

BSES Limited



**FINAL REPORT – SRDC PROJECT BSS231
DEVELOPMENT AND APPLICATION OF SPATIAL ANALYSIS TO IMPROVE
PRECISION IN SELECTION TRIALS**

by

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SUMMARY

In the early stages of selection, a sugarcane breeder tests a large number of genotypes. As the amount of planting material for each genotype for testing is typically limited, selections are usually made on small, unreplicated, single-row plots. Unfortunately, such designs are prone to errors arising from spatial variability and interplot competition, which, unless accounted for, can seriously bias variety estimates and reduce genetic progress.

In this project, an approach to the simultaneous modelling of spatial variability and interplot competition is developed. This approach combines nearest-neighbour techniques to model spatial variability, together with the genotypic and phenotypic interference models to estimate interplot competition. The joint modelling and standard approaches are compared using 23 sugarcane data sets for cane yield. Agreement between the two approaches varied from approximately 38% to 90%. Hence, for some trials there would be large differences in the selections to be advanced to final assessment trials. Additionally, for two trials, the joint modelling approach was applied to cane yield and CCS data. The number of selections in common for sugar yield for the two approaches was compared. Approximately 43% and 75% of the clones were in common, indicating that appropriate modelling of interplot competition and spatial variability can have a very large effect on the varieties to be advanced to final assessment trials.

This project has resulted in an improved selection system, and this is likely to result in increased genetic gain through the advancement of superior varieties to later stages.

The project has formed the basis of a PhD thesis submitted to the University of Queensland.

1 BACKGROUND

In the early stages of selection, a sugarcane breeder tests a large number of genotypes. As quantity of seedcane and space are limited, this usually results in the use of small, single-row unreplicated plots. Such trials may be subject to bias from spatial variability and interplot competition. Numerous studies on the efficiency of using single-row plots for sugarcane breeding have been conducted (Skinner 1961; Jackson and McRae 2001). They concluded that, in trials which use single-row plots, interplot competition could seriously bias clonal estimates and may reduce genetic progress.

Kempton (1984a) described the classical approaches for dealing with fertility trends in unreplicated trials and these included using replicated plots distributed over the trial as checks. These checks are used as a benchmark to assess the yields of test plots. An alternative approach for adjustment is to use spatial analysis or nearest-neighbour methods in which a plot is adjusted for spatial variability by using information from immediate neighbours. Such an approach is being used to analyse over 500 cereal trials in Australia annually and has resulted in increased accuracy and precision in the estimates of variety effects (Gilmour *et al.* 1997). This approach needed to be extended to clonally propagated perennial crops such as sugarcane.

Competition effects are also acknowledged to be substantial in early stage selection trials. Because resource limitations generally preclude the use of multi-row plots to reduce interplot competition, statistical approaches have been suggested to adjust for competition in the analysis phase. Where both spatial variability and interplot competition are important, a joint modelling approach is needed. Durban (1998) and Durban *et al.* (2001) presented a method that combines smoothing splines to model trend with the phenotypic interference model for competition. However, their model was limited by having no spatially dependent process to model fertility trends or random variety effects.

In this project, I developed a broad approach that was based on both the genotypic and phenotypic interference models and allows for random variety effects and correlated error structure.

2 OBJECTIVES

This project aimed to improve precision in sugarcane selection trials through the application of spatial analysis techniques.

The specific objectives of the project were to:

- evaluate the effectiveness of spatial modelling in early stage sugarcane selection trials;
- quantify the effects of competition in early stage selection trials;
- develop spatial analysis to minimise the effect of inter-plot competition within sugarcane breeding trials;
- utilise BLUPs from spatial analysis to predict genetic effects;
- develop spatial analysis to accommodate ratooning in sugarcane and incomplete data sets in ratoon crops.

All of the objectives of the project have been achieved.

Objective 1: Evaluate the effectiveness of spatial modelling in early stage sugarcane selection trials.

Using the methods developed by Gilmour *et al.* (1997), 28 BSES/CSIRO trials were analysed to determine the extent of spatial variation in early generation trials. For both tonnes cane per hectare (TCH) and commercial cane sugar (CCS), extraneous variation and global trend were present in many trials. Given this, it was not surprising that there were large differences in the selections based on the top 10% for the spatial and current methods. Agreement between the two methods ranged from approximately 26 to 85%.

