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TRENDS IN VARIANCE COMPONENTS AND OPTIMUM REPLICATIONS AND CROP-YEARS FOR VARIETY TRIALS AT DWANGWA SUGAR ESTATE IN MALAWI

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Abstract

The ability to detect significant differences between varieties in trials depends on the variance associated with the means. Dwangwa sugar estate, Malawi, imports varieties from the South African Sugarcane Research Institute (SASRI) for testing before release for commercial use. Currently, trials are planted in early, mid and late seasons. Each trial is planted to eight replications and harvested in the plant and four ratoon crops. The objectives of this study were to determine the trends in variance components that will optimise number of replications and crop-years, while providing adequate discriminating ability for yield and quality. Data for yield (cane and sugar) and quality (sucrose and fibre %) were collected from two series of trials. The data were analysed using the mixed procedure of the Statistical Analysis System (SAS) to estimate the variance components that were used to calculate broad sense heritabilities. The broad sense heritabilities were used to model the optimum number of replications and crop-years. In both series, yield traits produced more significant variance components for genotype by environment interaction (GxE) than quality traits. Quality traits produced larger broad sense heritabilities than yield traits. The changes in broad sense heritability indicated that four replications and harvesting in the plant and subsequent three ratoon crops provided adequate discriminating ability among clonal means.

Keywords: replications, crop-years, broad sense heritability, variance components, sugarcane, varieties

Introduction

In sugarcane variety improvement, the selection process requires that inferior varieties be identified and eliminated from further consideration or from being released for commercial planting (Kimbeng *et al.*, 2009). To declare a variety as superior to a control in a trial, there should be adequate discrimination between the mean of the test variety and that of the control. The ability to detect significant differences between these means depends on the variance associated with the means. The variance of the genotype means estimated from seasons, crop-years, and replications can vary, depending on the relative importance of the variance components of the variety interactions with seasons, crop-years and replications. The discriminating ability is enhanced as the variance decreases and the broad sense heritability increases. Broad sense heritability (H) refers to the extent to which the phenotype of a variety is determined by its genotype (Falconer, 1989).

Discriminating ability refers to the ability to detect significant differences between test varieties and the control.

The variety testing programme at Dwangwa sugar estate relies on varieties imported from the South African Sugarcane Research Institute (SASRI) that are planted in trials to test for adaptability to local growing conditions. The objective of the variety testing is to identify varieties that are high yielding, adaptable and low flowering for eventual planting in commercial fields. Importing varieties from other breeding programmes is also undertaken.

Currently, each series of variety trials is planted in the early, mid and late seasons (Isyagi and Khembo, 2009). Each trial is planted to eight replications and harvested in the plant and four ratoon crops. The eight replications in each trial are expected to increase the precision of identifying elite varieties. Harvesting each trial in the plant and four ratoon crops is expected to identify clones with excellent ratooning ability. An evaluation of the Dwangwa variety testing programme by visiting SASRI plant breeders in February 2010 considered that the eight replications in each trial were more than adequate and there was an opportunity to reduce them and subsequently reduce cost. As a reference, SASRI plant breeding trials generally use three replications (Nuss, 1998) and trials in rain-fed areas are harvested in the plant and two ratoon crops while the irrigated trials are harvested in the plant and three ratoon crops (Parfitt, 2000, 2005). Therefore, harvesting in the plant and four ratoon crops was also considered more than adequate and could be reduced. The evaluation concluded that a study should be carried out to determine the optimum number of replications and crop-years.

The objectives of this study were to evaluate the trends in variance components and determine the optimum number of replications and crop-years that would provide adequate discriminating ability for yield and quality in variety trials at Dwangwa sugar estate, Malawi.

Materials and Methods

Experimental design and data collection

The data were collected from variety trials at Dwangwa sugar estate in Malawi. The trials were designed as a randomised block with 12 varieties (Table 1) and eight replications. The trials were planted in the early, mid and late seasons in 2003 and 2004 and harvested at 12-month crop age in the plant and three or four ratoon crops. The varieties included in series 1 were made up of 11 SASRI varieties and one variety (ZN1L) developed by the Zimbabwe Sugar Association Experiment Station (ZSAES). In series 2, there were five SASRI varieties, one from Coimbatore, India (Co61275), one from Canal Point, USA (CP72-1312) and five from ZSAES (ZN2E, ZN3L, ZN4, ZN5, ZN6). Tons cane per hectare (TCH) was determined from nett plot weights of millable stalks divided by the nett plot areas. Estimated recoverable crystal (ERC) % cane was determined from a random stalk sample from the net plot area that was analysed in the factory sucrose laboratory using standard protocol (Shoonees-Muir *et al*., 2009). Tons sugar per hectare (TSH) was the product of TCH and ERC % cane.

