SHORT NON-REFEREED PAPER

USING QUANTITATIVE GENETIC PARAMETERS TO DETERMINE SAMPLE SIZE FOR SUCROSE CONTENT IN SUGARCANE BREEDING

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Introduction

Resource allocation is used to optimally determine design parameters and sample size in plant breeding trials. A larger sample size and more replications are expected to accurately estimate breeding parameters (Lorenz, 2013). Leite *et al*. (2009) investigated the minimum sample size needed to efficiently estimate genetic and phenotypic parameters of yield related traits while studies by Kimbeng *et al*. (2009) and Zhou *et al*. (2012) used resource allocation to determine optimum replications in sugarcane variety trials.

Brix % in juice of progenies measured using a hand held refractometer can be used to predict sucrose content of a genotype (Kimbeng and Cox, 2003). Hand held refractometer Brix can be measured on individual genotypes providing data to quantify progeny variability within families. Brix measurements, taken by using a hand held refractometer, is rapid to measure in the field and less expensive. However, there is no knowledge on the optimum number of genotypes (sample size) and replications required to get accurate estimates of breeding parameters in sugarcane breeding. Therefore the objectives of this study were to determine the optimum sample size and replication number using hand held refractometer measured Brix from stage 1 trials in sugarcane breeding.

Materials and Methods

The crosses used for this study were made in the SASRI glasshouse at Mount Edgecombe (29.7°S, 31.03°E, 96 m above sea level) Durban, South Africa. Brix % data were collected in stage 1 (mini-lines) trials from the irrigated, coastal short cycle average potential (CSCAP) and Midlands sandy soils breeding programmes. The trials were laid out as randomised complete block designs with three replicates per family. Brix was measured from one stalk of each of the first 20 genotypes per family plot using a hand held refractometer. A refractometer determines sugarcane Brix by measuring the refractive index of sugarcane juice. A stalk was cut from the base in each genotype plot. The stalk was cut at the centre. Sugarcane juice was squeezed from the centre cut end of the stalk onto the refractometer and a reading obtained. The hand held refractometer was calibrated after each reading using distilled water.

Data were analysed using the following linear mixed models on statistical analysis system (SAS) software (SAS institute, 2012):

$$Y_{ijk} = R_i + F_j + RF_{ij} + G(RF)_{ijk} \quad \text{Equation 1}$$

Where Y_{ijk} is the observation for the *kth* genotype in the *jth* family of the ith replication, R_i is the random effect of the *ith* replication, F_j is the random effect of the *jth* family, RF_{ij} is the random interaction effect of the *ith* replication by the *jth* family and is the error term for testing the fixed effects of the family effect and $G(RF)_{ijk}$ is the random effect of the kth genotype nested

within the random interaction effect of the *ith* replication by the *jth* family and is the residual error term.

The family variance was in turn used to calculate broad-sense heritability using the following formula:

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

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

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

$$SE = \sqrt{\frac{2(1-H)^2[1+H(q-1)]^2}{q(q-1)(n-1)}} \quad \text{Equation 3}$$

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

The optimum sample size and number of replications were determined by simulating broad-sense heritability for sample size ranging from one to 20 genotypes and replications one to five per family. Response surface graphs were used to visualise the simulated broad-sense heritability.

Results and Discussion

The family variance was significant (P<0.01) for the FML15, UML15 and SML15 trials (Table 1) suggesting Brix would benefit from family evaluation. Family by replication interaction variance (σ^2_{RF}) was highly significant for all three trials. Family by replication interaction variances were smaller than family variances for all three trials. Magnitudes of broad-sense heritability were 0.77 for FML15 and UML15 and 0.74 for SML15.

Table 1. Variance components, broad-sense heritability (H) estimates, and mean value determined for Brix.

Statistic	Irrigated (FML15)	CSCAP (UML15)	Midlands sandy soils (SML15)
σ^2_F	1.99±0.57**	1.33±0.40**	0.88±0.26***
σ^2_{RF}	1.33±0.30***	0.85±0.40***	0.75±0.16***
$\sigma^2_{G(FR)}$	8.88±0.27***	6.95±0.21***	3.31±0.10***
H	0.77±0.04	0.77±0.04	0.74±0.04

σ^2_F - family variance, σ^2_{RF} – family by replication variance, $\sigma^2_{G(FR)}$

The broad-sense heritability values for the FML15, UML15 and SML15 trials increased up to the fourth replication. After the fourth replication, the total increase in broad-sense heritability was less than 0.05 (FML15), 0.03 to 0.06 (UML15) and 0.06 (SML15). The simulated broad-sense heritability values increased with each additional genotype sampled up to a sample size of eight to 13 genotypes. At a sample size of greater than eight genotypes, the total increase in broad-sense heritability was less than 0.01. Sample sizes between 14 and 20 genotypes produced smaller increases in broad-sense heritability for the FML15 trial. For UML15 the

simulated broad-sense heritability values increased consistently with each additional genotype sampled up to a sample size of 10 to 12 genotypes. Beyond a sample size of 10 genotypes, the increase in broad-sense heritability was less than 0.01. Sample sizes between 13 and 20 genotypes per plot produced a smaller increase in broad-sense heritability. The simulated broad-sense heritability values increased with each additional genotype sampled up to a sample size of 10 genotypes for SML15. Beyond a sample size of 10 genotypes per plot, the increase in broad-sense heritability was less than 0.01. Sample sizes between 11 and 20 genotypes per plot produced smaller gains in broad-sense heritability.

