

## **RELATIVE EFFICIENCY OF SPATIAL ANALYSES FOR NON-REPLICATED EARLY-STAGE SUGARCANE FIELD EXPERIMENTS**

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### **ABSTRACT**

In the early stages of sugarcane (*Saccharum spp.*) selection programs, large numbers of clones are tested in non-replicated plots. Field trends are likely to affect the performance of these non-replicated experimental genotypes and mask their true genetic potential. The purpose of this study was to evaluate different spatial analyses for their relative efficiency in accounting for field trends in early-stage sugarcane selection trials. A Moving Means (MM) and Autoregressive Spatial (SP) analysis methods were used to adjust genotype values in the 2000 and 2002 non-replicated Stage-II field experiments within the Canal Point (FL, USA) sugarcane breeding program. Variogram plots showed more complex patterns for fields of larger size and suggested the existence of both local trends and gradients across the fields. Based on reduced error variance for the checks, the relative efficiency (RE) obtained with MM (quadratic method) was inconsistent and ranged from 4 to 188%. Based on the average standard error of differences, gains in RE ranged from 95 to 492% with the SP method. SP was, therefore, the preferred method for giving consistent and greater gains in RE, thereby indicating its suitability for analysis and selection of early-stage sugarcane trials. Of the 135 clones normally advanced to the next testing stage, 75% were congruent between the standard and SP. Adjusting for field trends also resulted in the improvement of phenotypic correlations among the traits, even annulling some of the negative associations between cane yield and quality traits. These results suggest that spatial methods could be suitable for the analysis and selection of sugarcane clones planted in non-replicated early-stage field trials.

### **INTRODUCTION**

Early-stage evaluation in sugarcane (*Saccharum spp.*) breeding is commonly practiced on large numbers of experimental clones that are derived from multiple crosses and field-tested without replications. Precision in estimating the genetic potential of these genotypes is crucial to the plant breeder at this stage to avoid committing a Type II error of losing a potentially valuable cultivar. The challenge is to accurately select the superior (in yield) clones in order to maximize genetic gains. However, heterogeneity among experimental plots and field trends, which are common occurrences in agricultural field experiments, are likely to affect yield and its components (Becker, 1995; Kirk et al., 1980). Spatial variation is usually manifested by substantial correlation among neighboring plots (Brownie et al, 1993; Federer, 1956; Lin et al., 1993), which affects the ranking of genotypes, replicated or not (Brownie et al, 1993; Stroup et al., 1994) and biases estimates of genetic variances and heritability (Magnussen, 1993; Rosielle, 1980) in plant breeding trials.

Different experimental designs (incomplete block, row-column, spatial) that are more

suitable and more efficient than the randomized complete block (RCBD) have been introduced and compared, along with proper statistical analyses, to account for and remove field trends and/or competition among experimental genotypes (Cochran and Cox, 1957; Federer, 1956; Grondona et al., 1996). Gains in efficiency are also possible when a spatial analysis is applied to a RCBD layout in the presence of field trends. However, proper designs and analyses, when used in tandem, are necessary to further reduce the experimental error variance (Cullis and Gleeson, 1991; Federer, 1998).

For non-replicated trials with a large number of genotypes, augmented designs, first suggested by Federer (1956, 1961) for use in sugarcane, have been recommended to control spatial variation, increase the precision of estimating genetic potential (Shunmughasundaram et al., 1980; Stringer and Cullis, 2002), and increase genetic gains from selection (Magnussen, 1993; Rosielle, 1980). Augmented designs use two types of treatments as replicated reference cultivars and non-replicated experimental genotypes. The reference cultivars are distributed across the field to sample any variation that may be present in order to adjust the values of the test genotypes. Non-replicated experiments, normally used in early stages of sugarcane breeding, may benefit from adjustments based on augmented designs, moving means or other spatial analytical methods, to accurately estimate the true genetic potential of the clones for advancement (Stringer and Cullis, 2002).

Profuse literature exists on the use and efficiency of designs that allow the recovery of block, trend, and other design information for a better discrimination of the genotypes under selection in non-replicated layouts (Federer, 1956, 1961, 1998; Federer et al., 1975; Federer and Raghavarao, 1975; Burgueño and Crossa, 2000). Comparing a simple lattice design with an augmented design, Shunmughasundaram et al. (1980) found that the two designs closely agreed on the top 10% of the 86 sugarcane clones under selection. The relative efficiency of the lattice design was much higher than that of the augmented design probably because of the relatively low number of clones included in the trials. More recently, Stringer and Cullis (2002) recommended introducing spatial analyses into the Australian sugarcane breeding program as a means of accounting for fertility trends and plot competition in non-replicated early-stage trials. The efficiency of the augmented or spatial designs and analysis relies on a better partitioning of the variance components for block and residual effects, allowing the breeder to better compare the new genotypes with the checks.