Table 1. The series, seasons, years of harvest for the different crops of the 12 varieties included in each trial.

Series	Season	Years of harvest	Crop-years	Varieties included in trials
1	Early	2005, 2006, 2007, 2008, 2009	P, 1R, 2R, 3R, 4R	86F0504, 87F2719, 90F0613, 90F3125, 91F0820, N19, N25, N35, N36, N38, NCo376, ZN1L
	Mid	2004, 2005, 2006, 2007, 2008	P, 1R, 2R, 3R, 4R	
	Late	2004, 2005, 2006, 2007, 2008	P, 1R, 2R, 3R, 4R	
2	Early	2005, 2006, 2007, 2008, 2009	P, 1R, 2R, 3R, 4R	82F2907, 91F1161, Co62175, CP72-1312, N19, N25, NCo376, ZN2E, ZN3L, ZN4, ZN5, ZN6
	Mid	2004, 2005, 2006, 2007	P, 1R, 2R, 3R	
	Late	2004, 2005, 2006, 2007	P, 1R, 2R, 3R	

Data analysis

All experimental design variables were considered random in this analysis. The varieties in these trials, although selected, were considered to represent a random sample of the several varieties that could be included in variety testing at Dwangwa sugar estate. The data were subjected to analysis of variance (ANOVA) using the linear statistical mixed model:

$$Y_{ijkm} = \mu + S_i + R(S)_{j(i)} + G_k + GS_{ik} + GR(S)_{jk(i)} + C_m + CS_{im} + CR(S)_{jm(i)} + GC_{km} + GSC_{ikm} + GCR(S)_{jkm(i)}$$

Equation 1

where Y_{ijkm} is the observation for variety k (k = 1, 2, ..., g) in crop-year m (m = 1, 2, ..., c) in replication j (j = 1, 2, ..., r) nested in season i (s = 1, 2, ..., s); μ is the overall mean; S_i is the random main effect of ith season; $R(S)_{j(i)}$ is the random effect of the jth replication nested within the ith season; G_k is the random effect of the kth variety; GS_{ik} is the random interaction effect of the kth variety by the ith season; $GR(S)_{jk(i)}$ is the random interaction effect of the kth variety by the jth replication nested within the ith season; C_m is the random effect of the mth crop-year;CS_{im} is the random interaction effect of the ith season by the mth crop-year; $CR(S)_{jm(i)}$ is the random interaction effect of the mth crop-year by the jth replication nested within the ith season; GC_{km} is the random interaction effect of the kth variety by the mth crop-year; GSC_{ikm} is the random interaction effect of the kth variety by the mth crop-year by the ith season; and $GCR(S)_{jkm(i)}$ is the random interaction effect of the kth genotype by the mth crop-year by the jth replication nested within the ith season and was the residual error.

The data were analysed using the mixed procedure of SAS (SAS Institute, 2009). The estimates of the variance components, their standard errors (Anderson and Bancroft, 1952) and their significant tests were calculated using the COVTEST option in the model statement (Littell *et al*., 2008). 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):

$$V_k = \frac{\sigma_E^2}{rsc} + \frac{\sigma_{GSC}^2}{sc} + \frac{\sigma_{GS}^2}{s} + \frac{\sigma_{GC}^2}{c}$$

Equation 2

where, σ_E^2 is the variance component for the residual term in the model; σ_{GSC}^2 is the variance component for the interaction between genotype, season and crop-year; σ_{GS}^2 is the variance component for the interaction between genotype and season and σ_{GC}^2 is the variance component for the interaction between genotype and crop-year.

From the above, it was possible to calculate H or genetic repeatability as:

$$BSH = \sigma_G^2/(\sigma_G^2 + V_k) \quad \text{Equation 3}$$

where, σ_G^2 is the variance component for genotype main effect. Using equations 2 and 3, the number of replications was increased from one to eight and the number of crop-years was increased from plant to fourth ratoon crop to model the changes in H. The change in H when the number of replications and crop-years increased was plotted graphically to determine the optimum replications and crop-years.

Results

The variance components that influence genotype by environment interaction (GE) (genotype by season (GS), genotype by crop-year (GC) and genotype by season by crop-year (GSC)) are used to evaluate genotype performance (Table 2). The other variance component effects are used to account for variability that occurs in the trials and remove that variability in the analysis and thus increase the precision of comparing GE. Data for TCH produced significant (P<0.05) variance components for genotype main effect (G), GS and GC in both series. GSC was significant (P<0.05) in series 1. The order of importance for TCH was G>GS>GC>GSC. The variance components for seasons (S) and crop-year main effects (C) and season by crop-year (SC) were non-significant (P>0.05) in both series.

