorerflikheid gebruik. Die resultate het aangedui dat vier lokaliteite, vier herhalings per proef en vier gewas-jare genoeg was vir cultivar toetsing binne die industrie. Terwyl die data wat gebruik is vir die gekombineerde analise van besproeide suikerriet cultivar proewe van Swaziland en Suid Afrika grootliks

ongebalanseerd was, het die analise die potensiaal vir SSA getoon om die resultate van SASRI se na-vrystellingsproewe te gebruik om industrie cultivar aanbevelings te maak. Die alternatief is dat beide industrië dit kan oorweeg om evaluasie hulpbronne bymekaar te gooi en om cultivar evaluasies op streek vlak te doen. Die verwagting is dat hierdie aanpassings sal sorg dat SSA maksimum voordeel trek uit die hulpbronne wat gebruik word om ingevoerde cultivars te evalueer.

Sleutelwoorde: cultivars, genotipe by omgewing interaksie, hulpbronne, opbrengs, proewe, sukrose, suikerriet, Swaziland