Optimal Plot and Sample Sizes for Sugarcane (Saccharum spp. Hybrids) Varietal Assessment

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ABSTRACT

A uniformity trial was conducted using the variety SL 95 4430 at the Sugarcane Research Institute, Uda Walawe Sri Lanka to determine the optimal plot sizes and sample sizes of sugarcane for their efficient assessment in different stages of the varietal selection program in Sri Lanka. The optimal plot sizes were determined for the characteristics; plot weight (WT), number of millable stalks (ST), hand refractometer brix (HB), laboratory brix (Brix), stalk length (SL), stalk diameter (SD), rind hardness (RD), pol in juice (Pol), purity (Pur), pure obtainable cane sugar (POCS) and fibre content (Fib) by determining the point of inflection of the coefficient of variation (CV) verses plot size graph through split line regression and maximum curvature methods. The optimal sample sizes were found for the prescribed margin of error of 10% of the plot mean for the above-mentioned variables except for the number of millable stalks. Three-metre long single-row plots and five-metre long two-row plots were found as the optimum for sugarcane variety evaluation in stages 2 and 3 of the selection program, respectively. For assessing the varieties for hand refractometer brix and rind hardness at stage 1, samples of three and four millable stalks, respectively from a clump was found as the optimum. The optimal sample sizes to appraise varieties in variety selection stage 2 for stalk length, field brix and rind hardness were six, three and four millable stalks; respectively Six millable stalks for stalk length and 3 millable stalks for laboratory brix, purity and fibre content were found to be the optimum sample sizes for assessing the varieties at stage 3. For the evaluation of varieties in Preliminary Yield Trials and Replicated Yield Trials, a sample of 12 stalks should be obtained and eight stalks should be used for extracting mixed juice for the analysis of brix, pol, and purity and the remaining four stalks for the analysis of fibre content.

Keywords: Crop improvement, Optimum plot size, Optimum sample size, Sugarcane, Variety selection

INTRODUCTION

New sugarcane varieties are produced primarily by hybridisation and subsequent clonal propagation of the progenies. Each year, a population consisting of many thousand seedlings of new varieties is produced. These varieties are passed through a series of stages of selection and the selected varieties in each stage are evaluated for their performance in larger plots in the subsequent stage. At the latter stages of selection, the varieties are replicated to reduce the effect of environmental variation, and evaluations are carried out in the sites representing sugarcane-growing areas with different environmental conditions. Subsequently, the varieties superior to the existing commercial varieties are propagated and released for commercial cultivation (Skinner et al., 1987).

In Sri Lanka, sugarcane variety selection starts from stage 1 where more than sixty thousand seedlings of different varieties produced by true seeds of crosses are assessed for sucrose and fibre contents approximated by hand refractometer brix and rind hardness, respectively. Each clump of an individual variety is evaluated visually for its morphology, infection of diseases and cane yield at this stage. Annually, a fairly large tissue culture sub-clone population produced by exploiting somaclonal variation is also assessed in similar manner, parallel to selection stage 1. The better varieties consisting of nearly ten percent of the population are selected in stage 1 and are advanced into stage 2 where stem cuttings of

each selected variety are planted in a 5 m-long single-row plot for evaluation. The better varieties selected in stage 2 are advanced into stage 3 for evaluation in 10 m-long 2-row plots. Selection stage 3 usually consists of about 10 percent of the varieties at stage 2, and the better varieties selected in stage 3 are screened for resistance to major pests and diseases. The varieties found to be resistant to the major pests and diseases are advanced for testing in Preliminary Yield Trials (PYTs) in plots of 5 m x 4 rows. Replicated Yield Trails (RYTs) are conducted using the plots of size, 10 m x 5 rows to evaluate the promising varieties selected from PYTs. The varieties in both PYT and RYT stages are tested for cane yield and biochemical characteristics (sugar and fibre) in each sugar industry site. In RYTs, the varieties are tested up to second ratoon crop. The experimental designs for PYTs and RYTs are decided based on the number of varieties to be tested and the type of the lands where the trials are established.