The G for ERC % cane was significant (P<0.05) in both series whereas the GSC was significant (P<0.05) in series 2 (Table 2). G was the largest variance component followed by GSC, GS and GC for both series. The S and C were non-significant (P>0.05) while SC was significant (P<0.05) in series 2.

For TSH, G was significant (P<0.05) in series 1 (Table 2). GS was significant (P<0.05) in series 2 while GSC was significant (P<0.05) in both series. G was the largest while the GC was the smallest. S and C were non-significant (P<0.05) while SC was significant in series 1.

The G and GSC for Fibre % cane were significant (P<0.05) in both series. GS was significant (P<0.05) in series 2. G was the largest followed by GSC. The S, C and SC were not significant (P>0.05).

Series 1 variety trials produced larger H than series 2 (Table 2). Quality (ERC % cane and Fibre % cane) produced larger H than yield (TCH and TSH) in both series. TCH produced larger H than TSH in both series.

The optimum number of replications was determined by plotting the trends in H as the number of replications increased from one to eight (Figures 1 and 2). TCH produced marginal increases in H after replication three in series 1 and replication four in series 2 (Figure 1). TSH produced marginal increases in H beyond replication four for both series. Quality (ERC % cane and Fibre % cane) produced marginal increases in H beyond the third replication for both series (Figure 2). Figure 1 show that the H for TCH and TSH were larger in series 1 than in series 2. Figure 2 show that the H for ERC % cane was larger in series 1 than in series 2. The trends in H for Fibre % cane remained largely unchanged in both series.

Table 2. Variance components and broad sense heritabilities (H) for tons cane per hectare (TCH), estimated recoverable crystal (ERC) % cane, tons sugar per hectare (TSH) and Fibre % cane for series 1 and series 2 trials.

Effect	TCH	ERC%	TSH	Fibre%
Series 1				
S	834.45±850.01	0.584±0.601	11.31±11.62	0.029±0.042
R(S)	25.65±10.73**	0.016±0.012	0.48±0.24*	0.000±0.000
G	61.77±32.67*	0.268±0.126*	0.96±0.55*	0.579±0.278*
GS	20.03±9.58*	0.037±0.0.024	0.38±0.25	0.045±0.051
GR(S)	23.36±6.42**	0.046±0.027*	1.15±0.26**	0.127±0.070*
C	137.93±111.32	0.046±0.048	2.14±1.77	0.192±0.151
SC	46.35±26.19*	0.044±0.030	0.84±0.50*	0.000±0.000
CR(S)	17.41±5.50**	0.000±0.000	0.28±0.14*	0.000±0.000
GC	19.81±7.46**	0.005±0.016	0.21±0.16	0.091±0.060
GSC	15.23±6.50**	0.038±0.027	0.50±0.23**	0.128±0.076*
GCR(S)	206.33±9.79	0.992±0.047**	6.85±0.34**	2.692±0.128**
H	82.20	91.72	78.84	90.01
Series 2				
S	753.34±762.16	0.330±0.383	10.53±10.87	0.055±0.130
R(S)	10.54±9.27	0.010±0.013	0.27±0.24	0.023±0.034
G	47.06±29.11*	0.185±0.101*	1.42±0.92	0.865±0.418*
GS	31.62±15.17*	0.062±0.043	1.23±0.60*	0.169±0.095*
GR(S)	79.69±13.01**	0.000±0.000	1.63±0.35**	0.000±0.000
C	156.04±116.17	0.008±0.067	3.02±2.38	0.087±0.127
SC	12.51±10.60	0.184±0.109*	0.62±0.49	0.175±0.124
CR(S)	32.70±9.04**	0.005±0.021	0.62±0.24**	0.067±0.057
GC	17.64±7.02**	0.020±0.040	0.23±0.27	0.044±0.069
GSC	3.55±6.35	0.138±0.057**	1.15±0.40**	0.199±0.100*
GCR(S)	275.92±13.35**	1.296±0.060**	8.28±0.44**	3.005±0.139**
H	73.92	80.50	70.27	89.32

The optimum crop-years were determined by plotting the trends in H from plant to fourth ratoon. There were marginal gains in H after the third ratoon for TCH in series 1 and the second ratoon in series 2 (Figure 3). TSH produced marginal gains in H after the third ratoon crops in both series. ERC % cane produced marginal increases in H after the second ratoon in series 1 and third ratoon in series 2 (Figure 4). Fibre % cane produced marginal gains in H after the second ratoon for both series (Figure 4). Trends in the magnitudes of H shown in Table 2, Figures 1, 2 were also apparent in Figure 3 and 4. From the results, the optimum number of replications is between three and four, while that for crop-years is between second and third ratoon.

