

MULTIVARIATE REPEATED MEASURES: A STATISTICAL APPROACH FOR ANALYSING DATA DERIVED FROM SUGARCANE BREEDING VARIETY TRIALS

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Abstract

Data from plots in sugarcane breeding variety trials are collected for several variables over several sequential crop cycles, creating a multivariate repeated measures (MRM) data structure. The MRM analysis accounts for correlations between variables (multivariate) and correlations between crop-years (repeated measures) when computing experimental errors. Currently, univariate analysis (split-plot in time) is used to analyse the data. This approach ignores the correlation between variables and the correlation between crop-years, assuming independence between variables and between crop-years. The assumption of independence could produce incorrect estimates of experimental errors and that could lead to incorrect interpretations. The objectives of this study were to demonstrate the use of MRM analysis on sugarcane breeding variety trial data by determining multivariate effects, covariance structure for crop-years, and comparing univariate to MRM analysis. Data for yield (cane and stalk dry matter), quality (ERC % cane and fibre % cane) and agronomic (stalk height and diameter) traits were collected from 16 genotypes planted in four blocks at Mkwazine location and five blocks at Triangle location, and harvested over eight crop-years. The UN@CS covariance structure was chosen because it used fewer parameters than UN@UN and produced lower Akaike information Criterion (AIC) and Bayesian Information Criterion (BIC) than UN@AR(1). The MRM produced better model fit than univariate analysis for yield traits and height leading to greater statistical efficiency. The MRM analysis produced greater discrimination of the differences between experimental genotypes and the control cultivar than univariate analysis for yield traits. The univariate method was adequate for quality traits.

Keywords: sugarcane, varieties, multivariate repeated measures, univariate, mixed model, model fitness

Introduction

Data from plots in sugarcane breeding advanced variety trials are collected for several variables (yield, quality and agronomic traits) for several sequential crop-years. The data are used to evaluate the differences between experimental genotypes and a control (usually the most widely grown cultivar). The data collected in each plot for the several sequential crop-years are also used to determine and compare the ratooning ability of the experimental genotypes to the control (Berding *et al.*, 2004). Ratooning ability is important in sugarcane production economics because

it is cheaper to maintain ratoon crops than to plant a new crop (Berding *et al.*, 2004; Clowes and Breakwell, 1998; Ellis and Merry, 2004; Salassi and Giesler, 1995).

Data of multiple variables measured from each plot resemble a multivariate structure (Johnson and Wichern, 2002). Values of the multiple variables measured from each plot may not be independent because they are influenced by the same factors existing in that plot. For example, a plot that produces high cane yield is also likely to produce taller and thicker stalks. Therefore multiple variables measured from the same plot could be correlated. The data of each variable measured from each plot over several sequential crop-years resemble repeated measures (Littell *et al.*, 2002, 2005). The measurements from sequential crop-years are unlikely to be independent because the crop-years cannot be randomised to the plots (as would be done in an ideal split plot design). Additionally, a plot that produced high cane yield in crop-year 1 is likely to produce high cane yield in crop-year 2 and subsequent crop-years. Cane yield in crop-year 1 can thus be correlated to cane yield in crop-year 2. As a result, the analysis of plant breeding data need to account for the within plot correlation of the multiple variables (multivariate structure) and the correlation of values of variables measured in sequential crop-years (repeated measures).

Currently, the univariate method that assumes a split-plot in time experiment design is used to analyse data from the advanced variety trials. The univariate method assumes independence between variables measured from the same plot and between values of a variable measured in sequential crop-years (Freund and Wilson, 2003). The assumption of independence between data from multiple variables from the same plot and between data measured from the same plot in sequential crop-years may not always be valid. If the multiple variables are significantly correlated, then the assumption of independence is violated. Violating the assumption of independence would lead to the underestimation or overestimation of experimental errors. Underestimating or overestimating experiment errors increases Type I or Type II errors, respectively, leading to inaccurate statistical tests and incorrect interpretations. The underestimation or overestimation is caused by the exclusion of the covariance between variables, as well as the covariance between crop-years in the computation of experimental errors. The covariance accounts for the correlation between multiple variables and the correlation between crop-years. The ideal analysis should combine multivariate and repeated measures, to create a multivariate repeated measures (MRM) analysis. The MRM analysis would account for the correlation between the multiple variables as well as the correlations between the sequential crop-years in a single analysis. It is hypothesized that combining the multivariate and repeated measures in one analysis will increase precision in the analysis of sugarcane breeding advanced variety trials data and therefore produce accurate tests and correct interpretation.