The selection index "I1 = 0.3 (rank of stalk length) + 0.4 (rank of hand refractometer brix) + 0.3 (rank of absolute deviation of rind hardness from the standard)" is employed in selecting varieties at stage 2 and the index "I2 = 0.3 (rank of stalk length) + 0.2 (rank of purity) + 0.2 (rank of laboratory brix) + 0.3 (rank of absolute deviation of fibre percent from the standard)" is used for selection of varieties at stage 3 (Wijesuriya et al., 1997). The use of indices that manipulate several attributes simultaneously in these stages of varietal selection is of great importance for selecting the varieties base on the overall performance.

The plot sizes used for the stages 2 and 3 of the variety selection programme are determined solely based on the planting material requirement for establishing the next stage of selection. The plot sizes used in these stages require a large land area to accommodate a large number of test varieties in the initial stages of variety selection. The variation in soil conditions in a larger experimental area causes bias over selection of varieties grown under better soil conditions and vice-versa under poor soil conditions. The use of optimal plot sizes and sample sizes assures obtaining reliable, accurate and precise parameter estimates of the variables used for selection of varieties in different selection stages and eventually improves selection efficiency.

There are not many findings in literature regarding the optimal plot sizes in sugarcane variety selection. Leite et al., (2009) has evaluated the number of rows and individual plants per plot required for the assessment of sugarcane families based on cane and sugar yield components. Barbosa et al., (2001) have studied a population of 500 sugarcane plants and concluded that 50 individual plants are sufficient to estimate the production of stalks, and ten individuals per plot are enough to estimate the mean Brix of the families. Those findings on optimal plot sizes were based on the sugarcane seedling families where each seedling varies genetically to each other. In such cases, within-family variation and genotype x environment variation warranted bigger plot sizes as optimal for estimating the parameters. Such situations arise only at stage 1 if combined selection (individual selection followed by family selection) is practised, and it does not happen in other stages of sugarcane variety selection as all the plants in a plot are genetically identical.

There is hardly any literature on optimal plot sizes for use in selection of varieties in different stages of sugarcane crop improvement. Therefore, this research is the pioneering attempt in this discipline for determining the optimal sample sizes together with the optimal plot sizes for more accurate assessment of sugarcane at different stages of selection with efficient utilisation of resources; land, labour and funds.

MATERIALS AND METHODS

Layout of the uniformity trial

The field experiment for the determination of optimal plot and sample sizes for sugarcane variety selection was carried out at the Research Farm of the Sugarcane Research Institute (SRI), Uda Walawe, Sri Lanka. This

area belongs to agro-ecological zone DL1a. The sugarcane field was established in February 2013 using ridge and furrow system with the recommended spacing of 1.37 m apart. A block of uniform sugarcane field of the variety SL 95 4430 at harvestable maturity was selected for sampling to derive optimal plot and sample sizes.

Harvesting and collection of data for determining optimal plot sizes

Harvesting and collection of data were carried out in February, 2014. The whole block of the land was pegged along the cane rows to create a grid where one-meter-long cane harvesting units (plots) were demarcated before commencing the harvesting. This will accomplish amalgamation of harvesting units in both row and column directions in the grid to generate different plot sizes and shapes required for the analysis. Each unit was harvested separately, from one end to the other in each cane row. The canes were cut at the bottom and the tops were removed at the highest fully-formed internode of the stem. Only the millable stalks were used for recording the data.

