

REFEREED PAPER

## **INFLUENCE OF LOCATIONS AND SEASONS AND THEIR IMPLICATIONS ON BREEDING FOR SUGARCANE YIELD AND SUCROSE CONTENT IN THE IRRIGATED REGION OF SOUTH AFRICA**

ZHOU, M

*South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa  
University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa*

*Marvellous.Zhou@sugar.org.za*

### **Abstract**

Breeding programmes conduct multi-environment trials to evaluate the adaptability and performance of genotypes across variable agro-ecological regions. Genotype by environment interaction (GxE) causes the genetic values to vary across environments. When GxE is large, then greater genetic gains can be achieved through developing specific genotypes for each environment. The objectives of this study were to investigate location and seasonal effects on genotype performance for cane yield and sucrose content and their implication on breeding varieties for the irrigated regions of South Africa. Data were collected from a series of variety trials planted in 2009 and 2010 at Mpumalanga and Pongola locations in the early and late seasons. Randomised complete block designs with three replications were used for the variety trial at each site. Data for cane yield and sucrose content were collected in the plant, first, second and third ratoon crops. Data were analysed using the mixed procedure of Statistical Analysis System. 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 regions. Seasonal effects were larger for sucrose content than cane yield. Genotype by ratooning effects was non-significant, indicating that ratooning ability was less important in irrigated regions. High cane yield and low sucrose in early season and low cane yield and high sucrose in late season indicated the uniqueness of the early and late seasons. Establishing early and late season breeding programmes in the irrigated region would enhance genetic gains for yield and quality in each season.

*Keywords:* locations, seasons, breeding, irrigation, cane yield, sucrose content

### **Introduction**

Breeding programmes conduct multi-environment trials to evaluate the adaptability and performance of genotypes across agro-ecological environments (Yan and Tinker, 2006). Agro-ecological environments vary from one region to another, as well as within regions (Setimela *et al.*, 2005). Genotype x environment interaction (GxE) results in the genetic values varying from one environment to another. If GxE is large, then greater genetic gains can be achieved through

developing specific genotypes for each environment (Windhausen *et al.*, 2012). Developing broadly adapted varieties implies the absence of significant GxE.

The influence of GxE on yield and quality in sugarcane is known to be large (Kimbeng *et al.* 2002) and can influence the precision of selection. GxE in sugarcane is largely controlled by locations, time of planting or harvest (also known as seasons) and crop-years (Kimbeng *et al.* 2009). Genotype testing trials need to be established across representative locations and seasons, and harvested for several crop-years to increase precision in determining genetic values. Increasing the accuracy of determining genetic values improves the estimation of genotype differences and improves selection efficiency. Accurate selection leads to advancement of high yield and quality genotypes to later testing stages and eventually commercial release.

Increasing selection and genetic gains are key measures of the success of breeding programmes. Niche breeding by nature allows for the development of varieties with adaptation to specific environments. Niche breeding is also acknowledged to increase selection and genetic gains because it reduces GxE, and more specifically, GxL. South African Sugarcane Research Institute (SASRI) restructuring in the late 1990s was designed to increase selection and genetic gains (Nuss, 1998). Quantifying the environmental niches prevalent in breeding programmes provides for accurate determination of correct testing sites in order to develop varieties specific to the requirements of those agro-ecological regions.

This study was based on data derived from the irrigated breeding programme of the South African Sugarcane Research Institute (SASRI) at Pongola and Mpumalanga research stations. The early stages of selection and testing are conducted at Pongola research station, while the final testing stage is conducted at Pongola and Mpumalanga locations. All crops are irrigated and harvested at 12 month crop age. The early and late seasons are distinct. The early season represents the period from January to May of the year, and the late season represents the period from October to December of the year. The mid-season represents the period from June to September of the year.

The weather at Mpumalanga and Pongola is characterised by low rainfall and high temperatures in summer, and cool and dry weather in winter. Rainfall is also poorly distributed and irrigation is used to supply crop water requirements. Early crop harvesting starts during the late rains in summer when the crop is still actively growing, resulting in low sucrose content.