The efficiency of one analysis over another is usually measured in terms of reduced error variance, expected error mean square, or average standard error of the difference between genotype means (Binns, 1987; Cochran and Cox, 1957; Magnussen, 1990). The average standard error of the difference (SED) was reported to be more appropriate since it is used for comparison among genotypes using the same scale as the traits (Binns, 1987; Cullis and Gleeson, 1991; Gleeson, 1997). The objectives of this study were 1) to assess if field trends are present in Stage II trials in the USDA-ARS Canal Point sugarcane breeding program 2) to compare the relative efficiency of spatial models over a standard analysis of variance in selecting the best clones for advancement and 3) to assess the effects of field trends on the relationships among different traits used for advancement in the Canal Point breeding program.

## **MATERIALS AND METHODS**

Five stages (Seedling, Stage I-IV) of selection characterize the Canal Point sugarcane breeding program and Stage II is the second non-replicated clonal stage and the first to be sampled for assessment of cane and sucrose yield components. Stage II involves selection among 1,000-1,500 clones, of which 135 (about 10%) are advanced to Stage III. A total of 1166 (yr 2000) and 1532 (yr 2002) Stage II clones were planted in November on three to four contiguous fields at the USDA Sugarcane Field Station, in Canal Point, FL. Only data collected from the two fields which carried the majority of the clones were considered for this study. The fields were different each year and arbitrarily designated A and B for 2000 and C and D for 2002. The soil type of these fields was classified as Torry muck (*euic hyperthermic typic haplosaprist*), a minor but rich organic soil type in the Everglades Agricultural Area.

In 2000, 688 and 352 experimental clones were arranged in 16 blocks of 43 and of 22 plots in fields A and B, respectively. In 2002, Fields C and D were planted with 744 and 672 experimental clones in 16 blocks of 46 and of 42 plots, respectively. Five reference cultivars were planted in each field in 2000 and six in 2002, randomly distributed (4% and 5% of the test clone plots, respectively) across with unequal frequencies or replicates (Table 1). These cultivars represent different maturity groups, ranging from early ('CP 70-1133'; Rice et al., 1978), to intermediate [( 'CP 65-357' (Breux et al., 1974), 'CP 72-1210' (Miller et al., 1981), 'CP 72-2086' (Miller et al., 1984)], to late ('CP 57-603'; Dunckelman et al., 1969). In 2002, CP 72-1210 was replaced by two other important cultivars, CP 78-1628 (Tai et al., 1991) and CP 89-2143 (Glaz et al., 2000) in Florida (Glaz and Vonderwell, 2004). Maturity in sugarcane generally corresponds to the time of maximum sucrose content in the stalks during the growth cycle.

The experimental units were two-row plots, spaced 1.52 m apart and 4.56-m long. The blocks were planted end-to-end and separated alternately with 1.52-m and 4.56-m alleys. The plots were treated similarly to a commercial sugarcane field in terms of soil preparation, irrigation, and other cultural practices, but no fertilization was applied. A scouting of the fields was made in September as usual, which eliminated a few clones with naturally occurring diseases, lodging, and poor establishment. Stalk counts (STN; July) and 10-stalk samples (September) were taken on the remaining clonal and check plots to estimate stalk weight (STW; kg), sucrose content (SC; %), cane yield (TCH; Mg ha<sup>-1</sup>), sugar yield (TSH; Mg ha<sup>-1</sup>), and theoretical recoverable sugar (TRS; kg sugar Mg<sup>-1</sup> of cane) based on procedures formulated by Arceneaux (1935) and Legendre (1992).

### Statistical analyses

Augmented designs use replicated checks to assess the performance of non-replicated genotypes in incomplete block designs (Federer, 1998). The experimental genotypes are usually considered as random effects and the check cultivars as fixed effects, evaluated according to the following mixed model:

$$y_{ijk} = \mu + g_i + c_j + \beta_k + \varepsilon_{ijk}$$

where  $\mu$  is the general mean,  $g$  is the experimental genotype (or clone) considered with block ( $\beta$ ) and error ( $\epsilon$ ) as the random effects, while the reference cultivars ( $c$ ) are the fixed effects

**Table 1.** Frequency of the five check cultivars used to sample the variation in the fields used to test non-replicated Stage II sugarcane clones in the Canal Point, FL breeding program. (Years 2000-01 and 2002-03).

	Fields (Yr 2000) <sup>†</sup>		Fields (Yr 2002)	
	A	B	C	D
Clones (no.)	688	352	744	672
Checks				
	-----no. replicates-----			
CP 57-0603	3	3	4	4
CP 65-0357	3	3	4	4
CP 70-1133	4	4	6	6
CP 72-1210	2	2	-	-
CP 72-2086	4	4	6	6
CP 76-1628	-	-	6	6
CP 89-2143	-	-	6	6
Total	16	16	32	32
Percent <sup>‡</sup>	2.3	4.3	4.1	4.5

<sup>†</sup>Two fields out of three in 2000 and two fields out of four in 2002 that carried the majority of the clones were used for the analysis. Different fields are used each year.