Objective 2: Quantify the effects of competition in early stage selection trials.

Approximately 79% of the trials analysed indicated the presence of large competitive effects for TCH. In only one trial were there significant competition effects for CCS.

Objective 3: Develop spatial analysis to minimise the effect of interplot competition within sugarcane breeding trials.

As both spatial variability and interplot competition were found to be important in early stage selection trials, an approach that simultaneously accounts for both sources of bias needed to be developed. Previous work by Durban (1998) and Durban *et al.* (2001) was limited by not having a spatially dependent process to model spatial variability and random variety effects to minimise the effects of selection bias. They argued that it may be difficult to estimate competition and spatial variability and developed an approach using modified profile likelihood. I developed a very broad approach that was based on both the genotypic and phenotypic interference models and allows for random variety effects and correlated error structure. This approach uses REML (restricted estimate maximum likelihood approach) for parameter estimation.

Objective 4: Utilise BLUPs from spatial analysis to predict genetic effects.

The joint modelling approach was applied to TCH data from the early selection outlined in objective 1. The percentage of clones in common in the top 10% was compared with the standard methods of analysis. Agreement between the two methods varied from approximately 38% to 90%. Additionally, for two trials the joint modelling approach was applied to TCH and CCS data. From this, an adjusted tonnes sugar per hectare (TSH) was obtained by applying the formula $(TCH \cdot CCS) / 100$ to the predicted values for each clone. This was then compared to the relative TSH, which is used by BSES/CSIRO plant breeders in their net merit grade (NMG) calculations. The percentage of clones in common for the top 10% was compared. Approximately 43% and 75% of the clones were in common which indicated that appropriate modelling of interplot competition and spatial variability can have a very large effect on the varieties to be advanced to the next stage of selection.

Objective 5: Develop spatial analysis to accommodate ratooning in sugarcane and incomplete data sets in ratoon crops

Spatial analysis can easily be extended to accommodate ratooning in sugarcane. The correlation between different harvests can be taken into account in the error structure. When only selected plots are harvested in later ratoons, the power of spatial analysis is limited by having to adjust a plot for missing neighbours.

3 RESEARCH METHODOLOGY, RESULTS AND DISCUSSION

The following summarises material to be presented in my PhD thesis. A copy of the thesis will be sent to SRDC on completion of the degree. A draft of the thesis is contained in Appendix 1 (electronic version only).

3.1 Preliminary analysis of stage 2 trials

3.1.1 Materials and methods

Gilmour *et al.* (1997) partition spatial variability within a field trial into three additive components:

1. *local trend*, which reflects small changes in fertility, soil moisture and light. If local trend is present within a field trial, then plots that are closer together will be more related than ones further apart. A correlation between pairs of plots, either in the row or column direction, that decays towards zero as the distance increases is characteristic of an autoregressive (AR) process. Gilmour *et al.* (1997) usually model local trend using a first-order separable autoregressive process in the row (AR1) and column (AR1) direction.
2. *large scale variation or global trend* is usually aligned with the rows and columns of a field trial. Global trend can be accommodated in the model by design factors such as linear row and/or linear column effects or by fitting polynomial or spline functions to the row and/or column co-ordinates.
3. *extraneous variation* arises from experimental procedures or management practices that have a recurrent pattern, such as direction of harvesting or method of planting. Such procedures may result in systematic and/or random row/column effects in the data. For example, serpentine harvesting up and down the rows causes plots in the 'up' direction to be consistently higher/lower than in the 'down' direction. Extraneous variation is often modelled by design factors such as a fixed 'harvesting effect'.

3.1.2 Statistical analyses

The modelling process presented in Gilmour *et al.* (1997) was applied to TCH and CCS data from 28 early selection sugarcane trials conducted by BSES in the 1999 and 2000

harvest seasons. The trials were analysed to identify and adjust for spatial variability and to detect the presence of interplot competition. The potential impact that this bias has on the choice of clones for the next stage of selection is discussed.

After completion of analyses on TCH and CCS, a calculated value for TSH was obtained by applying the formula $(TCH*CCS)/100$ to the predicted values for each clone. This spatially adjusted TSH was then compared to the relative TSH used by BSES/CSIRO plant breeders in their NMG calculations. As NMG is not statistically analysed by BSES/CSIRO plant breeders, this makes a comparison in precision between the spatial and current BSES/CSIRO method difficult. In my study, the percentage of clones in common in the top 10% from the two methods was compared.