The objectives of this study were to investigate the location and seasonal effects on genotype performance for cane yield and sucrose content and their implication on breeding sugarcane varieties for the irrigated regions in South Africa.

## **Materials and Methods**

### *Experiment materials*

Data were collected from the series of variety trials planted in 2009 and 2010 (Table 1) at two locations, namely Mpumalanga and Pongola. At each location, the trials were planted in the early and late seasons. At Mpumalanga, one trial each was planted for the early and late season. At

Pongola, one trial was planted in the early season and two trials were planted in the late season. Trials FVR09 and FVS10 were established in the early season in 2009 and 2010, respectively, while FV2R09 and FV2S10 were established late season in 2009 and 2010, respectively, all at Pongola research station. Trials NPV2R09 (2009), NPV2R10 (2010) and NPV2S10 (2010) were established late season at the Pongola off-station site. Trials NNVR09 and NNVS10 were established early season in 2009 and 2010, respectively, at Mpumalanga research station. Trials NTV2R09 and NTV2S10 were established late season in 2009 and 2010, respectively, at Mhlati, an off-station site in Mpumalanga. Data were collected in the plant, first, second and third ratoon crops. All the trials were harvested at 12 months crop age.

#### *Experimental design and data collection*

Each trial was laid out as a randomised complete block designs with three replications. Plot sizes were five rows by 8 metres long spaced 1.4 m apart. The number of genotypes planted in each trial ranged from 32 to 36. Different genotypes were planted to trials each year in each trial series. At harvest, all millable stalks in the plots were cut and weighed. From each plot, 12 stalks were randomly picked to provide a sample for estimating sucrose content (Schoonees-Muir *et al.* 2009). The sucrose content (%) was expressed as estimable recoverable crystal (ERC) using an empirical formula that accounts for losses via bagasse and molasses. The cane weights in a plot were converted to cane yield (tons/ha) by dividing by the plot size.

**Table 1. Series, locations, seasons, trials, crops and years harvested.**

Series	Location	Season	Trials	Crops	Years harvested
2009	Mpumalanga	Early	NNVR09	P, 1R, 2R, 3R	2010-2013
		Late	NTV2R09	P, 1R, 2R, 3R	2010-2013
	Pongola	Early	FVR09	P, 1R, 2R, 3R	2010-2013
		Late	FV2R09	P, 1R, 2R, 3R	2010-2013
			NPV2R09	P, 1R, 2R	2010-2012
			NPV2R10	P, 1R, 2R	2011-2013
2010	Mpumalanga	Early	NNVS10	P, 1R, 2R, 3R	2011-2014
		Late	NTV2S10	P, 1R, 2R	2011-2014
	Pongola	Early	FVS10	P, 1R, 2R, 3R	2011-2014
		Late	FV2S10	P, 1R, 2R	2011-2014
			NPV2S10	P, 1R, 2R	2011-2014

#### *Data analysis*

The data were subjected to analysis of variance using the linear mixed model:

$$Y_{ijklm} = \mu + Li + Sj + LS_{ij} + R(LS)_{ijk} + Gl + GL_{il} + GS_{jl} + GLS_{ijl} + GR(LS)_{ijkl} + Cm + LC_{im} + SC_{jm} + GC_{im} + LSC_{ijm} + GLC_{ilm} + GSC_{ilm} + GLSC_{ijlm} + E_{ijklm} \quad \text{Equation 1}$$

Where  $Y_{ijklm}$  = observation for genotype  $l$ , in location  $i$ , season  $j$  of replication  $k$  nested with the interaction of the  $i$ th location and  $j$ th season, in Crop-year  $m$ ;  $\mu$  = overall mean;  $Li$  = fixed effect of the  $i$ th location;  $Sj$  fixed effect of the  $j$ th season;  $LS_{ij}$  = fixed interaction effect of the  $i$ th