The number of millable stalks per plot (ST) and their weight (WT) were taken after removing water shoots and unproductive tillers. Having counted the number of millable stalks and weighing canes in each plot, a sample of randomly-selected 5 millable canes was obtained and tagged. These 5-stalked samples were used to measure hand refractometer brix (HB), stalk length (SL), stalk diameter (SD) and rind hardness (RD) in the field. After recording these data, the cane samples were analysed forbrix (Brix), pol in juice (Pol) and, fibre content (Fib) at the Laboratory of the Sugarcane Research Institute. The variables, purity (Pur) and pure obtainable cane sugar (POCS) content in juice were estimated based on the results of these analyses.

Harvesting of canes for determining optimal sample sizes

The individual stalks of a randomly-selected 5 m-long cane plot in the uniformity trial was harvested from one corner to the other, and the harvested canes were labelled in numerical order up to the last stalk in the plot. HB, WT, RD, SL, SD were recorded, Brix, Pol and Fib were measured and Pur and POCS were estimated for each millable stalk.

Analysis of Data

Determination of optimal plot sizes

Amalgamation of basic harvesting units in both row and column directions in the trial block can form plots of different sizes. Co-efficient of variation (CV) values for each characteristic for these plot sizes were calculated using the computer programme developed through R programming language by Jeewanthi et al., (2014). Subsequently, the estimated CV values were plotted against corresponding plot sizes. This relationship was estimated in exponential form (Figure. 1); $Y = aX^b$, where Y is the CV for the characteristic concerned and X is the plot size and a and b are the estimated coefficients. The optimum plot size for each variable was found by finding the point of inflection of this graph through split-line regression and maximum curvature methods, suggested by Ryan and Porth (2007) and Vallejo and Mendoza (1992), respectively. The methodology proposed in www.mathworld.wolfram.com/curvature.html was used for the maximum curvature method (Wolfram Math World. (Undated) Retrieved January 1, 2016 from http://www.mathworld.wolfram.com/curvature.html), and the software Genstat 17 was used in split-line regression method.

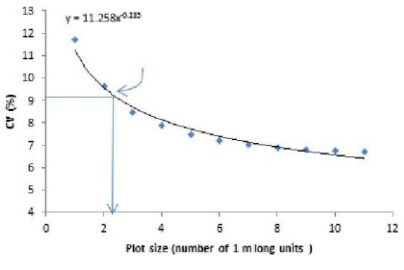

Figure 1: The typical relationship between plot size and Coefficient of Variation (CV) of the variable concerned, derived through the sugarcane uniformity trial

The variation of CV against plot size in single-row plots was used in determining the optimum plot size in variety selection stage 2 and that was used in the determination of the optimum plot size in variety selection stage 3.

Determination of optimal sample sizes

Using the estimate of the sampling variance, the required sample size can be determined based on a prescribed margin of error of the plot mean, or the treatment mean (Gomez and Gomez, 1984). The sample size for a random sampling design that can satisfy a prescribed margin of error of the plot mean is computed as:

$$n = \frac{(z_\alpha^2)(v_s)}{(d^2)(\bar{x}^2)}$$

Where, n = sample size, z_α= value of the standardized normal variate corresponding to the level of significance α (the value z_α can be obtained from Cumulative Normal Frequency Distribution Table), v_s = sampling variance, x = mean and d = margin of error expressed as a fraction of the plot mean. In this study, the margin of error was taken as 0.1.

RESULTS AND DISCUSSION

Optimal plot sizes for different characteristics used in sugarcane varietal assessment

The sugarcane variety selection programme conducted at the Sugarcane Research Institute, Sri Lanka, begins with the selection of individual sugarcane plants (clump) generated through true seeds in selection stage 1. Therefore, the plot size at this stage is invariably a single plant.

Optimal plot sizes for selection stage 2 of sugarcane

The selected plot sizes that determined through both split-line regression method and maximum curvature method (Table 1) indicated that the use of 3 m-long plots in the selection stage 2 is sufficient for accurate assessment of varieties for hand refractometer brix, stalk length, stalk diameter, rind hardness, laboratory brix, Pol, Purity and pure obtainable cane sugar. The results showed that 5 m-long plots are required to appraise varieties for number of stalks and plot weight at this stage and the results proved that these two characteristics, among the characteristics studied, possessed the highest variability. Two-metre-long plots are sufficient for evaluation of varieties for fibre at this stage.

