

POTENTIAL METHODS OF IMPROVING THE DETECTION OF PROMISING SUGARCANE CLONES IN EARLY SELECTION TRIALS

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Abstract

Field effects complicate the efficiency of making selections from early stage trials in the sugarcane selection programme at Mount Edgecombe. Since the plots are small and unreplicated, it is difficult to obtain accurate estimates of the yield parameters of individual clones. This is important if the best clones are to be advanced to the next selection stage.

Various methods for correcting data are suggested in the literature, but the technique that holds the most potential is spatial analysis. Using a 'nearest neighbour' technique, each clone is adjusted according to a measure of the general performance in the area containing the clone, relative to the rest of the field or fields. A variation of this method is to use data from regularly spaced control clones within the trial to adjust the plots. As the control clones are all genetically identical, the only differences between their plots should be environmental.

These methods are currently being evaluated for use in the second and third stages of selection. They also hold potential for use in later stages of the selection programme, but their main value lies in the adjustment of small, unreplicated plots.

Keywords: clone, spatial analysis, selection, sugarcane

Introduction

Accurate clonal evaluation and the ability to differentiate between clones are crucial to a successful plant breeding programme. In the early stages of the sugarcane selection programme at the South African Sugar Association Experiment Station (SASEX), limitations of seedcane and space result in the use of small, unreplicated plots. When a large number of clones are tested, growing conditions are often not uniform throughout the trial area, due to such factors as variation in soil fertility, previous land use, weeds and pests. These field effects hamper the reliable estimation of yield characteristics of individual clones. Estimation of, and adjustment for, fertility trends within a trial increase the precision of prediction of superior clones (Clarke, Baker and DePauw, 1994; Stroup, Baenziger and Muiltze, 1994).

Many statistical methods have been devised in order to minimise the effects of field variation. These are mainly limited to detecting broad trends in the trial data, but variation often occurs on a much smaller scale than can be accommodated by conventional methods. Because of the large number of clones in the early stages of the selection programme and the selection of relatively few of these clones for further testing, reliable selection of those clones with the highest potential is important. Therefore a more precise method to overcome field effects and

give more accurate values for each clone's performance is desirable.

Layout of Single Line trial

Each of the 300 000 new clones produced annually by the SASEX breeding programme enters the selection programme, where it undergoes rigorous screening in a series of field trials. The Single Line stage is selected from Single Stools, the first stage of the selection programme. In Single Stools, each new clone is represented by a single stool, which has been propagated from a genetically distinct new seedling. Because of the lack of seedcane, only a family evaluation of sucrose content can be conducted, and the clones are advanced to the next stage based on this family sucrose, as well as on a visual assessment.

Each plot in the Single Line trial consists of an unreplicated 8 m row, planted from each selected Single Stool. Yield and sucrose estimates are obtained from each clone, both in the plant crop and in the first ratoon. The plant crop is harvested to obtain the yield estimate, while the ratoon yield estimate is calculated from a sample only, as seedcane is required for planting of the next stage. Based on a combination of the results from the plant and ratoon crops, clones are chosen for advancement.

Typically, 4 000 Single Lines are evaluated annually at each of the seven selection sites. Therefore at each site the Single Lines are, of necessity, planted over a large area, usually incorporating several fields. Most fields used for trial purposes have fertility gradients to a greater or lesser degree. In these large trials, it is particularly important that an effective method of local yield control is applied to improve the estimation of the yield potential of the individual clones.

Current method of field adjustment

Various techniques, both physical and statistical, are used at SASEX to minimise the effects of field variation on the results of Single Line trials. Utilising the most uniform sites is a key, but no trial is totally free from fertility gradients. The clones are randomised within a trial in an endeavour to remove spatial effects but, because of the small plot size and lack of replication, the results are still subject to a high degree of variance. Further statistical methods are therefore necessary.

One such method is family evaluation. Family evaluation considers all the offspring of a particular family, regardless of location in the trial, and determines which families perform better than others. A weighting is then applied to clones from the better families. Family evaluation can be more accurate than individual evaluation in small, unreplicated plots, but it cannot

be relied upon as the only method of evaluation. A method of field adjustment is also required.