location by the  $j$ th season;  $R(LS)_{ijk}$  = random effect of the  $k$ th replication nested within the interaction effect of the  $i$ th location by the  $j$ th season and was the experimental error for testing fixed effects of location, season and location by season interaction;  $Gl$  = fixed effects of the  $l$ th genotype;  $GL_{il}$  = fixed interaction effect of the  $l$ th genotype by the  $i$ th location;  $GS_{jl}$  = fixed interaction effect of the  $l$ th genotype by the  $j$ th season;  $GLS_{ijl}$  = fixed interaction effect of the  $l$ th genotype by the  $i$ th location by the  $j$ th season;  $GR(LS)_{ijkl}$  = the random interaction effect of the  $l$ th genotype by the  $k$ th replication nested with the interaction of the  $i$ th location by the  $j$ th season and was the experimental error for testing the G, GL, GS and GLS fixed effects;  $C_m$  the fixed effect of the  $m$ th crop-year;  $LC_{im}$  = fixed interaction effects of the  $i$ th location by the  $m$ th crop-year;  $SC_{jm}$  = fixed interaction of the  $j$ th season by the  $m$ th crop-year;  $GC_{im}$  = the fixed interaction effect of the  $l$ th genotype by the  $m$ th crop-year;  $LSC_{ijm}$  = fixed interaction effects of the  $i$ th location by the  $j$ th season by the  $m$ th crop-year;  $GLC_{ilm}$  = fixed interaction effects of the  $l$ th genotype by the  $i$ th location by the  $m$ th crop-year;  $GSC_{ilm}$  = the fixed interaction effect of the  $l$ th genotype by the  $j$ th season by the  $m$ th crop-year;  $GLSC_{ijlm}$  = fixed interaction effects of the  $l$ th genotype by the  $i$ th location by the  $j$ th season by the  $m$ th crop-year;  $E_{ijklm}$  = residual error and was also the experimental error for the fixed effects of C, LC, SC, GC, LSC, GLC, GSC and GLSC fixed effects. The least square means for the LS fixed effect were separated using least significant difference (Freund and Wilson, 2003). The data were analysed using the mixed procedure of SAS (SAS Institute, 2009). The combined analysis across locations and crops for each series was used to identify the top five and bottom five genotypes for cane yield and sucrose content. The location x season least square means of the top and bottom five genotypes was estimated and plotted to determine the trends.

## Results

There were significant differences in cane yield for location effects in 2009 ( $P < 0.01$ ) and 2010 ( $P < 0.0001$ ) (Table 2). Seasonal effects produced highly significant differences ( $P < 0.0001$ ) for both series. The location by seasons (LxS) interaction effects was significant ( $P < 0.001$ , 2009) and ( $P < 0.0001$ , 2010). Genotype effects were highly significant ( $P < 0.0001$ ) for both series. The genotype by location interaction (GxL) effects were significant ( $P < 0.01$ ) in 2009 but non-significant ( $P > 0.05$ ) in 2010. The genotype by seasons interaction (GxS) effect was significant ( $P < 0.001$ ) for both series. The genotype by location by season interaction (GxLxS) effect was significant ( $P < 0.01$ ) in 2009 and 2010. The Crop, Location by Crop (LxC) and Seasons by Crop (SxC) interaction effects produced highly significant F-values ( $P < 0.0001$ ) in both series. The Genotype by Crop (GxC) interaction effect was non-significant ( $P > 0.05$ ) across both series. The Location by Seasons by Crop (LxSxC) interaction effect was significant ( $P < 0.0001$ ) in 2009 but non-significant ( $P > 0.05$ ) in 2010. The Genotype by Location by Crop (GxLxC), Genotype by Season by Crop (GxSxC) and Genotype by Location by Seasons by Crop (GxLxSxC) interaction effects were non-significant ( $P > 0.05$ ) for both series. The seasonal effects produced consistently larger F-values than the location effects. GxS effects F-values were consistently larger than GxL effects. The  $R^2$  values were 0.72 and 0.80 for 2009 and 2010, respectively while coefficient of variation (CV%) were 16.14 and 17.00%, for 2009 and 2010, respectively.