Currently, at this stage, data on stalk length, hand refractometer brix and rind hardness are recorded from five randomly-selected millable stalks from 5 m x 1-row plots for calculating of index values of the varieties under evaluation. The results of optimal plot sizes for characteristics under study (Table 1) indicated that 3 m-long plots are sufficient to evaluate varieties for these three variables in the index.

Therefore, it is evident that the plot size used currently (5 m x 1 row) is larger than the optimum size, i.e., 3 m x 1 row in selection stage 2. Accordingly, 3 m-long plots in the establishment of varieties in selection stage 2 is recommended for adoption in the future. Adoption of this new recommendation provides space for 1606 plots per hectare as compared to 1095 plots per hectare in the current practice.

Optimal plot sizes for stage 3 of sugarcane variety selection programme

The selected plot sizes determined through split-line regression method and maximum curvature method (Table 2) indicated that 3 m-long, 2-row plots in the selection stage 3 are sufficient for accurate assessment of varieties for stalk length, hand refractometer brix, laboratory brix, pol, purity, pure obtainable cane sugar and fibre. The results indicated that 4 m-long 2-row plots are required to appraise varieties for number of stalks and 5 m-long 2-row plots are needed for evaluating varieties for stalk diameter and rind hardness. At this stage, 6 m-long 2-row plots are required to assess varieties for plot weight. The results proved that, among the

Table 1: Optimal plot sizes determined by split line regression method and maximum curvature method and selected plot sizes for different characteristics in sugarcane variety selection stage 2

Characteristic	Optimum plot size [single row (m)]		Selected plot size for stage 2 [m]
	Split line regression	Maximum curvature	
Hand refractometer brix	3	2	3
Stalk length	3	2	3
Stalk diameter	3	2	3
Rind hardness	3	3	3
Number of stalks	2	5	5
Plot weight	2	5	5
Laboratory brix	3	1	3
Pol % in juice	3	2	3
Purity	3	1	3
Pure obtainable cane sugar	3	2	3
Fibre content	2	2	2

Table 2: Optimum plot sizes determined by split line regression method and maximum curvature method and selected plot sizes for different characteristics in sugarcane variety selection stage 3

Characteristic	Optimum plot size [m x 2 rows]		Selected plot size for stage 2 [m x 2 rows]
	Split line regression	Maximum curvature	
Hand refractometer brix	3	2	3
Stalk length	3	2	3
Stalk diameter	5	2	5
Rind hardness	5	4	5
Number of stalks	3	4	4
Plot weight	3	6	6
Laboratory brix	3	2	3
Pol % in juice	3	2	3
Purity	3	2	3
Pure obtainable cane sugar	3	2	3
Fibre content	3	2	3

characteristics studied, plot weight possesses the highest variability. Two-metre-long 2-row plots are sufficient for evaluation of varieties for fibre at this stage.

The index used in this stage comprised four measurable variables, namely; stalk length, purity, laboratory brix, and fibre content. The results obtained for optimum plot sizes relevant to these four characteristics indicated that 3 m x 2-row plots (Table 2) are sufficient to evaluate varieties for these four characteristics in stage 3 of the variety selection programme. Therefore, the plot size used at present, i.e., 10 m x 2 rows is larger than the required plot size for variety evaluation at selection stage 3. However, sufficient quantities of planting materials from the selected varieties are required to establish trials in the variety multiplication stage that is followed by stage 3 of the variety selection programme and to provide planting materials of the selected varieties for establishing field experiments for screening those varieties for resistance to major sugarcane diseases. As such, considering the level of precision of the results of evaluation and the amount of planting materials of the selected varieties required to be provided, the plot size, 5 m x 2-row is proposed in the establishment of the trials in stage 3 of the sugarcane variety selection programme. Adoption of 5 m x 2-row plots in the establishment of variety selection stage 3 allows establishing 1095 plots per hectare of land as compared to 584 plots in the current practice of 10 m x 2-row plots.