<sup>‡</sup>Percent of the number of test clones planted in the respective fields and year.

(Wolfinger et al., 1998). An autoregressive spatial analysis (thereafter referred to as spatial or SP) via the Mixed procedure (SAS, 1996; Wolfinger et al., 1997) incorporated in the SAS macro ‘UNREP’ (Burgueño and Crossa, 2000) was used to estimate effects and to obtain variance components for the parameters of interest (clones and residual) through the restricted maximum likelihood (REML) method. Adjusted means for both clones and checks were computed as well as the standard errors of differences among them. Separate analyses were performed for each field, since the size and number of rows and of columns were different from field to field. The Variogram and Krige2D procedures (SAS, 1996) were used to create the semi-variogram and standard errors of prediction plots, reported here for TCH, TSH, and TRS in yr 2002 only.

The data structure of Stage II is also amenable to a moving means analysis that makes simultaneous use of the frequency of the reference cultivars and of neighboring plots in the fields. A moving means (MM) analysis with the Agrobases software (Agronomix, 2000) was also

used to account for any trend in the fields. The data were subjected to three different methods of analyses (linear, quadratic, and row x column) to estimate the genotypic values of the clones after removing potential field trends revealed by the variance of the reference cultivars. The best method was chosen as the one that accounted for the largest variance of trends and that reduced the variance of the checks. Data are presented only for the Quadratic method, which was consistently the best across fields and years.

Since no statistical analysis is normally applied on Stage II data for selection, a simple ANOVA (by means of the SAS GLM procedure; Federer and Wolfinger, 1998) was used for comparison with the spatial analysis. The standard errors of the differences or of the predictions (SED) were used to calculate the relative efficiency (RE) of the spatial method over the standard ANOVA, based on the formula given by Magnussen (1990) and Cullis and Gleeson (1991):

$$RE = [100 \times (SED_{\text{Standard}}/SED_{\text{SP}})] - 100,$$

where 'Standard' stands for the conventional method based on a simple ANOVA and 'SP' for the autoregressive spatial analysis based on a REML-mixed model. For MM, RE was based on the reductions of error variances of reference cultivars after adjusting for field trends.

Congruence between the ANOVA and either spatial method was evaluated with Spearman rank correlation coefficients. Relationships among the traits before and after adjustments were appraised with Pearson correlation coefficients using a combined dataset of the two fields within the year.

Similarity indices between the spatial and standard analyses in selecting the top 10%, 15%, and 20% of the experimental clones were assessed by means of the Czekanowski coefficient ( $IS_C$ ) as follows:

$$IS_C = 2x / (2x + y + z)$$

with x being the number of clones selected by both methods, y being the number of clones selected by the spatial analysis only, and z being the number of clones selected by the conventional method only. For a fixed selection intensity,  $y = z$  and the coefficient becomes:

$$IS_C = x / (x + y) = x / (x + z)$$

In the calculation of  $IS_C$ , adjusted yield (TCH, TSH, and SC) values, obtained from both fields in the corresponding year, were first appended and then ranked in descending order to separate the best performing clones.

## RESULTS AND DISCUSSION

### Trend assessment

In assessing the performance of clones with the Moving Means analysis, the Quadratic method (two neighbors on either side) was preferred to other linear combination of neighbors or row x column (data not shown). It explained the largest variance of potential field trends in all traits, while reducing the variance of the reference cultivars. The analysis lost efficiency as the number of sides or neighboring plots increased. This indicated that plots closer together were more correlated than plots further apart. Moreover, the trend and check variances were much larger in fields A and C than in fields B and D (Tables 2 and 3). Fields A and C, being larger in size and carrying more clones, were more variable than the other two fields. Wider and longer layouts, receiving more clones than smaller layouts, are expected to be more heterogeneous, and taking this variation into account should result in a more efficient selection. However, this does

not eliminate plot competition as an important factor worth considering when selecting clones from early-stage non-replicated sugarcane trials (Stringer and Cullis, 2002).

The variogram or trend plots (given only for TCH, TSH, and TRS) portray portions of the fields with high or low yields in both years (Fig. 1, presented for yr 2002). These surface trends showed similarity of yield values for neighboring plots. The variogram plots indicate some aggregated patterns, i.e. spatial dependence (with an autoregressive spatial variation in the residuals), of yield over the lengths of the fields. These plots suggest the existence of local trends with gradients in both rows and columns across the fields. While the organic soils in the cane growing area of the Everglades tend to be uniform for a particular field in terms of soil composition, variations could occur from year to year as a result of cultivation practices, bedrock slope, moisture, field history, etc. These year-to-year variations have the potential to affect the performance of clones under selection, particularly when tested as single replicates.