The current method of field adjustment is based on the principle that differences between the mean performances of clones in different fields, rows and banks in a trial are mainly due to fertility trends in the trial. The mean yields of the fields, banks and rows are compared to the overall mean of the trial, according to the ratio of the overall mean over the appropriate local mean. If all clones within the field, row or bank are adjusted by this ratio, it has the effect of changing the mean of that field, bank or row to the same as the overall mean. Trends that exist across the field, in the direction of the rows and banks, and between fields, are therefore removed. The field means are calculated using a large number of clones, and are therefore not much affected by individual values. A full adjustment towards the mean can therefore be used between fields. However, rows and bank means have fewer individuals, sometimes very few, and are therefore more affected by individual values. Some of the difference between rows or banks may therefore be genotypic differences. Row and bank adjustments are therefore reduced by using a coefficient, usually 0.75, giving a partial adjustment towards the overall mean and not removing all genotypic variation. Such coefficients are found in many trend-removal techniques (Stroup, Baenziger and Mulitze, 1994).

If the current method of adjustment, described above, is to be effective, rows and banks must be correctly arranged, in both direction and scale, in relation to any patterns of variation that are known to occur (Ainsley, Dyke and Jenkyn, 1995). However, these can seldom be predicted with confidence. Furthermore, patterns of variation often occur on a small scale, and are undetected by the row and bank groupings.

Method

Nearest neighbour

The Nearest Neighbour (NN) technique shows potential for providing a field adjustment that overcomes trends that the present method does not cater for (Cullis and Gleeson, 1991). The NN adjustment is a relatively simple, yet effective, method of accounting for variation caused by field conditions. It measures a smaller area of the trial and is therefore more sensitive to smaller trends. It can also accommodate trends in any direction, as it does not rely on values that were calculated across the trial in a particular direction.

The NN technique makes use of those clones that immediately surround the particular clone being evaluated. Various alternatives are suggested by the literature. The primary options involve calculating a fertility index, using a single neighbour in the same bank (Binns, 1987), utilising a second neighbour in the adjacent bank (Robinson, Kershaw and Ellis, 1988; Kempton, Seraphin and Sword, 1994), or using several neighbours on either side of the clone, but in the same bank (Townley-Smith and Hurd, 1973; Lawrence and Townley-Smith, 1975). The use of control plots as neighbours has also been evaluated (Mak, Harvey and Berdahl, 1978; Lin, Poushinsky and Jui, 1983; Cullis, Gleeson and Thomson, 1992). The fertility index is then subtracted from the clone's value, giving a value that

reflects how much greater or lower that clone's value differs is from its surroundings.

For use in Single Line trials, it was decided that a comparison with one or two neighbours was inadequate. Even a mean of two clones does not overcome the effect of each of those clones' own values. Because of the layout of the trial, the neighbouring clone in the same bank would also likely be a sibling of the clone under evaluation, thereby biasing the index value. Too large a number of neighbours is also undesirable as the size of the block would be increased, making the estimate less sensitive to local changes in field variation (Mak, Harvey and Berdahl, 1978). The decision was made to include the neighbour on each side in the same bank, the neighbours on the two adjacent banks, and the diagonals on the adjacent banks (Figure 1), giving a total of eight neighbours. An additional ring of neighbours was initially included, but showed no improvement over the original eight, and was discarded for computational simplicity.

This design also partly overcomes the effect of competition between the clone and its neighbours. If only the two neighbours in the same bank were used, a clone which yielded less because of competition with taller neighbours would be penalised even further. A clone benefiting from less competition would be incorrectly adjusted upwards. A moderating effect is introduced by including the neighbours from adjacent banks, as these clones are not interacting closely with the clone under consideration.

Including only the neighbours in the same bank, which may be related, also affects the ability of the technique to detect superior crosses. If the clone and its neighbours are superior because the cross is good, then the use of only the related neighbours causes the entire cross to be adjusted downwards. Neighbouring banks do not contain related individuals, and therefore are free of this bias.