Optimal sample sizes for different characteristics used in sugarcane variety assessment

The optimal sample sizes derived through calculations together with descriptive statistics for the characteristics studied are given in Table 3. Among the characteristics studied, plot weight showed the highest variability. It needs a sample of more than 22 millable stalks for more accurate assessment of cane weight. Measurements from 7 and 6 millable stalks are needed for the assessment of stalk diameter and stalk length, respectively. Hand refractometer brix needs 3 millable stalks as the sample and a 4-stalked sample is needed for rind hardness. All characteristics that are measured in the laboratory required less number of stalks for a sample compared to those measured in the field. Single-stalked sample is enough to measure laboratory brix, pol and purity while a two-stalked sample is needed to measure pure obtainable cane sugar. The required sample size for fibre was determined as four, which is separately used for fibre analysis. The variable, pure obtainable cane sugar is an estimation based on the sample analysis of laboratory brix, pol and fibre content. Therefore, to achieve the required accuracy for pure obtainable cane sugar, laboratory brix and pol should be measured from juice extracted at least from a 2-stalked sample.

Optimal sample sizes for different characteristics in Selection stage 1

Genotypes in this stage are evaluated on two measurable variables namely; hand refractometer brix and rind hardness. Visual estimations on clump weight (cane yield), plant architecture and morphology and free from diseases and pests are used for selection of genotypes in this stage.

At present, one juice sample extracted from one mature cane is taken for assessing of hand refractometer brix and one rind hardness value is taken for measurement of fibre. The results show that 3- and 4-stalked samples are required for accurate assessment of hand refractometer brix and rind hardness, respectively. Therefore, it is proposed to use 3 and 4 stalks in a clump respectively, for determining the average values of hand refractometer brix and rind hardness in evaluating genotypes at this stage of selection.

Optimal sample sizes for different characteristics in Selection stage 2

The index used for this selection stage comprised three variables; namely, stalk length, hand refractometer brix and rind hardness. At present, average values of 5 millable stalks for each variable is used for

Table 3: Descriptive statistics and optimal sample sizes under the margin of error = 0.1 for each characteristic of sugarcane

Characteristic	Unit	Average	CV	Calculated sample size	Sample size (No. of stalks)
Hand refractometer brix	Degrees	19.33	8.18	2.57	3
Stalk length	cm	260.84	12.39	5.89	6
Stalk diameter	mm	26.93	13.40	6.90	7
Rind hardness	mm	20.00	10.50	4.23	4
Plot weight	kg	1.28	23.65	21.49	22
Laboratory brix	degrees	20.10	4.50	0.78	1
Pol % in juice	%	17.18	5.94	1.36	1
Purity	%	85.42	2.84	0.31	1
Pure obtainable cane sugar	%	12.98	6.97	1.86	2
Fibre content	%	11.17	9.35	3.36	3

estimating the index values for each variety at this selection stage. The optimal sample sizes for more accurate assessment of stalk length, hand refractometer brix and rind hardness are 6, 3 and 4 millable stalks, respectively. Thus, the sample size currently being used is sufficient for assessing hand refractometer brix and rind hardness, but is not adequate for the assessment of stalk length. As such, this study proposes to use average values of stalk length, hand refractometer brix, and rind hardness from 6, 3 and 4 stalks respectively, from varieties under evaluation.