### **Relative efficiency**

The superiority of MM over the standard practice was consistent for cane and sugar yields in both years (Tables 2 and 3). The Moving Means analysis efficiently reduced the variances of checks by 4% (STW, yr 2000) to 188% (STN, yr 2000). However, this approach was not able to improve on the estimates of quality traits (Brix, Pol, SUC, and TRS) and was inconsistent, i.e. field-dependent, for stalk number and stalk weight when all four fields were considered. The MM analysis may be too sensitive to missing plot data since, normally in the CP breeding program, data are not collected on plots with diseases, poor stand, and other defects that render the clone inferior. For this reason, the quadratic method was more likely to be more efficient than any linear combination of neighbor plots.

The SP analysis was more efficient than the standard ANOVA in both years at analyzing the eight traits of interest in this study (Tables 2 and 3). Gains in precision ranged from 95% (TCH) to 492% (TSH) in 2000 and from 177% (TCH, TSH) to 282% (SUC) in 2002. The gains appeared to be more substantial for fields B and D (with the smallest layout) than for fields A and C (with the largest number of plots in rows and columns). In the presence of an underlying field trend, fitting a model that accounts for spatial variation can improve the estimates of clonal performance in sugarcane non-replicated trials. Grondona et al (1996) gave evidence that the most efficient methods at modeling field trends are those that account for this variation in two dimensions (row and column).

The relative efficiency can be taken as an indication of the presence of spatial heterogeneity or field trends in field experiments (Vollmann et al., 2000). The effects of field and seasonal variation were apparent in the 2000 and 2002 Stage II trials in all four fields. While greater gains in efficiency were consistently obtained with the SP method, it was also evident that yield and quality traits were differentially affected by the trends (Tables 4 and 5). Greater precision was achieved in analyzing cane yield (TCH) in fields of larger size (A and C) and quality traits in field of smaller size (B and D) both years. However, in 2000, RE was larger for STN, Brix, SUC, and TSH, and in 2002, STW, Pol, TRS, and TCH were measured with greater precision. These results suggested that clonal advancement from non-replicated Stage II trials at

**Table 2.** Relative efficiency of spatial analyses (Moving Means and autoregressive) over a standard ANOVA performed on non-replicated Stage II sugarcane clones tested in two contiguous fields at the Canal Point Sugarcane Field Station, FL. Yr 2000.

Traits <sup>¶</sup>	Moving Means				ANOVA		Spatial <sup>†</sup>		
	$\sigma^2_{Trends}$	$\sigma^2_{Check}$		RE <sup>‡</sup>	$\sigma^2_{Standard}$	SED <sup>§</sup>	$\Sigma^2_{SP}$	SED	RE <sup>‡</sup>
		Unadjusted	Adjusted						
<b>Field A</b>				---%---					---%---
STN	527.33	952.02	330.38	188.16	970.62	44.06	747.2	14.34	207.25
STW	25.12	7.90	6.08	29.93	50.1	10.01	16.94	3.44	190.99
Brix	-	-	-	-	2.70	2.32	0.69	0.73	218.33
Pol	-	-	-	-	78.14	12.50	32.13	5.22	139.49
SUC	-	-	-	-	4.33	2.94	1.01	0.88	234.41
TCH	136.75	348.14	209.97	65.80	1362.56	52.20	1148.11	20.38	156.15
TSH	1.87	1.94	1.47	32.27	18.67	6.11	8.95	2.41	153.55
TRS	-	-	-	-	1191.07	48.80	569.09	21.36	128.46
<b>Field B</b>									
STN	394.96	438.91	349.10	25.73	813.55	40.34	377.93	14.27	182.67
STW	17.57	9.97	9.55	4.40	34.98	8.36	20.36	2.99	179.74
Brix	-	-	-	-	2.62	2.29	0.49	0.66	246.83
Pol	-	-	-	-	93.35	13.66	24.31	4.40	210.54
SUC	-	-	-	-	5.19	3.22	0.45	0.66	388.15
TCH	96.42	129.90	117.30	10.74	962.41	43.86	185.36	22.52	94.76
TSH	1.18	1.07	1.01	5.76	12.93	5.09	1.65	0.86	491.86
TRS	-	-	-	-	1531.62	55.35	280.54	16.25	240.62

<sup>†</sup>Spatial analysis as performed by the SAS “Unrep” macro published by the Biometrics Department at CIMMYT.

<sup>‡</sup>RE=Relative efficiency. For Moving Means, RE is based on the unadjusted and adjusted check variances for field trends. For the spatial analysis, RE is based on the average standard errors of the difference among clones calculated by the standard ANOVA and spatial (autoregressive) methods.

<sup>§</sup>SED=Average standard error of the difference among the experimental clones.

<sup>¶</sup>STN=Stalk number; STW=stalk weight; SUC=sucrose content; TCH=cane yield; TSH=sugar yield; TRS=theoretical recoverable sugar.