Multiplication of the individual values by the ratio of the overall mean over the local mean, calculated from the neighbours, was selected in preference to the subtraction of the local mean. This is a similar adjustment to the current method, and is preferred as it gives a yield estimate expressed in the same magnitude as the raw data, rather than a value representing an increase or decrease over the surrounding area. Because the current method uses a partial adjustment towards the mean, various

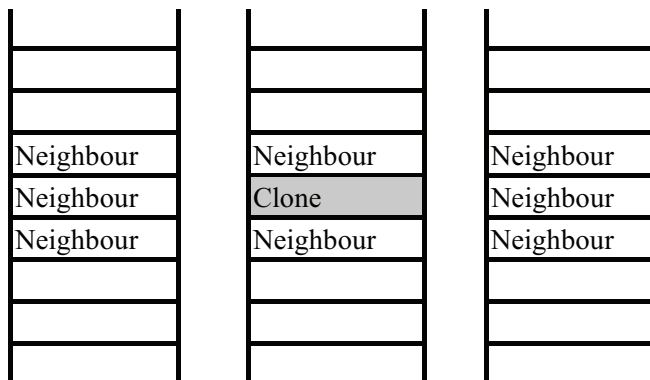


FIGURE 1. Demonstration of which neighbours are selected for calculation of local mean.

coefficients were also evaluated with the NN technique. This is an important consideration as NN methods are known to overadjust (Mak, Harvey and Berdahl, 1978; Besag and Kempton, 1986; Binns, 1987).

Border plots have fewer neighbours than those within the body of the trial. Plots on the edges of trials have always been a problem and there is no consensus on the best way to deal with them. In the developed NN method, missing neighbour plots were disregarded, and the calculations made with fewer individuals (Stroup, Baenziger and Mulitze, 1994; Ainsley, Dyke and Jenkyn, 1995).

Control Neighbour

In a trial that is to be analysed by NN, all the plots must be sampled. This is not a problem in the plant crop of Single Lines. In the ratoon crop, however, only the best third of the population is sampled. An alternative suggested by the literature is to make use of regularly spaced control cultivars to represent the field effects (Mak, Harvey and Berdahl, 1978; Besag and Kempton, 1986). Because the controls are all a single genotype, any differences should be due to environmental variation.

The controls are planted every eleventh line, throughout the field. Once again the controls in the same bank and those in the adjacent banks were used, to give a more accurate estimate of the fertility index, i.e. a total of six control neighbours (CN).

Various methods were used to compare the current adjustment, NN and CN, in order to determine which is the superior method of field adjustment. Evaluation of the spread of selections across the area of the trial gives a measure of the effectiveness of the different methods, as selections should be spread randomly over the trial area. This spread was estimated by comparing numbers of superior clones in each field. A comparison with the expected values (using a chi-squared technique) enabled the similarity of each technique to the generated data to be determined. Visual evaluation of the patterns of the selected clones was also a useful measure of the performance of each method, although difficult to quantify.

Simulation study

The values of the simulated clones were randomly generated to ensure that all locations in the simulated trial had equal probabilities of being assigned a clone of a particular value. Estimates of the field effects, trends and random error were made, based on actual trial data. The field adjustment was simply an adjustment of all the clones by a factor, either positive or negative. The random error was randomly generated and represented uncontrollable non-trend changes in the data. The trend within each of the four fields of the trial was generated by quadratic equations to ensure that there was a smooth trend effect. The trends applied to the fields were representative of some of the effects that can occur in real trials that the current method is unable to compensate for. These included:

- (1) a decrease in yield in the center of the banks throughout the field, lessening towards one side of the field;
- (2) a slant of yield potential from one side of the field to the

other, on a diagonal;

- (3) a yield decline on one side of the field and an increase on the other, reversing across the field;
- (4) an area of yield decline running diagonally across the field, representing such situations as compaction from an old road or water logging from a natural waterway.

The simulated data were evaluated with both the current method of adjustment and the new NN method. Correlations of the different data adjustment methods with generated data were calculated. The simulation study served as a guide as to whether it was worthwhile investigating the new methods. Based on these results, real data was evaluated with NN and CN.