Multivariate repeated measures analysis using the mixed procedure of SAS

The mixed model procedure of SAS can perform both the multivariate and repeated measures analysis. The linear mixed model equation is,

$$Y = X\beta + Zu + \varepsilon, \quad \text{Equation 1}$$

where Y is the column vector of the response variables, X is the fixed effects design matrix, β is the column vector of the fixed effects parameters, Z is the random effects design matrix, u is the

column vector of the random effects parameters and ε is the column vector of the residual errors (Littell *et al.*, 2005). The linear mixed model (Equation 1) combines the analysis of fixed ($X\beta$) and random (Zu) effects as well as modelling covariance parameters (ε). The ability of the mixed models to perform multivariate and repeated measures analysis and, to model covariance parameters, was utilised in this study to perform the MRM analysis.

The direct (Kronecker) product structures allow the implementation of the MRM analyses (Galecki, 1994). The unstructured (UN) (representing the multivariate component) and the repeated measures covariance structures are merged by the direct product. In SAS, the products are coded TYPE=UN@AR(1), modeling the first order auto-regressive, TYPE=UN@CS, modeling the compound symmetry, and TYPE=UN@UN modeling the unstructured structure in the repeated measures. The direct product of the two matrices has rows equal to the product of rows for, say, UN and AR(1) and columns equal to the product of the columns for UN and AR(1). The UN@UN models unequal covariance, UN@CS models equal covariance, and UN@AR(1) models covariance decay over time.

Covariance structure selection

The objective of covariance structure selection is to identify the most parsimonious structure (Moser, 2005). Information criteria are used to select and measure the relative fit of two or more competing models. The Akaike Information Criterion (AIC) (Akaike, 1974) and the Bayesian Information Criterion (BIC) (Schwarz, 1978) are used to compare the competing models. The AIC is calculated using

$$AIC = -2 \log(L) + 2k, \quad \text{Equation 2}$$

where L is the maximum likelihood function of the model and k is the number of effective covariance parameters; that is, those that enter the optimisation process, are not held fixed by the user, and are not zero.

The BIC method was developed using the Bayesian approach and is not sensitive to prior distributions when the sample size is large. The BIC is calculated as

$$BIC = -2 \log(L) + k \log(n), \quad \text{Equation 3}$$

where n is the sample size. Studies by Guerin and Stroup (2000) found that larger values of BIC were associated with larger Type II errors. Lower values for AIC and BIC indicate better model fit.

The objectives of this study were to demonstrate the use of the MRM analysis method for analysing data from sugarcane advanced variety trials using the linear mixed model procedure of SAS (SAS Institute, 2007). Specifically determined were multivariate effects, the appropriate covariance structure for crop-years, and compared the univariate and MRM analysis methods for yield (cane and stalk dry matter), quality (sucrose % cane and fibre % cane) and agronomic (stalk height and stalk diameter) traits.

Materials and Methods

Locations, experimental design, and crop management

Data were collected from advanced variety trials grown at the Mkwesine and Triangle estates in the South-East Lowveld of Zimbabwe. The plots were arranged as a randomised block design, blocking across irrigation furrows. The Mkwesine location had four blocks, and the Triangle location had five. Each block was divided into 16 plots and each plot was planted to one of the 16 genotypes available. The plots were made up of 6 rows that were 12 m long and spaced 1.5 m apart. The trials were planted on April 25, 1995 (Mkwesine) and April 26, 1995 (Triangle) and were harvested at 12 months crop age every year for eight crop-years. At both locations, water was applied using furrow irrigation. Planting, fertiliser application, irrigation, and weed, disease and insect pest control were done according to standard recommendations for the commercial crop (Clowes and Breakwell, 1998).