**Table 3.** Relative efficiency of spatial analyses (Moving Means and autoregressive) over a standard ANOVA performed on non-replicated Stage II sugarcane clones tested in two contiguous fields at the Canal Point Sugarcane Field Station, FL. Yr 2002.

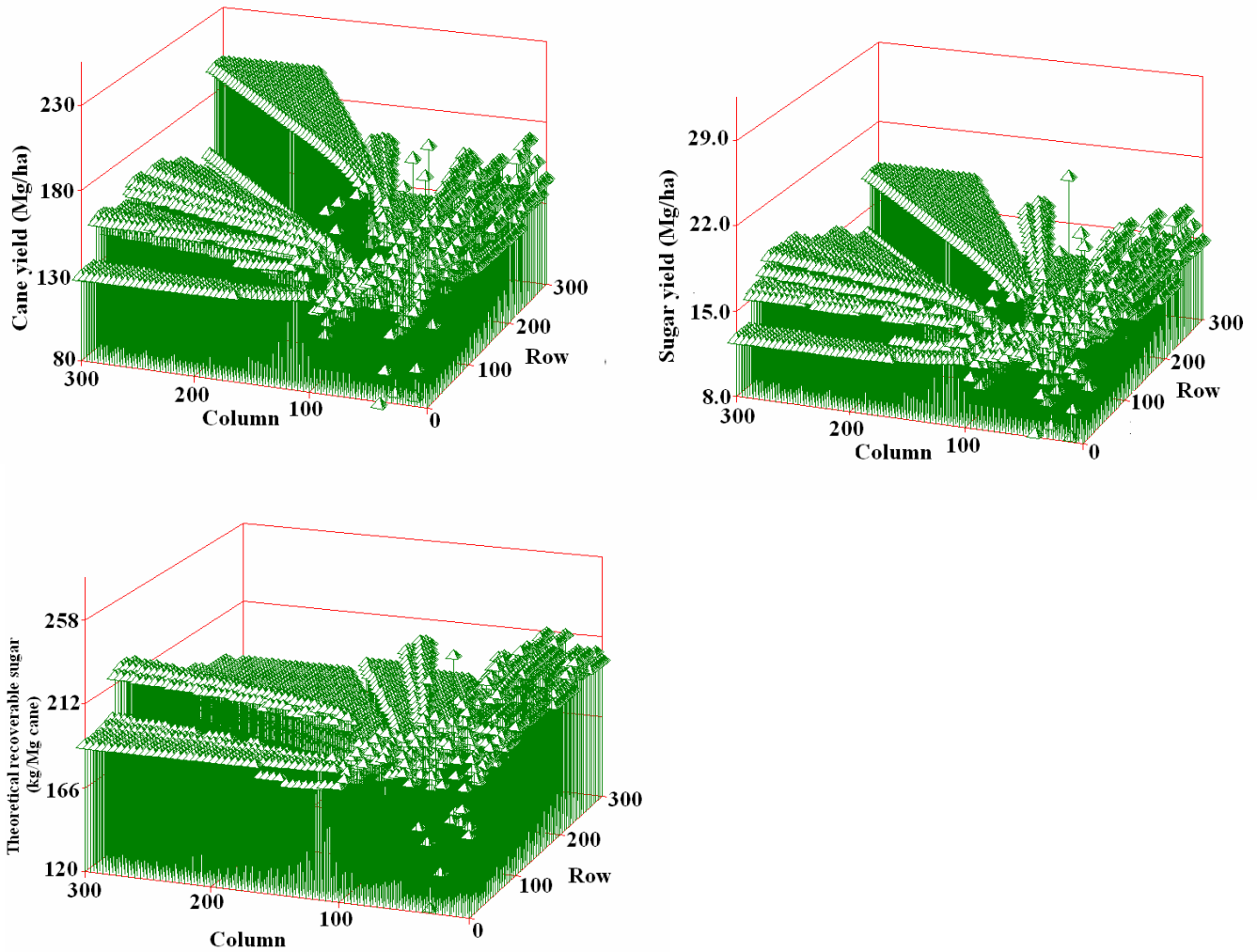
Traits <sup>¶</sup>	Moving Means				ANOVA		Spatial <sup>†</sup>		
	$\sigma^2_{Trends}$	$\sigma^2_{Check}$		RE <sup>‡</sup>	$\sigma^2_E$	SED <sup>§</sup>	$\Sigma^2_E$	SED <sup>§</sup>	RE <sup>‡</sup>
		Unadjusted	Adjusted						
Field C				---%---					---%---
STN	487.95	456.58	178.84	155.30	883.95	42.05	491.38	15.06	179.19
STW	34.23	20.31	27.46	-26.04	64.55	11.36	39.69	4.04	181.24
Brix	1.13	0.37	0.66	-43.94	2.21	2.10	0.53	0.65	223.44
Pol	35.61	10.60	20.03	-47.08	69.96	11.83	15.25	3.49	238.93
SUC	1.94	0.58	1.10	-47.27	3.82	2.76	0.84	0.82	237.08
TCH	837.80	1397.55	886.44	57.66	1456.77	53.98	257.25	14.92	261.78
TSH	12.08	23.10	10.24	125.59	22.74	6.74	19.92	2.31	191.94
TRS	134.84	40.79	75.93	-46.28	1060.85	46.06	288.04	14.90	209.14
Field D									
STN	568.47	208.24	267.79	-22.24	1089.22	46.67	343.23	15.74	196.53
STW	33.77	8.82	7.25	21.66	59.15	10.88	12.43	3.24	235.70
Brix	1.41	0.49	0.83	-51.96	2.56	2.26	0.66	0.72	214.27
Pol	40.47	8.53	23.41	-64.56	72.10	12.01	10.95	3.18	277.62
SUC	2.18	0.45	1.27	-65.57	3.89	2.79	0.58	0.73	282.09
TCH	709.03	538.17	318.27	69.09	1440.29	53.67	699.03	19.37	177.08
TSH	12.16	9.86	5.77	70.88	24.03	6.93	12.13	2.50	177.30
TRS	150.24	31.36	88.69	-35.36	1054.60	45.93	159.83	12.20	276.44