The current sample size (three replications by 20 genotypes) produced broad-sense heritability ranging from 0.74 to 0.77. The simulated broad-sense heritability using response surface graphs showed a sample size of four replications by 10 genotypes would produce broad-sense heritability ranging from 0.77 to 0.80 indicating greater precision of selection (Figure 1). The results indicated a sample size of four replications and 10 genotypes would be an ideal sample size to accurately determine sugarcane breeding parameters. Adopting four replications by 10 genotypes (40 samples) will reduce samples by 33 % compared to three replications by 20 genotypes (60 samples). The cost and time saved can be used to evaluate more families.

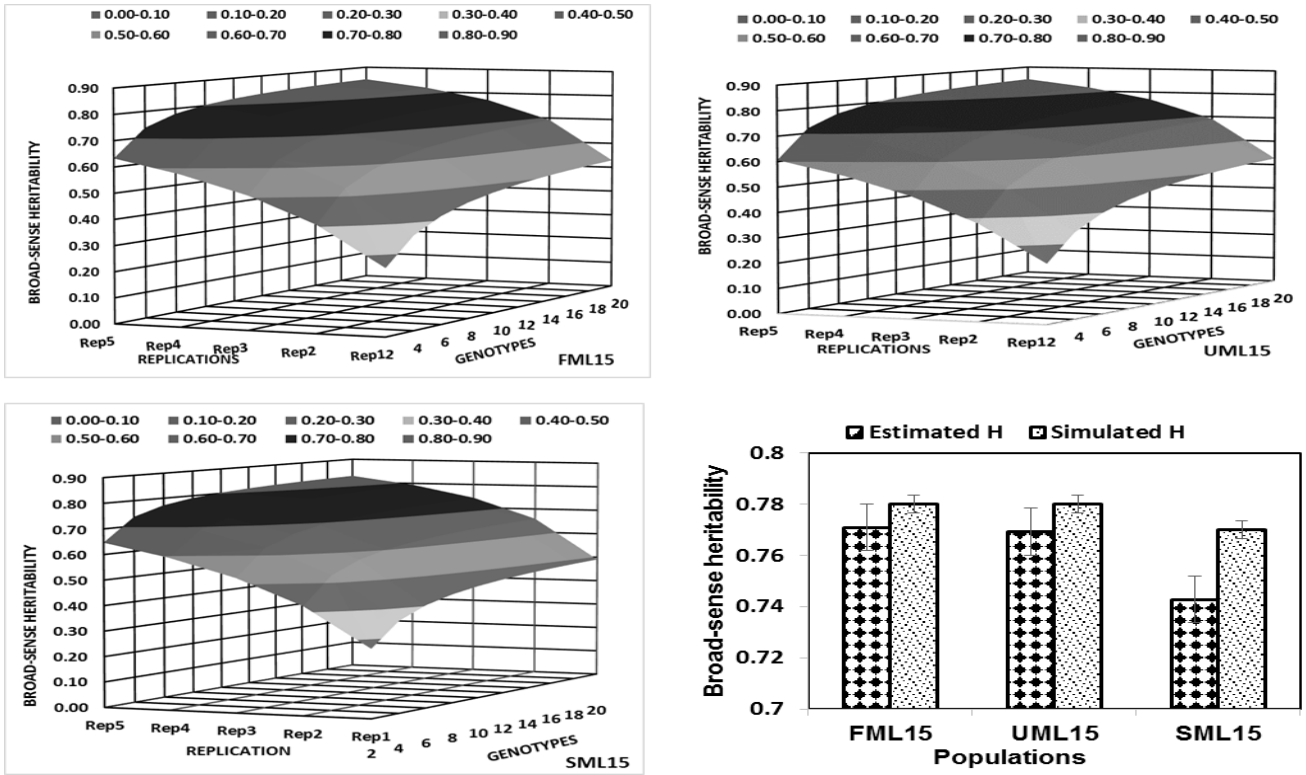

Figure 1. The change in broad-sense heritability (H) with an increase in number of replications and sample size for the irrigated, CSCAP and Midlands sandy soils breeding programmes.

Conclusion

The study suggested a sample size of four replications and 10 genotypes is adequate for family evaluation for brix. Reducing sample size to 10 genotypes and increasing replications to four will reduce the overall sample size by 33 % (40 versus 60 samples). The reduced costs associated with data collection and transport of samples as well as time can be used to evaluate more families in order to increase efficiency of breeding.

REFERENCES

Kimbeng CA and Cox MC (2003). Early generation selection of sugarcane families and clones in Australia: A review. J Am Soc Sug Cane Technol 23: 20-39.

Kimbeng CA, Zhou MM and da Silva JA (2009). Genotype x Environment Interactions and Resource Allocation in Sugarcane Yield Trials in the Rio Grande Valley Region of Texas. *J Am Soc Sug Cane Technol* 29: 11-24.

Leite MS de O, Peternelli LA, Barbosa MHP, Cecon PR and Cruz CD (2009) Sample size for full‐sib family evaluation in sugarcane. *Pesquisa Agropecuária Brasileira* 44(12): 1562-1574.

Lorenz AJ (2013). Resource allocation for maximizing prediction accuracy and genetic gain of genomic selection in plant breeding: A simulation experiment. *G3-Genes Genomes Genetics* 3: 481-491.

SAS Version 9.2 (2012). SAS/STAT user's guide, version 9.2 SAS Institute, Cary, North Carolina, USA.

Zhou MM, Chihana A and Parfitt RC (2012). Optimum replications and crop-years for sugarcane genotype trials at Dwangwa sugar estate in Malawi. *S Afr J Plant Soil* 29: 31-38.