Shakaskraal Single Lines

A Shakaskraal Single Line trial, WSL97, from which selections had already been advanced to the next stage, was re-evaluated using NN and CN. A comparison was made between the clones that were selected, particularly those chosen from the poorer areas, and those that were ranked the highest by NN and CN. This was done visually, by superimposing the selections on a map containing shadings that represented the yield values of the clones. The correlations between the different data sets were calculated, in order to determine how much each adjustment method amended the original data.

Pongola Single Lines

A Pongola Single Line trial, FSL98, was evaluated by both the current method of adjustment and NN, and field selections were carried out. The Pongola Single Lines had a different layout from the Single Lines on other sites. They did not have guard rows, and were therefore subject to edge effects. The control cultivars were not planted as every eleventh line throughout the field, but as blocks placed at regular intervals. The placement of controls meant that CN could not be evaluated.

Correlations between data sets were calculated. A comparison of adjustment methods with family evaluation was also performed. The mean performance of each cross was compared to the number of clones selected from each cross.

Gingindlovu Single Lines

Two current trials, GSL98 and USL98, were evaluated with the different methods of field adjustment. Results were compared by correlation and by visual representation on shaded maps. The number of selections from each field was also evaluated.

AA40 population

An evaluation trial is underway on an unselected population derived from a single cross. These clones have been advanced to a late selection stage without any selection having been applied. The yield potential of each clone has therefore been accurately estimated. These clones were planted in a mock Single Line trial to evaluate the efficiency of the selection programme (*Thomas, personal communication). They were also analysed by NN, in order to determine which evaluation method could detect the most superior clones.

Results and Discussion

Simulation study

The correlations between the generated data and the various adjustment methods are presented in Table 1. Although various coefficients were attempted, only the three-quarter and the seven-eighths adjustment results are shown for the NN. These were the most promising coefficients, as full adjustment shows the fairly common tendency to overadjust, and smaller coefficients have little effect on the field trends. The results for both NN and CN are very similar. The neighbour methods show an improvement over the standard row and bank adjustment method, although the difference is slight.

The percentage of selections from each field were calculated for both NN and CN (Table 2). The differences between the expected and observed values from each field are given in the last column. These give a quantifiable value to the efficiency with which each method distributes the selections uniformly throughout the trial. The seven-eighths NN calculation seems to be the most effective technique in this regard. The three-quarter NN evaluation performed equally as well as the Row-Bank technique. The CN method did not perform as well as the NN, as expected, but it still functioned better than the current adjustment method. The imbalance between fields of the raw data is obvious from the unequal numbers of selections made from each field.

The performance rating based on numbers selected from each field was reinforced by examining the patterns of selected clones within each field (results not shown). The areas of poorer yield were still visible when using the current method, but were harder to discern when using the neighbour methods.

Shakaskraal Single Lines

The Shakaskraal Single Line trial was subject to high variability across the eight fields. Many poorer areas were totally free from selections, suggesting that the current adjustment method was inadequate at compensating for field variation. The results were studied on a map of the field, on which the yield values of the clones were plotted and then shaded according to yield. Poor areas were easily detected, as were those clones in the poor areas that yielded better than the surrounding clones. When the NN and CN techniques were applied to the data, more of these clones were identified. This gave a visual appraisal of the performance of the different methods. As the genotypic potentials of the clones are not known, a more in-depth evaluation was difficult.

The correlations between the different data sets are presented in Table 3. Upon examination, the similarities were high. This was reinforced by visual comparison of the selections made by the techniques. Many of the clones selected by one method were also selected by the others. The main differences were evident in the high and low yielding areas of the trial. The Row-Bank adjustment tended to select high numbers from the high yielding areas, and low or no numbers from the poor areas. Both NN and CN selected fewer clones from the high yielding areas. These techniques also selected some clones from the poorer areas, although not as many as expected.

Pongola Single Lines

The Pongola Single Line trials are laid out in highly uniform fields with drip irrigation. The amount of improvement from any adjustment method is therefore expected to be small, compared with highly variable fields. This is reflected in the high

Table 1. Simulation study: correlations between analysis methods.