<sup>†</sup>Spatial analysis as performed by the “Unrep” SAS macro published by the Biometrics Department at CIMMYT.

<sup>‡</sup>RE=Relative efficiency. For Moving Means, RE is based on reductions of check error variances after adjusting for field trends (Agronomix Software Inc., 2000). For the spatial analysis, RE is based on the average standard errors of the difference among clones obtained from the standard ANOVA and spatial (autoregressive) methods.

<sup>§</sup>SED=Average standard error of the difference among the experimental clones.

<sup>¶</sup>STN=Stalk number; STW=stalk weight; SUC=sucrose content; TCH=cane yield; TSH=sugar yield; TRS=theoretical recoverable sugar.



**Figure 1.** Variograms showing surface trends for cane yield (top left), sugar yield (top right), and theoretical recoverable sugar (bottom) measured in non-replicated Stage II sugarcane trials planted at Canal Point, FL. (Yr 2002-03)

the Canal Point station needs to be assessed with a method that accounts for the different spatial (field) variations which the experimental sites are exposed to each year. Stringer and Cullis (2002) arrived at the same conclusion for similar non-replicated sugarcane trials in the Australian breeding program.

**Relationships among traits**

In sugarcane trials, breeders need to compromise selections between STN and STW and between cane yield and quality traits to compensate for the negative associations that exist among them (Skinner, 1971). In the Canal Point breeding program, an index of selection (Deren et al., 1995) is used in the last two stages (III and IV) and this index puts more emphasis on TRS (similar to harvest index in cereals) than on TCH (heavier canes).

In this study, field trends affected phenotypic correlations among traits. Correlations among quality traits (Brix, Pol, Suc, and TRS) were similarly strong with all three methods and highly significant ( $r = 0.90-0.99$ ;  $P \leq 0.001$ ), irrespective of field or year (Tables 4 and 5). The usually negative correlation between STN and STW was still present and was relatively similar across fields and years. The SP method reduced, more than the MM analysis, the typically negative correlations between STN (or STW) and quality traits. The correlations between TCH and quality traits were negative (ranging from  $r = -0.02$  to  $r = -0.23$ ) under the standard method of analysis (as usually found in sugarcane field trials). These relationships were negated in 2002, particularly under the SP method, becoming non-significant and at times significantly positive (ranging from  $r = -0.001$  to  $r = 0.17$ ) in 2000. While no changes in the associations between TSH and quality traits or TCH were noted for MM, stronger associations (from  $r = 0.54$  to  $r = 0.60$  in 2000; from  $r = 0.47$  to  $r = 0.50$  in 2002) in absolute values resulted from adjustments by the SP method. The TSH-TRS association was improved (in absolute values) with the spatial method (from  $r = 0.48$  to  $r = 0.51$  in 2000; from  $r = 0.56$  to  $r = 0.59$  in 2002). The improved correlations between TCH or TSH and quality traits, obtained after adjusting for field trends, imply that response to selection may improve by basing selections more on the genetic component of these traits after removing much of the residual variance associated with field trends. A stronger association between protein content and soybean yield was also reported by Vollmann et al. (2000) after adjusting for field trends and effects. The spatial adjustment of the Stage II data, with improvement of the associations among the traits, yielded clones with combined high-tonnage and high-sucrose content in the top 10-20% selected for advancement in the Canal Point program.