**Table 2. The effects, numerator degrees of freedom (NDF), denominator degrees of freedom (DDF), F-values and P-values for cane yield for 2009 and 2010 series.**

Effect	NDF, DDF	2009		2010	
		F-value	P-value	F-value	P-value
L	1,8	16.41	0.0037	105.66	0.0001
S	1,8	327.11	0.0001	156.01	0.0001
LxS	1,8	26.92	0.0008	59.02	0.0001
G	35,268	14.14	0.0001	6.85	0.0001
GxL	34,268	1.81	0.0055	1.46	0.0604
GxS	33,268	2.18	0.0004	2.07	0.001
GxLxS	33,268	2.10	0.0007	1.90	0.0039
C	3,1421	44.72	0.0001	125.41	0.0001
LxC	3,1421	15.48	0.0001	67.09	0.0001
SxC	3,1421	75.96	0.0001	117.01	0.0001
GxC	105,1421	0.94	0.6575	0.82	0.9033
LxSxC	3,1421	60.06	0.0001	1.60	0.2031
GxLxC	102,1421	0.61	0.9991	0.65	0.9959
GxSxC	99,1421	0.64	0.9975	0.63	0.9901
GxLxSxC	97,1421	0.67	0.9934	0.79	0.8831
R <sup>2</sup>		0.72		0.80	
CV %		17.00		16.14	

For sucrose content, location effects were non-significant ( $P>0.05$ ) in 2009 and significant ( $P<0.05$ ) in 2010 (Table 3). Seasonal effects and LxS effects were highly significant ( $P<0.0001$ ) in both series. Genotype effects produced highly significant effects ( $P<0.0001$ ) in both series. GxL effects were significant ( $P<0.01$ ) in 2010 but non-significant ( $P>0.05$ ) in 2009. GxS effects were highly significant ( $P<0.0001$ ) in both series. GxLxS effects were significant ( $P<0.05$ ) in 2009 and 2010. The Crop, LxC and SxC produced highly significant effects ( $P<0.0001$ ) in both series. The GxC effect was significant ( $P<0.0001$ ) in 2010 but non-significant ( $P>0.05$ ) in 2009. The LxSxC effect was highly significant ( $P<0.0001$ ) in 2009 and 2010. GxLxC and GxSxC effects were significant ( $P<0.05$ ) in 2010 but were both non-significant ( $P>0.05$ ) in 2009. The GxLxSxC effect was non-significant ( $P>0.05$ ) in both series. Seasonal effects produced larger F-values and smaller P-values than location effects. GxS effects F-values were consistently larger than GxL effects F-values. The R<sup>2</sup> values were 0.77 and 0.92 for 2009 and 2010, respectively while coefficient of variation (CV%) were 9.15 and 13.56% for 2010 and 2009, respectively.

The least square means for LxS effects were estimated for cane yield and sucrose content and compared using least significant difference (Table 4). The early season crops produced significantly ( $P<0.05$ ) higher cane yield than late season crops for both Mpumalanga and Pongola locations in 2009 and 2010. The early season crops in Mpumalanga produced significantly ( $P<0.05$ ) more cane than the early season crops in Pongola. The late season crops in Mpumalanga and Pongola produced similar cane yields ( $P>0.05$ ) across all series. The late season crops for both Mpumalanga and Pongola produced significantly ( $P<0.05$ ) higher sucrose content than the early season crops in 2009 and 2010. The Mpumalanga late season crop produced significantly ( $P<0.05$ ) higher sucrose content than the Pongola late season crop in 2009 and 2010. The early season crop in Pongola produced significantly higher sucrose content than

the early season crop in Mpumalanga in 2010. In the Mpumalanga early season trials, genotypes produced lower sucrose content than the Pongola early season crop.

**Table 3. The effects, numerator degrees of freedom (NDF), denominator degrees of freedom (DDF), F-values and P-values for sucrose content for 2009 and 2010 series.**