Data collection

At harvest, the crop was burnt to remove dry leaves. All the millable stalks in each plot were hand cut, hand trashed to remove the green leaves and hand topped at the natural breaking point. The millable stalks were weighed using a digital scale mounted on a tractor operated hydraulic boom. The weights per plot were divided by the plot area to estimate cane yield (tons/ha). Twenty-four millable stalks were randomly picked from each plot, and bundled. The length of the bundle from the bottom to the top provided the stalk height of each plot. The stalk diameter of each of the 24 stalks was measured at the center of the stalk using a caliper without reference to the bud, and the average stalk diameter of the 24 stalks provided the values for each plot. After measuring the stalk diameter, the 24 stalks were divided into three groups of eight stalks each. From the first group, the bottom one-third of the stalk was cut. From the second group, the middle one-third was cut and from the third group, the top one-third was cut. The bottom, middle and top portions of the stalks were bundled together to form one sub-sample per plot. Each sub-sample was shredded to simulate milling. Two sub-sub-samples were collected from each shredded sub-sample. One sub-sub-sample was analysed for sucrose content, which was expressed as estimable recoverable crystal (ERC % cane) using an empirical equation determined from mill sugar recovery data derived from the previous seasons. The other sub-sub-sample was dried for 24 hours in an oven at a constant temperature of 100°C and used to determine the fibre % cane and moisture content. The moisture content (MC) was then used to estimate the stalk dry matter (SDM) from cane yield (Equation 4):

$$\text{SDM} = \text{Cane yield} * (100 - \text{MC}) \div 100 \quad \text{Equation 4}$$

Three groups of data emerged; that is, for yield, quality and agronomic traits. Yield traits (cane and SDM) were measured at the plot level, the agronomic traits (stalk height and stalk diameter) were measured from the 24 stalks sampled from each plot, and the quality traits (ERC % cane and fibre % cane) were measured from the sub-sub-sample derived from the shredded sub-sample of a third of the 24 stalk sample. As a result, the correlation within yield, quality and agronomic traits was assumed to be larger than the correlation between the trait groups. Each trait group was therefore analysed separately.

Data arrangement and analysis using multivariate mixed model procedure of SAS

The MRM analysis was done using the mixed procedure of SAS (SAS Institute, 2007). A response variable (Y) was created with all the response variables stacked. A class variable, RV, was created identifying each variable by stacking the corresponding variable names. The data was arranged as shown in Table 1. In Table 1, using yield data as an example, RV=1 referenced cane yield and RV=2 referenced SDM yield. Location=1 referenced the Triangle location and location=2 referenced the Mkwasi location. The effects nested in RV and, together with the NOINT option, produced the multivariate analysis and testing. The NOINT option allows each variable in RV (for example, cane and SDM) to be treated as unique. With the NOINT option, the levels of RV are not compared. The comparisons are done within the levels of RV and the effects within the RV are added up for both the variables in the multivariate structure to produce the multivariate tests.

Table 1. Data arrangement for the response class variable (RV), location, replication, genotype, crop-year, and measured values (Y) for the multivariate repeated measures analysis using the linear mixed model procedure of SAS.

RV	Location	Replication	Genotype	Crop-year	Y
1	1	1	1	1	Y ₁₁₁₁₁
1	1	1	1	2	Y ₁₁₁₁₂
1	1	1	1	3	Y ₁₁₁₁₃
.
.
1	1	1	1	c	Y _{1111c}
1	1	1	2	1	Y ₁₁₁₂₁
1	1	1	2	2	Y ₁₁₁₂₂
1	1	1	2	3	Y ₁₁₁₂₃
.
.
1	1	1	g	c	Y _{111gc}
1	1	2	1	1	Y ₁₁₂₁₁
1	1	2	1	2	Y ₁₁₂₁₂
1	1	2	1	3	Y ₁₁₂₁₃
.
.
1	1	r	g	c	Y _{11rgc}
1	2	1	1	1	Y ₁₂₁₁₁
1	2	1	1	2	Y ₁₂₁₁₂
1	2	1	1	3	Y ₁₂₁₁₃
.
.
1	2	r	g	c	Y _{12rgc}
2	1	1	1	1	Y ₂₁₁₁₁
2	1	1	1	2	Y ₂₁₁₁₂
2	1	1	1	3	Y ₂₁₁₁₃
.
.
2	1	r	g	c	Y _{21rgc}