### **Similarity index**

The agreement between the standard procedure with either spatial method was moderate, as indicated by the spearman rank correlations between adjusted and unadjusted means. These correlations were highly significant ( $P < 0.0001$ ) and were around 0.70 for all traits. This indicates that some disagreements existed in the top 10-20% clones selected for advancement. Similarity indices were calculated for the three most important traits (disease traits were not measured in this study) considered for advancing clones from Stage II in the Canal Point breeding program (Table 6). The similarity index is a measure of the percent agreement among the methods on the top 10-20% clones selected for advancement. This index revealed that congruence between the standard procedure and MM was in general lower (from 0.24 to 0.61) than that between the former and SP (from 0.16 to 0.87). Inconsistencies were greater for TCH than for TSH and TRS. Cane yield is usually more variable than these other traits since it is more susceptible to yearly changes in weather and since it was estimated as the product of stalk number and weight. With selections based mostly on TSH and TRS in the breeding program, a 25% disparity would exist in the clones selected by the standard and spatial methods. This translated to a lack of congruence for 35 out of the 135 Stage II clones usually selected each year for advancement. This strategy is being evaluated in the Canal Point breeding program. To make the 135 clones needed for advancement to Stage III, 35 clones exclusively selected by the SP method in the 2002-03 cropping season were added to the 100 clones selected in common. One of these 35 clones is actually in the last stage of selection and is being evaluated across locations with 12 other clones for release: this is a proof that the SP spatial analysis is something to

**Table 4.** Correlation coefficients among traits measured from non-replicated Stage II trials before and after adjustments for field trends in the Canal Point sugarcane breeding program. Data were combined for the two fields analyzed. (Yr 2000-01)

Traits	STW <sup>†</sup>			BRIX		Pol		SUC		TCH			TSH			TRS	
	UN <sup>‡</sup>	M M	SP	UN	SP	UN	SP	UN	SP	UN	MM	SP	UN	MM	SP	UN	SP
	----- r <sup>§</sup> -----																
STN	-0.29 **	-0.28 **	-0.25 **	-0.20 **	0.02 ns	-0.20 **	-0.01 ns	-0.20 **	-0.01 ns	0.64 **	0.62 **	0.59 **	0.40 **	0.43 **	0.45 **	-0.19 **	-0.01 ns
STW	-	-	-	0.18 **	0.18 **	0.18 **	0.19 **	0.19 **	0.19 **	0.53 **	0.44 **	0.46 **	0.54 **	0.46* *	0.51 **	0.18 **	0.17 **
BRIX				-	-	0.94 **	0.94 **	0.93 **	0.93 **	-0.01 ns	-	0.17 **	0.50 **	-	0.57 **	0.89 **	0.88 **
Pol						-	-	0.99 **	0.99 **	-0.00 ns	-	0.15 **	0.56 **	-	0.61 **	0.99 **	0.99 **
SUC								-	-	-0.00 ns	-	0.15 **	0.56 **	-	0.61 **	0.99 **	0.99 **
TCH										-	-	-	0.81 **	0.74 **	0.81 **	0 ns	0.14 **
TSH													-	-	-	0.56 **	0.59 **
TRS																-	-

\*, \*\* Significant at  $P \leq 0.05$  or  $P \leq 0.01$  levels, respectively. ns=not significant at  $P \leq 0.05$ .

<sup>†</sup>STN=Stalk number; STW=stalk weight in kg; SUC= sucrose content in %; TCH=cane yield in Mg ha<sup>-1</sup>; TSH=sugar yield in Mg ha<sup>-1</sup>; TRS=theoretical recoverable sugar in kg Mg<sup>-1</sup> ha<sup>-1</sup>.

<sup>‡</sup>UN=unadjusted means or standard procedure; MM=Moving means analysis; SP=spatial analysis; MM analyses were not performed in 2000 for Brix, Pol, SUC, and TRS and a dash (-) was inserted in the off-diagonals cells for these traits.

<sup>§</sup>Pearson coefficients obtained after combining data from both fields considered in this study for the Year 2000.

**Table 5.** Correlation coefficients among traits measured from non-replicated Stage II trials before and after adjustments for field trends in the Canal Point sugarcane breeding program. Data were combined for the two fields analyzed. (Yr 2002-03)