Effect	NDF/DDF	2009		2010	
		F-value	P-value	F-value	P-value
L	1,8	0.50	0.5012	8.95	0.0173
S	1,8	1030.77	0.0001	572.49	0.0001
LxS	1,8	75.98	0.0001	63.88	0.0001
G	35,268	11.82	0.0001	16.82	0.0001
GxL	34,268	1.15	0.2657	1.85	0.0049
GxS	33,268	4.10	0.0001	3.54	0.0001
GxLxS	33,268	1.56	0.0303	1.66	0.0187
C	3,1421	25.12	0.0001	127.66	0.0001
LxC	3,1421	122.87	0.0001	184.98	0.0001
SxC	3,1421	22.65	0.0001	8.94	0.0001
GxC	105,1421	0.95	0.6212	2.01	0.0001
LxSxC	3,1421	100.42	0.0001	139.62	0.0001
GxLxC	102,1421	0.76	0.9652	1.28	0.0417
GxSxC	99,1421	0.77	0.9505	1.35	0.0396
GxLxSxC	97,1421	0.95	0.6078	1.10	0.2897
R <sup>2</sup>		0.77		0.92	
CV %		13.56		9.15	

**Table 4. The least square means for cane yield and sucrose content for the location, seasons and series of trials.**

Location	Season	Cane yield		Sucrose content %	
		2009	2010	2009	2010
Mpumalanga	Early	141.11a	159.99a	9.56c	9.05c
	Late	97.60c	102.21c	14.75a	14.18a
Pongola	Early	123.83b	111.47b	10.74c	10.75b
	Late	98.28c	103.49bc	13.55b	13.82b

The top five genotypes generally produced higher cane yield than the bottom five across 2009 and 2010 (Figure 1). Generally, all genotypes produced higher cane yield in the early season than the late season. There was no crossover interaction in 2009 and only one genotype showed cross-over interaction in 2010 series.

The sucrose content of the top five and bottom five genotypes increased from early to late season (Figure 2). There was no cross-over interaction for both series. The lower sucrose content in Mpumalanga in early crops compared to Pongola early crops was apparent. The higher sucrose content in Mpumalanga late season crops compared to Pongola was also apparent. The negative association between cane yield and sucrose content is also apparent when comparing Figures 1 and 2 and Table 4. High cane yield is associated with low sucrose content.

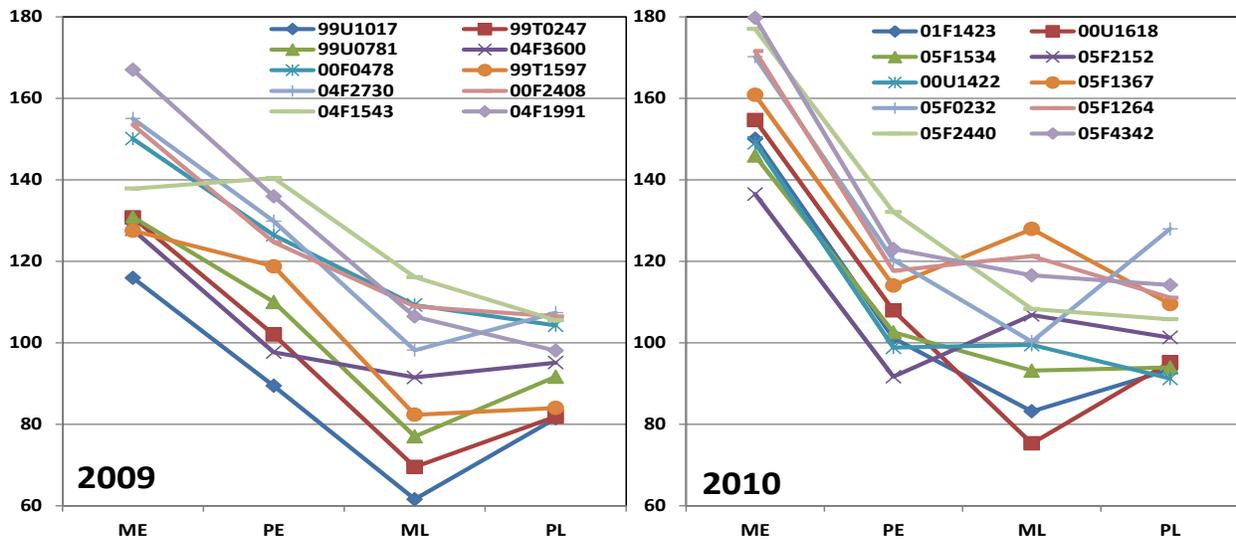


Figure 1. Trends in cane yield across location by season combinations.