RV	Location	Replication	Genotype	Crop-year	Y
2	2	1	1	1	Y ₂₂₁₁₁
2	2	1	1	2	Y ₂₂₁₁₂
2	2	1	1	3	Y ₂₂₁₁₃
·	·	·	·	·	·
·	·	·	·	·	·
2	2	r	g	c	Y _{22r_{gc}}

Multivariate repeated measures linear mixed model

The MRM linear mixed model for yield traits with two response variables (for example cane and SDM), two locations (1, 2), r blocks per location planted to g genotypes and harvested for c crop-years, is

$$Y_{ijkmn} = \pi_i + \alpha(\pi)_{j(i)} + \rho(\alpha(\pi))_{k(j(i))} + \gamma(\pi)_{m(i)} + \omega(\pi)_{n(i)} + \alpha\gamma(\pi)_{jm(i)} + \alpha\omega(\pi)_{jn(i)} + \gamma\omega(\pi)_{mn(i)} + \alpha\gamma\omega(\pi)_{jmn(i)} + \varepsilon_{ijkmn}$$

Equation 5

where Y_{ijkmn} is the response for the i th variable ($i=1,2$), j th location ($j=1,2$), k th block within j th location ($k=1,2,\dots,r$), m th genotype ($m=1,2,\dots,g$) by block (plot), and n th crop-year ($n=1,2,\dots,c$). The model effects are as follows: π_i is the fixed effect of the i th response variable (RV), $\alpha(\pi)_{j(i)}$ is the fixed effect of the j th location nested within the i th variable, $\rho(\alpha(\pi))_{k(j(i))}$ is the random effect of the k th block nested within the j th location that is in turn nested within the i th variable, $\gamma(\pi)_{m(i)}$ is the fixed effect of the m th genotype nested within the i th variable, $\omega(\pi)_{n(i)}$ is the fixed effect of the n th crop-year nested within the i th variable, $\alpha\gamma(\pi)_{jm(i)}$ is the interaction fixed effect of the j th location and the m th genotype nested within the i th variable, $\alpha\omega(\pi)_{jn(i)}$ is the interaction fixed effect of the j th location and the n th crop-year nested within the i th variable, $\gamma\omega(\pi)_{mn(i)}$ is the interaction fixed effect of the m th genotype and the n th crop-year nested within the i th variable, $\alpha\gamma\omega(\pi)_{jmn(i)}$ is the interaction fixed effect of the j th location by the m th genotype by the n th crop-year nested within the i th variable, and ε_{ijkmn} is the residual error. The above linear mixed model (Equation 5) was used for the quality and agronomic traits. All the effects in Equation 5 are nested within the response variable (RV) to create the multivariate analysis and testing.

Efficiency of univariate versus multivariate repeated measures

The MRM and univariate analysis were compared for their ability to account for the variability in the data (model fitness) using fit statistics. The MRM was compared to the univariate to evaluate the efficiency in discriminating the experimental genotype means for yield, quality and agronomic traits. Specifically, the two analytical methods were compared for their discriminating ability of the difference in trait values between the experimental genotypes and the control. The difference between the experimental genotypes and the control is routinely used by plant breeders to identify superior genotypes in variety trials. The experimental genotypes were compared to genotype 16, the control genotype, using Dunnett’s test for both the MRM and univariate methods. The P-value, which is the probability of obtaining a larger value of the difference between the experimental genotype and genotype 16, was the parameter interpreted.

Results

Multivariate repeated measures analysis of yield, quality and agronomic traits data

The MRM mixed model analysis for yield traits (cane, SDM) produced highly significant ($P < 0.01$) P-values for all the effects for the UN@CS and UN@AR(1) covariance structures (Table 2). The UN@UN covariance structure failed to converge. The MRM analysis for quality traits (ERC % cane and fibre % cane) and agronomic traits (stalk height and stalk diameter) produced highly significant ($P < 0.01$) P-values for all the effects and for all covariance structures.