Traits	STW <sup>†</sup>			Brix			Pol			SUC			TCH			TSH			TRS		
	UN <sup>‡</sup>	MM	SP	UN	MM	SP	UN	M M	SP	UN	MM	SP	UN	MM	SP	UN	M M	SP	UN	M M	SP
----	----- r <sup>§</sup> -----																				
STN	-0.34 **	-0.30 **	-0.36 **	0.06 ns	0.15 **	0.05 ns	0.06 ns	0.14 **	0.05 ns	0.05 ns	0.14 **	0.05 ns	0.15 **	0.55 **	0.58 **	0.34 **	0.50 **	0.51 **	0.06 ns	0.13 **	0.04 ns
STW	-	-	-	-0.19 **	-0.08 *	-0.04 ns	-0.22 **	-0.15 **	-0.07 *	-0.22 **	-0.15 **	-0.07 *	0.49 **	0.55 **	0.52 **	0.56 **	0.40 **	0.40 **	-0.24 **	-0.16 **	-0.08 **
Brix				-	-	-	0.96 **	0.94 **	0.94 **	0.96 **	0.94 **	0.94 **	-0.19 **	0.03 ns	0.02 ns	0.46 **	0.47 **	0.49 **	0.93 **	0.90 **	0.90 **
Pol							-	-	-	0.99 **	0.99 **	0.99 **	-0.23 **	-0.02 ns	-0.01 ns	0.48 **	0.48 **	0.51 **	0.99 **	0.99 **	0.99 **
SUC										-	-	-	-0.23 **	-0.02 ns	-0.02 ns	0.48 **	0.48 **	0.51 **	0.99 **	0.99 **	0.99 **
TCH													-	-	-	0.81 **	0.82 **	0.84 **	-0.28 **	-0.01 ns	-0.04 ns
TSH																-	-	-	0.48 **	0.47 **	0.51 **
TRS																			-	-	-

\*, \*\* Significant at  $P \leq 0.05$  or  $P \leq 0.01$  levels, respectively; ns=not significant at  $P \leq 0.05$ .

<sup>†</sup>STN=Stalk number; STW=stalk weight in kg; SUC=sucrose content in %; TCH=cane yield in Mg ha<sup>-1</sup>; TSH=sugar yield in Mg ha<sup>-1</sup>; TRS=theoretical recoverable sugar in kg Mg<sup>-1</sup> ha<sup>-1</sup>.

<sup>‡</sup>UN=unadjusted means or standard procedure; MM=Moving means analysis; SP=spatial analysis.

<sup>§</sup>Pearson coefficients obtained after combining data from both fields considered in this study for the Year 2002.

**Table 6.** Similarity indices calculated for different selection intensities on Stage II non-replicated sugarcane trials analyzed by a Moving Means and Autoregressive Spatial methods, both compared with a standard ANOVA procedure.

	Moving means								
	Common <sup>†</sup>			Different			IS <sub>C</sub> <sup>‡</sup>		
	10%	15%	20%	10%	15%	20%	10%	15%	20%
-----number of clones-----									
Yr 2000									
TCH <sup>§</sup>	39	63	81	30	40	54	0.57	0.61	0.60
TSH	34	53	78	35	50	57	0.49	0.51	0.58
Yr 2002									
TCH	20	27	35	63	81	100	0.24	0.25	0.26
TSH	37	57	76	46	51	59	0.45	0.53	0.56
TRS	31	47	67	52	61	68	0.37	0.44	0.50
	Spatial								
	Common			Different			IS <sub>C</sub>		
	10%	15%	20%	10%	15%	20%	10%	15%	20%
-----number of clones-----									
Yr 2000									
TCH	61	66	86	9	38	52	0.87	0.63	0.62
TSH	53	77	106	17	27	32	0.76	0.74	0.77
TRS	53	82	104	17	22	34	0.76	0.79	0.75
Yr 2002									
TCH	13	19	25	70	89	110	0.16	0.18	0.19
TSH	69	82	94	14	26	41	0.83	0.76	0.70
TRS	62	82	100	21	26	35	0.75	0.76	0.74

<sup>†</sup>Number of clones that were common or different between the Moving Means or autoregressive spatial analyses and the standard ANOVA.

<sup>‡</sup>IS<sub>C</sub>=Index of similarity between the Moving means or Autoregressive spatial analyses and the standard ANOVA.

<sup>§</sup>TCH=tons cane ha<sup>-1</sup>, TSH=tons sugar ha<sup>-1</sup>, TRS=sugar ton<sup>-1</sup>. TRS was not analyzed by Moving Means analysis in 2000.

consider.

## CONCLUSION

Spatial variation was present in 2000 and 2002 in the form of local trends with extraneous patterns in the fields used for selection of Stage II non-replicated sugarcane clones in the Canal Point breeding program. This field heterogeneity affected all the traits considered for selection, but quality traits were more stable. This spatial heterogeneity also altered the ranking of the clones (Spearman rank correlation coefficient  $r = 0.70$ ,  $P \leq 0.001$ ) and may result in disparity as high as 75% in the top 10-20% clones that would be selected for advancement. Substantial gains in efficiency were possible after accounting for the spatial dependence of the data on these trends. The autoregressive spatial method was statistically more efficient and consistent than the Moving Means analysis, which was somewhat hampered by the discarding of some plots prior to performing the analysis. Further research is considering collecting data on most of the discarded plots, comparing the effects on the predictions of the genotypic values of the clones, and evaluating the performance of SP-selected clones in Stages III and IV. The spatial method would be suited for selection in early-stage sugarcane trials with the potential to maximize genetic gains, as indicated by the improvement of the phenotypic correlations among the traits. An augmented or a modified augmented design or any spatial analysis should be used for selection in early stages of sugarcane breeding programs where there is no replication.

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