	Analysis method	Generated	Raw	Adjusted	NN ¾	NN ⅞
Excluding controls	Generated	1				
	Raw	0.64	1			
	Row-Bank	0.75	0.83	1		
	NN ¾	0.83	0.83	0.88	1	
	NN ⅞	0.83	0.76	0.85	0.99	1
Including controls	Generated	1				
	Raw	0.63	1			
	Row-Bank	0.75	0.83	1		
	CN ¾	0.82	0.83	0.88	1	
	CN ⅞	0.82	0.75	0.85	0.99	1

Table 2. Simulation study: percentage of selections derived from each field.

	Analysis method	Field 1	Field 2	Field 3	Field 4	χ^2
Excluding controls	Generated	25.33	23.00	25.00	26.67	
	Raw	2.33	4.00	29.00	64.67	91.36**
	Row-Bank	22.67	17.33	27.00	33.00	3.33*
	NN ¾	20.33	19.67	27.67	32.33	2.96*
	NN ⅞	26.33	22.33	28.00	23.33	0.84
Including controls	Generated	24.33	25.67	25.00	25.00	
	Raw	2.33	5.00	29.67	63.00	95.17**
	Row-Bank	23.33	16.67	25.33	34.67	6.94*
	CN ¾	24.00	19.33	27.33	29.33	2.54*
	CN ⅞	29.67	21.67	27.00	21.67	2.40*

* significant (50%), ** highly significant (99.9%)

correlations between the raw data and the adjusted values from the current technique and NN (Table 4). When investigated in the field, the results showed that the NN method did not compensate for the lack of guard rows, as was expected. Performance in the remainder of the trial was satisfactory. The NN technique adjusts effectively only for smooth changes in yield trends (Binns, 1987; Ainsley, Dyke and Jenkyn, 1995), while the outside row effect occurs in the space of a single row.

The comparison between family mean and number of clones selected from each cross produced interesting results. The correlation was 0.89 for NN and 0.67 for Row-Bank. Family performance is an important factor in evaluating clonal performance. Superior individuals within a poor family are possible, arising from fortuitous combinations of genes. Families that have high overall performance, however, contain greater numbers of superior clones, because of the accumulation of many beneficial genes. A high selection rate from these families is therefore desirable, preferably combined with individual selection in order to select only those clones within the outstanding cross that are superior.

The family mean is determined from clones distributed throughout the trial area, and is therefore less prone to environmental influences. Within each replication, however, selection is influenced by the field adjustment technique. Replications of superior crosses located in poor areas will have lower selection numbers if the field adjustment technique is unable to overcome field effects. The method that is best suited to overcoming environmental variation should therefore be better at detecting the superior crosses, and subsequently select higher numbers from these crosses. The number selected from each cross is more highly correlated with cross mean when using NN, suggesting that NN is better able to detect superior crosses, even if located in poor areas. At the same time, the ability to detect single clones in lower performance crosses will be improved by using NN, as detection of these clones depends entirely on the ability to overcome field effects.

Gingindlovu Single Lines

The Gingindlovu trials were evaluated for both correlation between data sets and percentage of selections from each field. Results from the long cycle trial GSL98 and from the short cycle trial USL98 are presented in Tables 5 and 6. Correlations between methods were lower than for Pongola, as is expected from fields with greater variability. The comparison between fields again showed that NN improved the spread of selections across the trial area. The CN method performed worse than the current adjustment method in both trials. Visual comparison of the patterns of selection for all methods in the GSL98 trial reinforced these conclusions (Figures 2,3,4 and 5). The trial was located on a slope, with the field at the bottom of the slope having the best conditions. In these figures, patches of higher and no selection can clearly be seen in the unadjusted and CN fields.

The CN technique did not perform well in these trials. In order to determine the reason for this, the yield values for the controls were examined. It was determined that there was a distinct difference in the yield of the controls compared with the rest of the field. The reason for this is unknown, although it may have been caused by a later planting date for the controls.

AA40 population

The AA40 trial had the potential to provide the best estimate of the performance of the various methods of adjustment. The trial was planted at Pongola as Single Lines, but the performance of the clones had been determined at Variety Trial level. Unfortunately, the CN method could not be evaluated due to the way in which trials are planted in Pongola, where the control cultivars are planted in blocks at regularly spaced intervals, rather than in lines across the field.