Optimal sample sizes for different characteristics in Selection stage 3

The index used for this stage possesses four measurable variables namely; stalk length, purity, laboratory brix and fibre content. At present, average length of 12 randomly-selected millable stalks from each variety is used to calculate the index. The same cane sample is used for laboratory analysis from which 10 millable canes are used to derive the values of laboratory brix and purity and 2 millable canes are used for analysis of fibre content. According to the study, 6-stalked sample for stalk length, 1-stalked sample for laboratory brix and purity and 3-stalked sample for measurement of fibre are found to be sufficient. Thus, the sample sizes used at present for stalk length, laboratory brix and purity are larger and that for fibre is smaller than the optimum. Therefore, the average values of 6-stalked samples are adequate for evaluation of varieties for stalk length at this stage. This 6-stalked sample can be divided into two samples of three stalks each at the laboratory and one can be used for analysis of laboratory, brix and purity and the other for analysis of fibre.

Optimal sample sizes for different characteristics in Preliminary Yield Trials (PYTs) and Replicated Yield Trials (RYTs)

The varieties selected from stage 3, which are resistant or moderately resistant to major diseases and pests are advanced to PYTs after variety multiplication for testing them in comparatively large plots. Plots of 5 m x 4 rows are used in this stage. Varieties at PYTs are basically selected on high cane and sugar yields and moderate fibre contents. Cane yields estimated based on the cane weight of the middle two rows of the plot are used for assessing the varieties for cane yield at this stage. The plot size used in Replicated Yield Trials is 10 m x 5 rows. Cane yield is

estimated based on the weight of cane in the middle three rows of the plot. In RYTs also, the varieties are evaluated on high cane and sugar yields and moderate fibre content

A sample of 12 randomly-selected stalks obtained from the middle rows in both PYTs and RYTs are used for testing varieties for sugar and fibre contents at the laboratory. From this sample, 10-stalks are separated for juice extraction for measuring laboratory brix, pol and estimation of purity and the remaining 2 stalks for the analysis for fibre content to determine pure obtainable cane sugar.

According to the results of this study, plot weight has the maximum variability, and therefore, the plot sizes used at present for the assessment of cane yield at these two stages should not be changed. Moreover, at these stages, cane weight of each plot is extrapolated to one hectare. However, the sample size used for fibre analysis at present is proven inadequate. Thus, it is proposed to mix the juice from 8 stalks of the 12-stalked sample for measuring laboratory brix and pol and to use the remaining 4 stalks for fibre analysis. Sugar yield (tonnes/ha) of the varieties should be estimated after estimation of pure obtainable cane sugar using the relevant formula.

CONCLUSIONS

The findings of this study indicated that the measurements from 3 and 4 stalks in a clump are required for accurate assessment of genotypes respectively for determining the average values of hand refractometer brix and rind hardness at selection stage 1. The selected plot sizes that determined through split-line regression method and maximum curvature method indicated that the use of 3 m-long plots in the selection stage 2 is sufficient for assessment of varieties for hand refractometer brix, stalk length and rind hardness measured from 6, 3 and 4 stalks, respectively. The average values of these characteristics should be used in the selection index for varietal evaluation. The plot size, 3 m x 2 rows is sufficient for assessing varieties for stalk length, laboratory brix, purity and fibre content, which are the index characteristics used in variety selection stage 3. This study proposes that the average values of 6 stalks should be used for stalk length and for measuring laboratory brix and purity from mixed-juice extracted from 3 stalks obtained from 6-stalked sample and for measuring fibre using the other 3 stalks should be used. Based on the planting material requirement for establishing subsequent varietal selection stage, the plot size 5 m x 2 rows was proposed for the establishment of variety selection stage 3.

This study did not propose any changes to the plot sizes used in Preliminary Yield Trials and Replicated Yield Trials. However, changes to sample sizes for accurate assessment of characteristics were proposed. Having recorded stalk length and stalk diameter from the currently-used 12-stalked sample in the field, mixed juice extracted from 8 stalks out of this 12-stalked sample is proposed for measuring laboratory brix and pol. The remaining 4 stalks are proposed to be used in fibre analysis.

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