The interpretation of the multivariate effects must recognise that the effects are computed within each of the variables making up the multivariate component. The effects computed within each variable are then added up to produce the values of the multivariate F-statistic that are tested. The multivariate P-values (Table 2) refer to the probability of obtaining a larger value of the multivariate F-statistic. The multivariate F-statistic of each multivariate effect follows the F-distribution with the numerator and denominator degrees of freedom shown in Table 2. The significant multivariate F-statistic would mean that at least one of the variables making up the multivariate structure produced significant effects.

Table 2. The numerator (N) and denominator (D) degrees of freedom (DF) and the probability of obtaining a larger Multivariate F-value (Multivariate P-values) for the multivariate effects for the yield traits (Cane (t/ha) and SDM (t/ha)) derived from the UN@UN, UN@CS and UN@AR(1) covariance structures.

Effect	DF		Yield traits			Quality traits			Agronomic traits		
	N	D	UN@UN	UN@CS	UN@AR(1)	UN@UN	UN@CS	UN@AR(1)	UN@UN	UN@CS	UN@AR(1)
RV	2	14	Did not converge†	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
L(RV)	2	14		<0.0001	0.0002	0.0004	0.0007	0.0006	0.0043	0.0014	0.0019
G(RV)	30	1778		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C(RV)	14	1778		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G*L(RV)	30	1778		<0.0001	<0.0001	0.0223	0.0170	0.0072	<0.0001	<0.0001	<0.0001
C*L(RV)	14	1778		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G*C(RV)	210	1778		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G*L*C(RV)	210	1778		<0.0001	<0.0001	<0.0001	0.0002	0.0002	<0.0001	<0.0001	0.0006

L=Location; G=Genotype; C=Crop-year; RV=Random variable; N=Numerator; D=Denominator

†The model did not converge because it was unable to make hessian positive definite matrix

The significant genotype within RV effects for the yield traits, for example, meant that the genotype effects were significantly different for cane or SDM or both. The significant location by genotype within RV effects for yield traits suggests that the location by genotype interaction effects were significantly different for cane or SDM or both. The significant crop-year within RV effects for yield traits meant that the crop-year effects were significantly different for cane or SDM or both. Significant genotype by crop-year within RV effects for the yield traits meant that the genotype by crop-year interaction effects were significantly different for cane or SDM or both. Significant location by genotype by crop-year within RV effects for the yield traits meant

that the location by genotype by crop-year interaction effects were significantly different for cane or SDM or both. The interpretation for the quality traits (ERC % cane and fibre % cane) and agronomic traits (stalk height and stalk diameter) followed the same pattern as that of yield traits.

Covariance structure selection

The covariance structure UN@CS was selected as the most appropriate because it used fewer parameters than the UN@UN covariance structure (simplicity) and produced lower AIC and BIC values than the UN@AR(1) (Table 3). The UN@AR(1) produced higher BIC values, indicating likely larger Type II errors particularly for the yield and agronomic traits than UN@CS (Guerin and Stroup, 2000). The UN@CS covariance structure was used in performing the Dunnett's tests comparing genotypes to the control. The probability values obtained from Dunnett's test were used to evaluate the efficiency of the univariate and MRM analysis in discriminating between the experimental genotypes.

Table 3. Number of fitted covariance parameters, the Akaike information criterion (AIC), and the Bayesian information criterion (BIC) derived from the multivariate repeated measures analysis for yield, quality and agronomic traits using UN@UN, UN@CS and UN@AR(1) covariance structures.

Covariance structure	Number of parameters	Yield traits		Quality traits		Agronomic traits	
		AIC	BIC	AIC	BIC	AIC	BIC
UN@UN	42	-	-	4967.7	4975.8	-363.5	-355.4
UN@CS	7	10403.0	10404.4	5047.4	5048.7	-321.6	-320.3
UN@AR(1)	7	10423.5	10424.9	5047.6	5048.9	-287.7	-286.4

Comparison of univariate versus multivariate repeated measures for model fitness

Model fitness determines if a statistical model adequately explains the variation in the data and can be used to compare two or more competing models (Littell *et al*, 2002, 2005). Three fit statistics, -2 Residual Log Likelihood (RLL), AIC (Akaike, 1974) and BIC (Schwarz, 1978) were used to compare the univariate and MRM. Smaller values of the fit statistics indicated a better model fit.