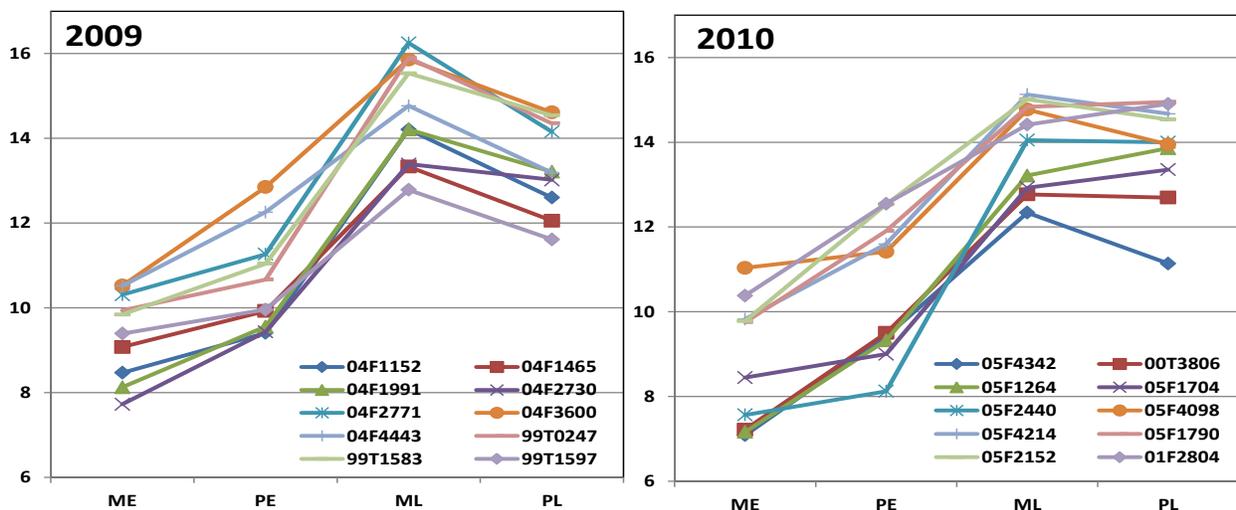


Figure 2. Trends in sucrose content % across location by season combinations.

### Discussion

Seasonal effects were larger than location effects, indicating that testing across seasons was more important than testing across locations. Seasonal effects F-values for sucrose content were larger than those for cane yield, indicating greater influence of seasons on sucrose content than cane yield. The larger F-values for location effects of cane yield compared to those for sucrose content indicated the larger variability in cane yield across locations compared to sucrose content and, therefore, sucrose content was more stable across locations. Generally, cane yield is known to be more variable across locations. Cane yield is controlled by many genes and therefore more sensitive to changes in environments (Mirzawan *et al.*, 1994). However, the larger F-values for sucrose content across seasons indicated the large sensitivity caused by the environment on

sucrose accumulation. In early season trials, genotypes produce lower sucrose content than late season. During early season, the high temperatures and high rainfall result in active growth of the sugarcane plants, and more photosynthates are allocated to growth than sucrose accumulation. In the late season, the dryer weather and rising temperatures act to reduce growth and increase sucrose accumulation. The macro-environments created by the early and late seasons provide a challenge for variety development.

The larger F-values for LxS effects for sucrose content than cane yield indicate the larger interaction of locations and seasons with sucrose accumulation and maturation of sugarcane in the irrigated region. Significant LxS effects F-values indicated potential existence of differences in sugarcane yield and maturity patterns across the irrigated regions. Further, these high values may suggest the need for further and more comprehensive testing of genotypes across the major production environments in the irrigated areas. Such testing may require trials set up to determine yield and quality responses in the Mpumalanga and Pongola regions.

The larger F-values for the G effect for both cane yield and sucrose content indicated that genotype differences were detectable in the trials. The F-values for cane yield and sucrose content were both highly significant indicating that selecting superior genotypes for both traits was effective. The larger F-values for sucrose content suggest that selection for sucrose content was carried out with higher precision than for cane yield. The lower F-values for cane yield than sucrose content highlight the complexity associated with breeding for cane yield, suggesting that cane yield is a more complex trait than sucrose content.