With the NN method there was a correlation of 0.33 with 'actual' values, while the current adjustment method was not correlated with the actual values. The number of clones in the top

Table 3. Shakaskraal Single Lines: correlations between analysis methods.

	Raw	Row-Bank	NN $\frac{3}{4}$	NN $\frac{7}{8}$	CN $\frac{3}{4}$	CN $\frac{7}{8}$
Raw	1					
Row-Bank	0.89	1				
NN $\frac{3}{4}$	0.90	0.91	1			
NN $\frac{7}{8}$	0.87	0.90	1	1		
CN $\frac{3}{4}$	0.94	0.87	0.89	0.87	1	
CN $\frac{7}{8}$	0.92	0.86	0.88	0.86	1	1

Table 4. Pongola Single Lines: correlations between analysis methods.

	Raw	Row-Bank	NN
Raw	1		
Row-Bank	0.89	1	
NN	0.91	0.89	1

Table 5. Gingindlovu Single Lines: correlations between analysis methods.

		Raw	Row-Bank	NN	CN
Long Cycle	Raw	1			
	Row-Bank	0.79	1		
	NN	0.78	0.85	1	
	CN	0.80	0.78	0.80	1
Short Cycle	Raw	1			
	Row-Bank	0.85	1		
	NN	0.83	0.88	1	
	CN	0.81	0.86	0.86	1

Table 6. Gingindlovu Single Lines: percentage of selections derived from each field.

	Analysis method	Field 1	Field 2	Field 3	Field 4	Field 5	χ^2
Long Cycle	Raw	7	9	22	25	137	278.29**
	Row-Bank	41	23	44	41	51	6.01*
	NN	52	30	45	38	35	3.57*
	CN	18	27	70	27	58	40.14**
	Expected	43	34	44	37	42	
Short cycle	Raw	78	98	24	- ^A	-	17.79**
	Row-Bank	68	95	37	-	-	3.92*
	NN	61	89	50	-	-	0.04
	CN	77	46	77	-	-	40.40**
	Expected	62	89	49	-	-	

* significant (50%), ** highly significant (99.9%), ^A only three fields in short cycle trial

ten percent that were selected by both methods was also determined: the current method predicted only one of the top clones, whereas NN detected five.

The AA40 trial was not well suited for either method, as the overall trial size was small. The last line of clones was also planted at the edge of the field, where yields were low. The lack of guard rows caused noticeable edge effects. The control cultivar performed much better than the unselected clones, and because it was planted in blocks, the fertility indices of clones adjacent to the blocks were severely biased. Despite this, the performance of NN was satisfactory. Its performance in normal

Plant Breeding selection trials should be even better.

Conclusion

In all the trials, the NN method performed better than the current adjustment technique. Results from the more variable sites, in particular, showed an improvement. The Pongola results, with inadequate adjustment for outside lines, showed the importance of avoiding edge effects. The AA40 results were particularly interesting, although they were not representative of a normal trial. This was the only trial where the yield potential

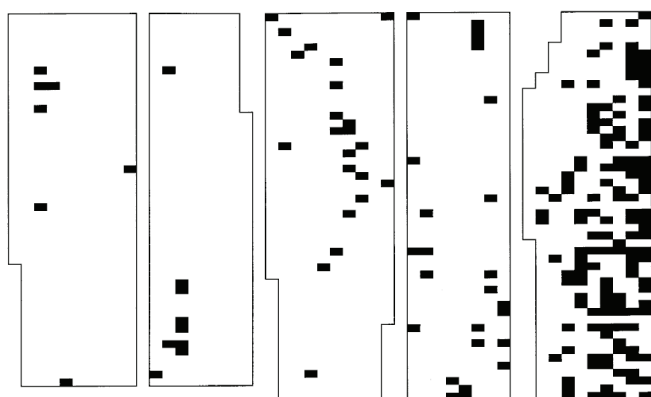


FIGURE 2. Location of selections in five fields at Gingindlovu using raw data.