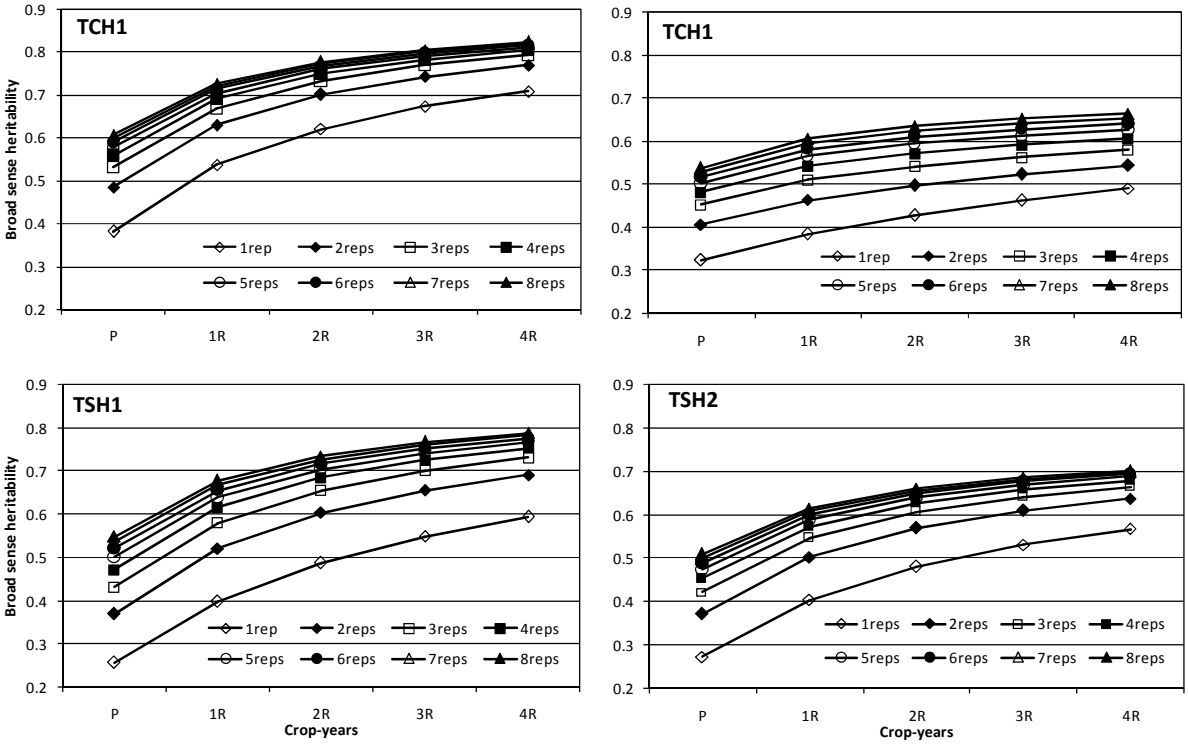

Figure 1. Replication effect on broad sense heritability (H) for tons cane per hectare (TCH) and tons sugar per hectare (TSH).