The MRM produced consistently lower values of the AIC, BIC and RLL for the yield traits (cane and SDM) and stalk height than univariate (Table 4). The quality traits (ERC % cane and fibre % cane) and stalk diameter produced similar values of the fit statistics for MRM and univariate. The lower values of the fit statistics of the yield traits and stalk height for MRM indicated better model fit than univariate (Guerin and Stroup, 2000).

Efficiency of univariate versus multivariate repeated measures in determining differences between experimental genotypes and the control cultivar

Sugarcane breeders are generally interested in comparing the experimental genotypes to a control (usually the dominant or a widely grown cultivar) using data from variety trials. Experimental genotypes that produce significantly greater yield than the control cultivar are recommended for release to growers, particularly if other important traits such as disease and pest tolerance are acceptable. The 15 experimental genotypes in this study were compared to the control (genotype

16, the most widely grown cultivar in Zimbabwe (Zhou, 2004)) using Dunnett's test for both the univariate and MRM analysis. At Triangle, the univariate method produced highly significant ($P < 0.001$) differences between the experimental genotypes and the control cultivar for cane for all genotypes (Table 5) while the MRM showed that six of the experimental genotypes were similar to the control. At Mkwesine, two experimental genotypes that were significantly ($P < 0.01$) different from the control using the univariate method were found similar to the control by MRM. The SDM yield of all genotypes at Triangle were significantly ($P < 0.001$) different from the control using univariate but seven genotypes were found similar to the control by MRM. The SDM yield of three genotypes at the Mkwesine location was similar to the control using MRM but showed significant ($P < 0.01$) differences using univariate. The correlation coefficients between the crop-years for yield traits produced by MRM analysis ranged from 0.35 to 0.42 and were highly significant ($P < 0.001$), indicating lack of independence among crop-years. Stalk height produced similar trends to yield traits.

Table 4. Model Fit Statistics (-2 Residual Log Likelihood (RLL), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC)) and the probability of obtaining a larger value of the likelihood ratio test statistic (P-value) for yield, quality and agronomic traits derived from the univariate (UNIV) and multivariate repeated measures (MRM) for the data from the Triangle and Mkwesine locations.

Fit statistic	Location	Method	Yield traits		Quality traits		Agronomic traits	
			Cane (t/ha)	SDM (t/ha)	ERC %	Fibre %	Height (m)	Diameter (cm)
RLL	Triangle	UNIV	4413.1	3215.2	1269.7	1795.1	96.2	-237.3
		MRM	4219.3	3047.2	1269.4	1793.9	-15.1	-239.9
	Mkwesine	UNIV	3065.2	2150.2	959.7	1256.0	10.1	-102.0
		MRM	3020.1	2112.0	959.3	1254.3	-12.7	-104.9
AIC	Triangle	UNIV	4417.1	3219.2	1273.7	1799.1	100.2	-233.3
		MRM	4225.3	3053.2	1275.4	1799.9	-9.1	-233.9
	Mkwesine	UNIV	3069.2	2154.2	963.7	1260.0	14.1	-98.0
		MRM	3026.1	2118.0	965.3	1260.3	-6.7	-100.9
BIC	Triangle	UNIV	4416.3	3218.5	1272.9	1798.3	99.4	-234.1
		MRM	4224.1	3052.1	1274.2	1798.7	-10.3	-235.0
	Mkwesine	UNIV	3068.0	2153.0	962.4	1258.8	12.9	-99.3
		MRM	3024.3	2116.1	963.4	1258.4	-8.5	-102.1

For the quality traits (ERC % cane and Fiber % cane), both the univariate and MRM produced similar trends in p-values for tests of the differences between experimental genotypes and the control (Table 6). The results in Table 6 followed the same trends shown by the fit statistics (Table 4). The correlation coefficients between the crop-years for quality traits produced by MRM ranged from -0.01 to 0.04 (data not shown) and were not significant ($P > 0.05$), indicating independence among crop-years. When the crop-years are independent, then the univariate and MRM are expected to produce similar results. Stalk diameter produced a similar trend to quality.

Table 5. Significance levels of the difference between the experimental genotypes and control cultivar for cane and stalk dry matter (SDM) yield when the data from Triangle and Mkwasi locations was analysed using the univariate (UNIV) and multivariate repeated measures (MRM).