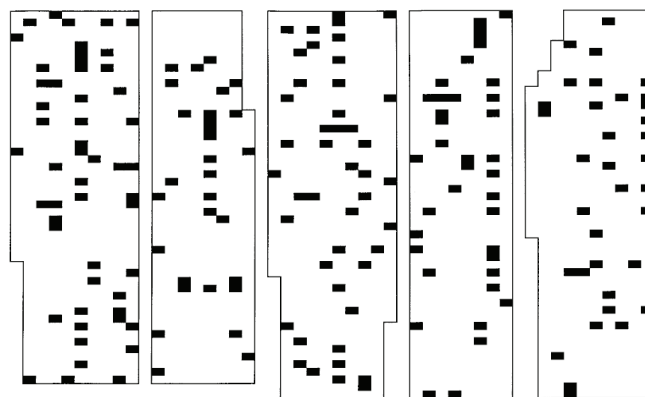


FIGURE 4. Location of selections in five fields at Gingindlovu using NN data.

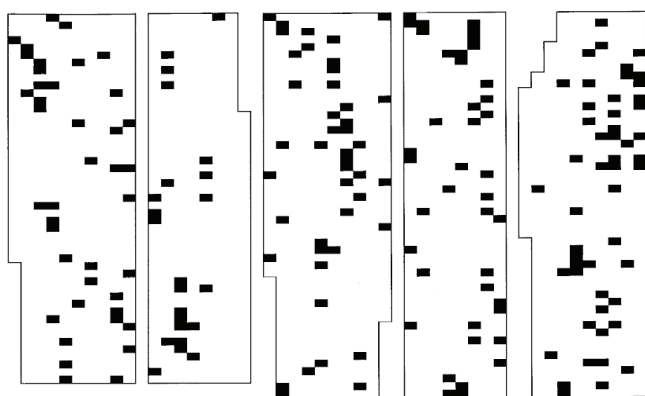


FIGURE 3. Location of selections in five fields at Gingindlovu using Row-Bank data.

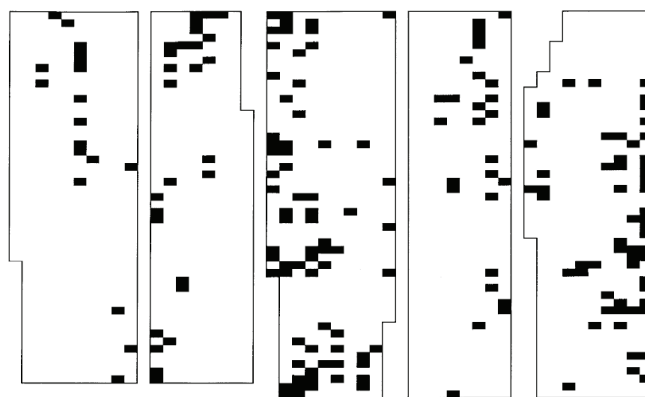


FIGURE 5. Location of selections in five fields at Gingindlovu using CN data.

of each clone had been determined with some accuracy. From these results, it is recommended that the NN technique be used for Single Line field adjustments.

The CN method did not perform as well as expected. Some results suggested that it would be ideal for adjusting data in the ratoon crop, where all of the neighbours are not sampled. Other results, however, indicated that use of the CN technique would provide less reliable results than the raw, unadjusted data. The CN method therefore is not recommended. Further study is required, to determine why this neighbour adjustment technique did not work as expected. The two Gingindlovu trials will be sampled in full in the ratoon crop, to re-evaluate all methods. Improvements in the CN method will also be considered.

The NN technique has another advantage over the current method, other than the improvement in accuracy. The current adjustment technique is fairly time-consuming and error-prone, requiring manual entering of information such as field, row and bank. Both neighbour techniques, on the other hand, are conducted using a MicroSoft Excel computer program, written in Visual Basic, and require only the map of the trial and the raw results.

The NN technique shows promise for improving the accuracy of predicting superior clones in the early selection stage of Single Lines. It also has potential for the next stage of selection, namely Observation trials. These trials consist of three-row plots and are replicated twice, making them slightly less susceptible to environmental influences than the Single Line trials. Trends are still evident in the data, however. Because NN has performed well in Single Lines, it will be evaluated for use in the Observation trials.

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