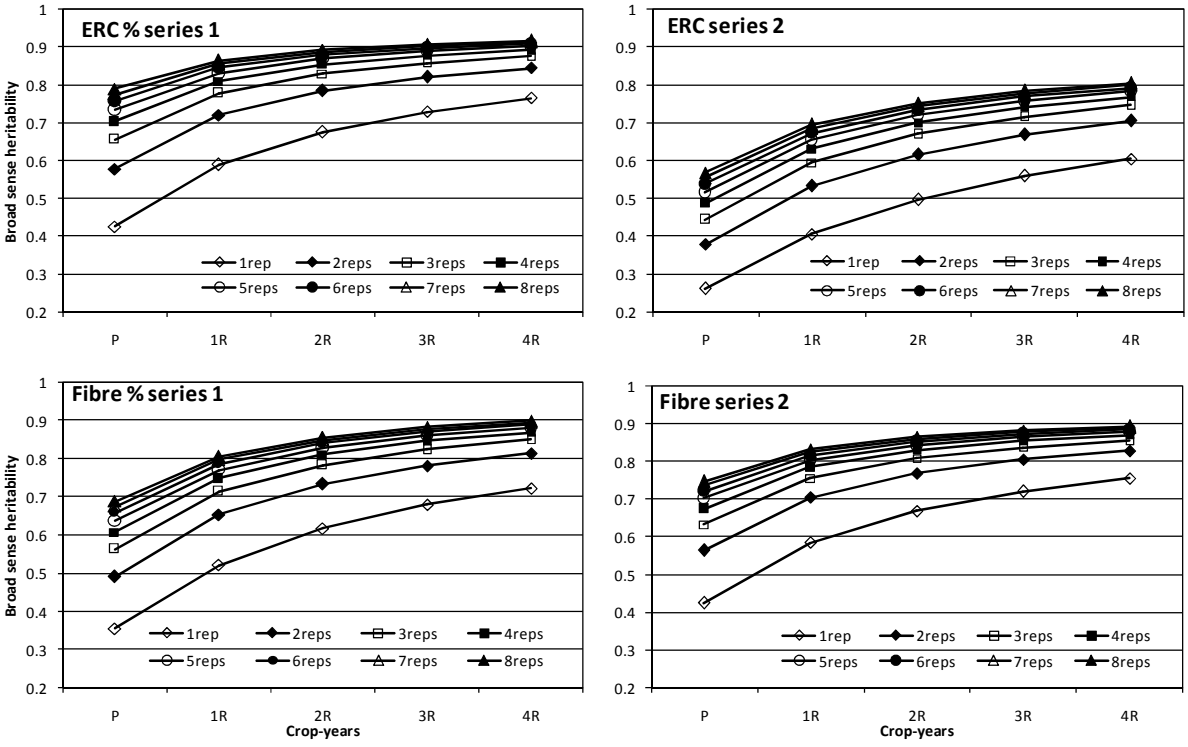

Figure 2. Replication effect on broad sense heritability (H) for estimated recoverable crystal (ERC) % cane and Fibre % cane for different number of crop years.

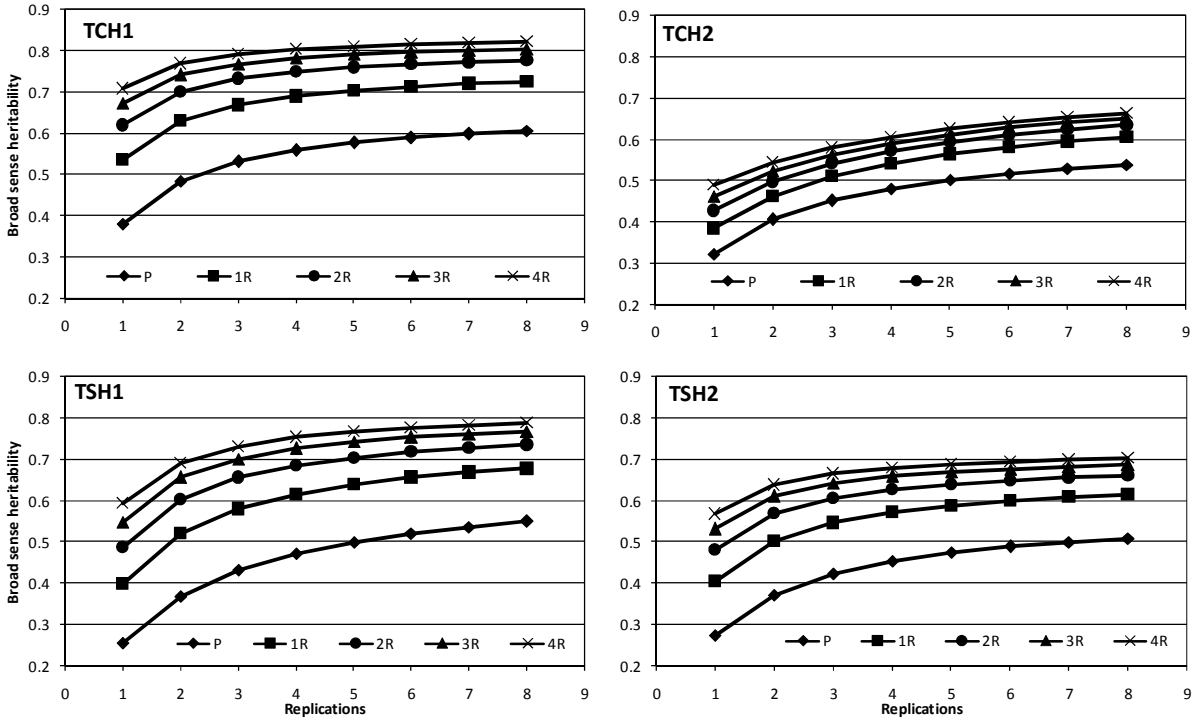

Figure 3. Crop-years effect on broad sense heritability (H) for tons cane per hectare (TCH) and tons sugar per hectare (TSH) for different numbers of replications.