Genotype	Cane yield (t/ha)				SDM (t/ha)			
	Triangle		Mkwasi		Triangle		Mkwasi	
	UNIV	MRM	UNIV	MRM	UNIV	MRM	UNIV	MRM
1	***	***	***	***	***	***	***	***
2	***	***	***	***	***	***	***	***
3	***	***	***	***	***	***	***	***
4	***	***	***	***	***	***	***	***
5	***	*	***	***	***	NS	***	***
6	***	NS	NS	NS	***	NS	**	NS
7	***	**	***	***	***	NS	***	***
8	***	**	***	NS	***	***	***	***
9	***	NS	***	**	***	**	***	***
10	***	NS	NS	NS	***	NS	NS	NS
11	***	NS	**	NS	***	*	***	**
12	***	*	***	**	***	NS	***	NS
13	***	***	***	***	***	***	***	***
14	***	NS	NS	NS	***	NS	NS	NS
15	***	NS	NS	NS	***	NS	NS	NS

*, **, *** = significant at 0.05, 0.01, 0.001, respectively; NS = not significant at P=0.05.

Table 6. Significance levels of the difference between the experimental genotypes and control cultivar for the ERC % cane and fibre % cane when the data from Triangle and Mkwasi locations was analysed using the univariate (UNIV) and multivariate repeated measures (MRM).

Genotype	ERC % cane				Fibre % cane			
	Triangle		Mkwasi		Triangle		Mkwasi	
	UNIV	MRM	UNIV	MRM	UNIV	MRM	UNIV	MRM
1	***	***	NS	NS	NS	NS	NS	NS
2	***	***	***	***	NS	NS	NS	NS
3	***	***	***	***	***	***	***	***
4	***	***	***	***	***	***	***	***
5	***	***	***	***	***	***	***	***
6	***	***	**	**	***	***	***	***
7	***	***	***	***	NS	NS	NS	NS
8	***	***	***	***	***	***	***	***
9	***	***	***	***	***	***	***	***
10	***	***	***	***	***	***	***	***
11	***	***	***	***	***	***	***	***
12	***	***	***	***	NS	NS	NS	NS
13	***	***	***	***	***	***	***	***
14	NS	NS	NS	NS	NS	NS	NS	NS
15	NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** = significant at 0.05, 0.01, 0.001, respectively; NS = not significant at P=0.05.

Discussion

The objective of the sugarcane breeding advanced variety trials is to evaluate the performance of experimental genotypes for yield, quality, agronomic traits and ratooning ability and to determine the potential of these genotypes for release as commercial cultivars as well as their potential as parents in future crosses. The sugarcane breeder is interested in evaluating genotype yield across locations and crop-years. The genotype within RV, genotype by location within RV, genotype by crop-year within RV and genotype by location by crop-year within RV effects are used to evaluate genotype yield potential, determine the influence of locations, crop-years, and location by crop-year interactions, respectively, on the genotype yield potential. The location effects test environmental adaptation to factors such as soil type, changes in temperature and rainfall across locations while the crop-year effects test the ratooning ability of the genotypes, which is the fluctuation in yield across crop-years.

The MRM produced greater discrimination of the differences in cane and SDM between the experimental genotypes and the control than the univariate method. The univariate method declared that most experimental genotypes were significantly different from the control but these genotypes were found to be similar to the control when the data was analyzed using MRM. The univariate method is widely and exclusively used by plant breeders to analyze advanced variety trials data. The implication of this result to sugarcane breeders using the univariate method is that genotypes similar to the control could be erroneously declared significantly superior or inferior to the control. The implication of erroneously declaring that a genotype was significantly higher yielding than the control when it was similar to the control is that some genotypes that are released as higher yielding would produce no yield gains in commercial crops. Such genotypes would show no yield benefits to the growers compared to the current cultivars they are intended to replace. This scenario has occurred many times in the sugarcane industries where released varieties have produced no yield gains in commercial crops. Conversely, erroneously rejecting genotypes as inferior when they are similar to the control could also result in the loss of parental germplasm that would be similar to the control but excelling in other important traits such as disease and insect pest resistance. An example could be the case with the sugarcane borer (White *et al.*, 1996) where very few sugarcane borer resistant genotypes are advanced because of low yield. Erroneously discarding potential parental genotypes could also narrow genetic diversity in breeding populations.