The larger GxS compared to GxL for both cane yield and sucrose content indicated the larger influence of seasons than that of locations on the performance of genotypes. These results suggest that breeding for seasonal effects was more important than that for locations. Therefore, identifying genotypes adaptable to early or late season was more important than identifying genotypes suitable for the different locations. The smaller GxL effects for sucrose content compared to those for cane yield suggest that genotype sucrose content was a more stable trait across locations than genotype cane yield. The larger GxS F-values for sucrose content than for cane yield suggested that genotype sucrose content was more unstable across seasons than genotype cane yield. The result indicates the difficulty associated with achieving higher genetic gains for sucrose content with the current set-up of the breeding trials for the irrigated regions. Currently, there is no testing and selection for early or late season adaptability in the first three testing stages. Testing for adaptability to seasons is only done in the final stage where few genotypes are included. The current approach could result in more genotypes with specific adaptability to the seasons being discarded, depending on the season in which the trial is established. These results may therefore suggest the potential of developing two breeding programmes, one for early and the other for late season in the irrigated environment. Such an approach is expected to accelerate breeding for adaptability to early and late seasons.

The larger GxLxS effects F-values for cane yield rather than sucrose content highlights the complex GxE for cane yield rather than sucrose content. Generally, in sugarcane breeding, it is acknowledged that cane yield has the most complex GxE (Jackson *et al.*, 1991). The complex GxE is caused by the large number of genes with small individual additive effects that control cane yield. The cumulative effects of these larger numbers of genes with small additive effects

result in several interactions with environments, which are different and are thus causing larger and more complex GxE.

Non-significant GxC, GxLxC, GxSxC, GxLxSxC effects for both cane yield and sucrose content suggest that genotype yield across crop-years was more stable in the irrigated regions. Previous studies by Ramburan *et al.* (2012) based on data from released variety trials concluded that management and other variables were more important than ratooning ability. This study observed non-significant differences among the released varieties for ratooning ability. Previous studies by Zhou *et al.* (2011) also showed that ratooning ability was the least important variance component for the irrigated regions of South Africa.

Non-crossover interactions for cane yield and sucrose content of the top and bottom five genotypes suggest that selection for high sucrose and high cane yield with adaptability to both early and late season was effective. However, there were distinct differences between the performance of the genotypes in early and late season. Generally, all genotypes produced high cane yield and low sucrose in the early season and low cane yield and high sucrose content in the late season. The results suggest that there is an opportunity to increase the gains for sucrose content in the early season and increase cane yield in the late season. Such a targeted breeding approach is expected to significantly increase sugar production in the irrigated regions of South Africa. Generally, sugarcane growers in the irrigated regions produce low sucrose content in the early season and need to use ripeners to improve their crop quality. However, the challenge of low cane yield in the late season has no easy intervention, and requires development through plant breeding of adapted and high cane yield genotypes. These results further suggest the need to restructure the irrigated region into early and late season breeding programmes. Generally, the higher cane yield and lower sucrose content in Mpumalanga compared to Pongola in the early season indicates the more favourable sugarcane growing conditions in Mpumalanga in the early season.

### Conclusion

Seasonal effects were larger and more important than location effects. GS effects were larger than GL effects, suggesting that seasons affected genotype selection more than locations. The GS effects influenced more sucrose content than cane yield. Generally, cane yield had more complex GxE than sucrose content. Generally, the early season was characterised by high cane yield and low sucrose content, whereas the late season produced low cane yield and high sucrose content. The seasonal trends for cane yield and sucrose content were similar for Mpumalanga and Pongola, indicating that performance of genotypes across the locations was stable. The two environments represented by the early and late season may require that the irrigated breeding programme be split into early and late season. Such a strategy is expected to enhance the exploitation of recurrent selection and lead to higher genetic gains for yield, quality and other commercial traits. Combined testing of the elite genotypes at the final stage would be maintained. Ratooning ability is not important in the irrigated regions.

## Acknowledgement

The author would like to thank management and staff at Pongola and Mpumalanga research stations for trial management and data collection.

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