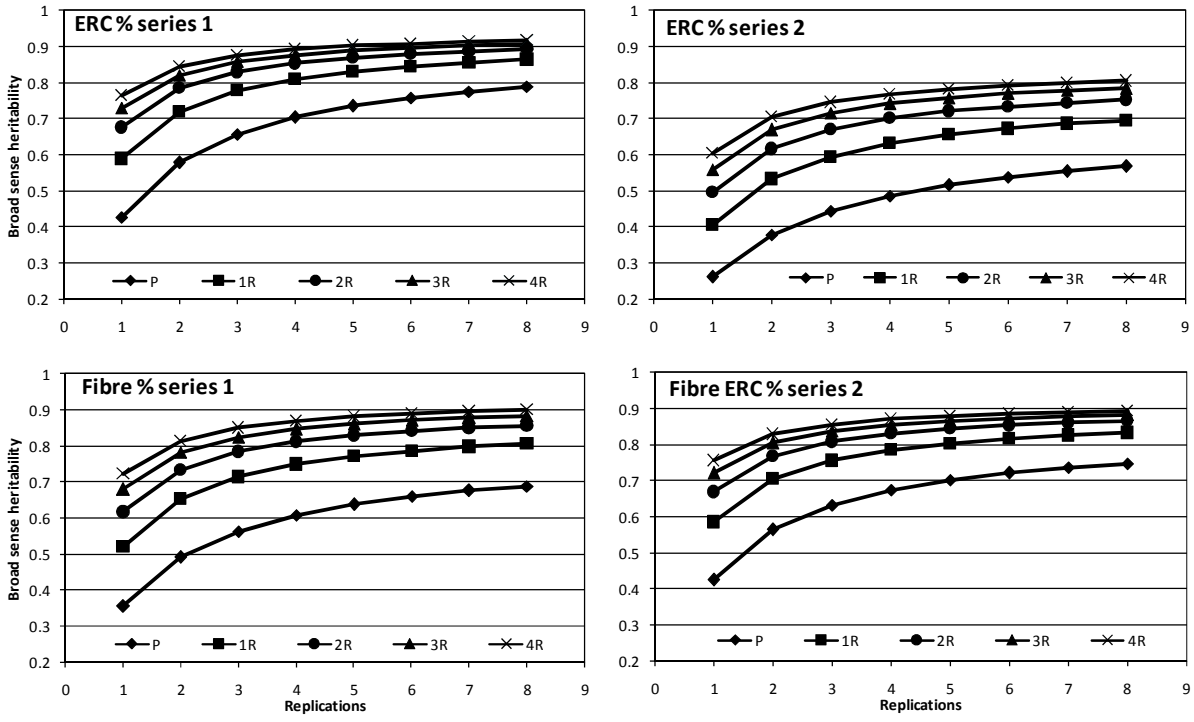

Figure 4. Crop-years effect on broad sense heritability (H) of estimated recoverable crystal (ERC) % cane and Fibre % cane for different number of replications.

Further identification of the optimum number of replications and crop-years was done by evaluating the unit increases in H (Tables 3 and 4). Using the number of crop-years (plant

and three ratoons) determined from Figures 1, 2, 3, 4, the gains in H showed that a maximum of four replications per trial was adequate. Similarly for crop-years, there were marginal gains in H of two units or less when harvesting the crops after the third ratoon.

Table 3. Units increase in broad sense heritability for every additional replication when crop-years were kept at plant, first, second, and third ratoon.

Rep	TCH		ERC % cane		TSH		Fibre % cane	
	Series 1	**Series 2**	**Series 1**	**Series 2**	**Series 1**	**Series 2**	**Series 1**	**Series 2**
1	67	46	73	56	55	53	68	72
2	74 (7)	52 (6)	82 (9)	67 (11)	66 (11)	61 (8)	78 (10)	81 (9)
3	77 (3)	56 (4)	86 (4)	72 (5)	70 (5)	64 (3)	82 (3)	84 (3)
4	78 (1)	59 (3)	88 (2)	74 (2)	73 (2)	66 (2)	85 (2)	86 (2)
5	79 (1)	61 (2)	89 (1)	76 (2)	74 (2)	67 (1)	86 (2)	87 (1)
6	80 (1)	63 (2)	90 (1)	77 (1)	75 (1)	68 (1)	87 (0)	87 (0)
7	80 (0)	64 (1)	90 (0)	78 (1)	76 (1)	68 (0)	88 (1)	88 (1)
8	80 (0)	65 (1)	91 (1)	78 (0)	77 (1)	69 (1)	88 (1)	88 (0)

Table 4. Units increase in broad sense heritability for every additional crop-year when replications were kept at four replications.