The MRM produced significantly better model fit to the data than the univariate method. The better model fitness suggests that the MRM is explaining more of the variation within the data than univariate. Better model fitness also indicates that the variances used for computing the tests of the effects are less inflated or deflated. The MRM achieved better model fitness by accounting for the correlation between the variables as well as the correlations between crop-years. The correlation between variables and between crop-years is ignored by the univariate method because of the assumption of independence. The covariance that measures the correlations between the variables and the correlations between the crop-years are added to the variances of each variable during the computation of experimental errors that are used to perform test of effects by the MRM. The test of the differences between the experimental genotypes and the control cultivar are likely inflated by the univariate method because of the exclusion of the

covariance in the computation of the experimental errors. The statistical power of the MRM comes from the inclusion of covariance in the computation of experimental errors.

The yield traits showed the greatest difference between the tests of the difference between the experimental genotypes and control between the univariate and MRM. Yield traits are generally more difficult to improve through plant breeding and selection compared to other traits because they are controlled by quantitative genes and therefore more susceptible to the influence of genotype by environment interaction (GxE) effects (Falconer and Mackay, 1996; Mirzawan *et al.*, 1993). Because of the large GxE effects, more accurate statistical methods are required to separate the genotype effects from the environment effects and thereby identifying true genotype differences. The poor model fit of the univariate method would decrease the precision of the tests further when GxE effects increase. The negative effects of GxE are increased by the poorer model fit of the univariate method compared to MRM. In a study of GxE and resource allocation by Kimbeng *et al.* (2009), differences in cane yield of less than 15-20% was proven to be more difficult to detect in advanced variety trials. However, contrary to the findings reported by Kimbeng *et al.* (2009), in this study, the univariate method showed significant differences for cane yield between the experimental and control ranging from 5-10%, a result that is likely to be caused by the high Type I errors. The MRM found such differences not significant, a result likely to be correct. Therefore the MRM method offers a more statistically powerful method for identifying true differences between the experimental genotypes and also for reducing the effects of GxE for yield traits.

Significant gains have been achieved and continue to be achieved for sucrose content using the univariate method. This study also explains one of the reasons why gains in sucrose content have remained higher than those for cane yield. Univariate analysis was shown to be similar to the MRM in this study, indicating that these gains can partly be attributed to correct statistical analysis for quality traits using the univariate method. The study by Kimbeng *et al.* (2009) also showed that the influence of GxE effects was lower for sucrose content than for cane yield. Studies in Australia have shown that the effects of GxE (Bull *et al.*, 1992) and competition between genotypes (Jackson and McRae, 2001) were lower for sucrose content than for cane yield.

The multivariate component of the MRM is important in determining the validity of the significance of further tests including univariate tests. Because the multivariate tests include the covariance between variables, they are more precise than the univariate tests. When multivariate tests are not significant, significant univariate tests should not be interpreted because they are likely to be due to Type I errors (Johnson and Wichern, 2002). Significant univariate tests should only be interpreted when the multivariate tests are significant when analysing data that comprise a multivariate structure. Therefore the MRM provides a quality control for the statistical analysis that includes multiple response variables measured from experimental units such as is the case with sugarcane breeding data and other crops with similar data structure.

Conclusions

The MRM produced better model fit than the univariate method for yield traits. MRM method was more discriminating for the differences in yield between the experimental genotypes and the control than univariate. Greater discrimination would result in correct selection decisions during variety testing for yield traits. MRM produced correct computation of experiment errors by including the covariance between variables as well as the covariance between crop-years leading to correct tests particularly for yield traits. Univariate analysis was likely to have larger Type I errors because of the violation of the assumption of independence that would result in the underestimation of experiment errors. MRM would reduce the erroneous interpretations for the yield traits likely to be associated with univariate method. MRM was a potentially powerful statistical tool for controlling the influence of GxE effects generally associated with complex traits such as cane yield that are controlled by quantitative genes and more subject to competition effects than quality traits. Quality traits showed that univariate analysis was adequate in identifying the true differences between genotypes.

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