Crops	TCH		ERC % cane		TSH		Fibre % cane	
	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2
P	56	48	70	49	47	45	61	67
1R	69 (13)	54 (6)	81 (11)	63 (14)	62 (15)	57 (12)	75 (14)	79 (12)
2R	75 (6)	57 (3)	85 (4)	70 (7)	69 (7)	63 (6)	81 (6)	83 (4)
3R	78 (3)	59 (2)	88 (3)	74 (4)	73 (4)	66 (3)	85 (4)	86 (3)
4R	80 (2)	61 (2)	89 (1)	77 (3)	75 (2)	68 (2)	87 (2)	87 (1)
5R	82 (2)	62 (1)	90 (1)	79 (2)	77 (2)	69 (1)	89 (2)	88 (1)
6R	83 (1)	63 (1)	91 (1)	80 (1)	79 (2)	70 (1)	90 (1)	89 (1)
7R	84 (1)	64 (1)	91 (0)	81 (1)	80 (1)	71 (1)	91 (1)	90 (1)
8R	84 (0)	65 (1)	92 (1)	82 (1)	81 (1)	72 (1)	91 (0)	90 (0)

Discussion

The important variance components used for interpreting GE are GS, GC and GSC (Jackson and Hogarth, 1992; Kang and Miller, 1984; Kimbeng *et al*., 2002, 2009; Mirzawan *et al*., 1993). GS was used for determining the influence of season on the performance of varieties. Significant GS indicated that genotype variability was significantly influenced by seasons. The significant GS indicated that varieties tested produced large and significant variability to identify varieties suitable for the different seasons. Significant GC indicated that genotype variability within crop-years was sufficient to discriminate varieties for ratooning ability. The significant GSC indicated that genotype specific to SC combination existed among the genotypes under variety trials. The GSC is complex (Brown and Glaz, 2001) and suggests that variety testing needs to identify varieties that would significantly achieve yield gains in each season for the differing ratooning abilities.

The G was significant in all series for all traits, indicating that varieties that excel in all the trait values could be identified under these variety trials conditions. GS was

significant in both series for cane yield, indicating that some varieties being tested could be adapted to specific times of planting and harvesting. Identifying varieties specifically adapted to seasons would mean that the optimum yields could be achieved for each season of harvest and therefore lead to greater profitability. GC was significant for both series, indicating that genotypes with different ratooning abilities could be identified among the varieties in these trials. The significant GSC in series 1 indicated that some varieties in that series could be identified that were specifically adapted to certain times of harvesting where they ratoon well. The significant complex interaction effect variance components for yield also indicate the complex genetic control that exists for yield traits. Yield traits are known to be controlled by multiple genes, each with small additive effects (Falconer, 1989). Quantitative traits are greatly influenced by the environment (Kimbeng *et al*., 2002; Mirzawan *et al*., 1993), hence the significant variance for the components of GE for yield observed in this study. The trends in variance components for sugar yield followed similar trends as those for cane yield, indicating that cane yield was a stronger component of sugar yield than sucrose content. The results suggest the importance of testing for seasonal effects as well as ratooning ability at Dwangwa and justify the planting of early, mid and late trials as well as harvesting several ratoon crops. Similar findings were reported by Kimbeng *et al*. (2009) for the Texas Sugarcane Improvement Program, Rattey and Kimbeng (2001) for the Burdekin region of Queensland, Australia and Zhou (2004a) for Zimbabwe. A common feature shared by Texas, the Burdekin in Australia, Zimbabwe, and Dwangwa in Malawi, is that sugarcane is grown under irrigation.

Quality traits (sucrose content and fibre % cane) produced significant G, indicating greater genetic control for quality traits. The largely significant GSC indicated the possibility of complex environmental effects on quality traits. This higher order interaction is more difficult to interpret, as noted by Brown and Glaz (2001) and Milligan *et al.* (1990). Milligan *et al*. (1990) demonstrated that although the high order three-way interaction can be significant, it would generally contribute very little to the overall variance. Better understanding of this interaction could identify genotypes that produce greater quality for specific seasons. The non-significant GS and GC indicated that the effect of season and crop-year alone was less important individually but in combination could guide variety positioning.

The G was the most important for all traits, indicating that the genetic variance in this population of varieties was significant. For cane yield the order of importance for the variance components was GS>GC>GSC, indicating that seasonal effects were the most important component of GxE. Significant differences in cane yield across seasons were also reported by Zhou and Shoko (2011) and such trends play an important role in variety positioning for maximising cane yield (Zhou, 2003). The second most important variance component was GC, indicating the importance of ratooning ability in the Malawi production systems. Generally, longer ratooning is preferred, and this study indicates that there is a potential to select for ratooning ability. For TSH, the GS and GSC appeared interchangeably important in both series, indicating importance of seasonal effects on sugar yield and variety positioning. The GSC indicates that positioning varieties for sugar yield for both ratooning and seasons was more complex and also indicated that ratooning ability of some genotypes was influenced by seasons.

The seasons and ratooning ability influenced the quality of the genotypes. The seasons influenced ERC % cane more than crop-years. Generally, early season harvested crops

produce lower quality (Zhou, 1996; Zhou, 2004a,b) necessitating the need for artificial ripening. The mid-season crops produce the best quality while the late crops are intermediate. Studies by Isyagi and Kembo (2009) also showed the seasonal trends in yield and quality at the Nchalo sugar estate in Malawi. There was no consistent trend for fibre but generally, there was lower fibre in early than late season. This trend always leads to a shortage of bagasse for co-generation in early crops, while surplus stockpiles exist at the end of harvest and can be used to start off the next season.

The yield traits (TCH and TSH) produced lower H than quality traits. The influence of GE on yield traits is known to be large because these traits are controlled by quantitative genes (Falconer, 1989; Mirzawan *et al*., 1993). Quality traits, although also controlled by quantitative genes, are generally known to be stable and can also be measured with greater precision compared to yield traits (Kimbeng *et al*., 2001; Jackson and McRae, 2001). Higher H values meant that selection for quality traits would be done with greater precision than for yield traits.

Series 1 group of trials produced larger H than series 2 trials indicating greater accuracy of selection in series 1 trials than series 2 trials. This result showed that H could also be used as a parameter to compare the precision of trials either spatially or temporally. Such information would help in further investigating the possible causes of the low accuracy of selection and providing solutions to improve the efficiency of variety testing.

The optimum number of replications was four per trial. The marginal increases in H after increasing replications beyond four indicated that the resources for the extra replications could be saved. Additionally, planting a trial with eight replications resulted in a very large trial that would be spatially spread in a large area that is unlikely to be uniform. Such a scenario could result in greater variability being introduced, and reduce the precision of the trials in addition to uneconomic use of resources. The analysis showed that plant and three ratoon crops were adequate to determine the ratooning ability potential for the genotypes. Brown and Glaz (2001) found that three crops were adequate for testing ratooning ability in Florida. Similar studies done in Louisiana (Milligan, 1994) showed marginal gains in H beyond two replications. Studies by Brown and Glaz (2001) recommended reducing the number of replications from eight to four for the Canal Point breeding programme in Florida, USA. Rattey and Kimbeng (2001) found that two replications were optimum for Bureau of Sugar Experiment Stations breeding programmes for the Burdekin region in Queensland, Australia.

The implication of reducing the number of replications and crop-years is that resources saved could be reallocated to additional trials. Studies done in Australia (Jackson and Hogarth, 1992; Mirzawan *et al*., 1993; Bull *et al*., 1994) found that locations were the most important source of variation. Therefore increasing the number of trial sites to capture the soil types that are predominant at Dwangwa sugar estate could yield more useful information that would also help in variety positioning. The four replications saved for each of the three seasons can be reallocated to establish further trials to capture the prevailing soil types in the Dwangwa sugar estate at no extra cost. Such trials would provide additional valuable information that would identify the best varieties for the different soil types matched by season, allowing for a more economic allocation and variety positioning for greater profitability. Such benefits need to be validated experimentally.

Conclusions

TCH and TSH were more affected by GE, as evidenced by large number of the components of variance (GS and GC) that were significant compared to quality traits. Additionally, quality traits produced larger H, mainly because they were less influenced by GE. Quality traits are measured with greater precision in the laboratory. Because of the minimum gains achieved by additional replications and crop-years, planting a maximum of four replications and harvesting each trial for the plant and three ratoon crops should provide adequate discriminating ability to determine the best yielding and high quality varieties. Studies of the benefits of planting more trials on different soil types using resources saved by reducing the number of replications and harvesting fewer crops is needed.

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