3.1.3 Results

Global trend in the form of linear column effects was present in many of the trials and was particularly dominant for both TCH and CCS in 1999 (Table 1). This was taken into account in the modelling process by the linear regression of TCH and CCS on column number. Extraneous variation that arises from experimental procedures or management practices that have a recurrent pattern were not present very frequently. However, in one of the Herbert trials, extraneous variation that was due to serpentine harvesting caused plots in the 'up' direction to be consistently higher for TCH than in the 'down' direction.

The correlation between adjacent plots in the same row (column to column) or column (row to row) is presented in Table 2. An unusual pattern is evident from this table. The quote made by Fisher (1960) 'that patches in close proximity are commonly more alike, as judged by the yield of crops, than those which are further apart', suggests that both ρ_c and ρ_r should be positive. There were 11 out of 15 values of ρ_r that were negative and significantly different from zero in 1999 and 11 out of 13 values in 2000 for TCH. This correlation relates to the correlation between plots that share the longest side (10 m) and it is proposed that is due to competition effects. On the other hand, there was only one correlation that was negative and significantly different from zero for CCS.

Table 2 gives the percentage of clones in common in the top 10% based on spatial analysis and the current method used by BSES/CSIRO plant breeders. Agreement between the two methods ranged from approximately 26% to 85%.

Table 1 Summary of global and extraneous spatial effects fitted to 28 sugarcane trials from Queensland

l_r and l_c represent the linear regression of tonnes cane per hectare or commercial cane sugar on the row and column index; spl_r and spl_c represent the random spline effects associated with rows and columns; Fixrow, fixed row code effect with values of 0 and 1 being assigned; Ranrow, random row effects based on row indices; Locn, fixed location effect; Ranrow, random row effects based on row indices.

Trial code	Tonnes cane per hectare					Commercial cane sugar				
	l_c	l_r	spl_c	spl_r	Extra effects	l_c	l_r	spl_c	spl_r	Extra effects
<i>1999 harvest</i>										
TUL98-32	✓	✓	✓	✓		✓	✓	✓	✓	
TUL98-33	✓		✓			✓	✓			
MUL98-31	✓				Fixrow		✓		✓	
MUL98-32		✓								
MUL98-33	✓		✓				✓		✓	
BAB98-31	✓	✓			Ranrow	✓				
PIO98-3132	✓		✓		Fixrow	✓		✓		
PIO98-3334	✓	✓			$l_r * l_c$	✓				
MAR98-3132	✓				Ranrow					Ranrow
RAC98-3132	✓	✓	✓	✓		✓	✓	✓	✓	
RAC98-35	✓				Ranrow	✓	✓		✓	
BIN98-31	✓		✓							
MQN97-39	✓					✓				
MQN98-31					Ranrow					
MQN98-32	✓					✓				
<i>2000 harvest</i>										
VIC99-3132	✓	✓				✓	✓		✓	
VIC99-33										Ranrow
VIC99-34						✓				
VIC99-3639					Locn, Fixrow*Locn					Locn
PIO99-3132	✓		✓							
PIO99-3334					Ranrow			✓		Ranrow
RAC99-31							✓			Ranrow
RAC99-3234					Locn					
RAC99-33					Ranrow					Ranrow
BIN99-31					Ranrow					Ranrow
MQN99-31					Ranrow					Ranrow
MQN99-32										
MQN99-39							✓			

Table 2 Results of spatial analyses applied to 28 sugarcane trials from Queensland

ρ_c and ρ_r are the correlation between adjacent plots in the same row and same column respectively

Selection program	Trial code	Tonnes cane per hectare		Commercial cane sugar		% of clones in common ^A
		ρ_r	ρ_c	ρ_r	ρ_c	
<i>1999 harvest</i>						
Northern	TUL98-32	-0.12*	0.14*	0.36**	-0.21*	28.6
	TUL98-33	0.05	0.31**	-0.10	0.37*	53.3
	MUL98-31	-0.27**	-0.04	0.15*	0.14*	57.5
	MUL98-32	-0.26*	0	0.03	-0.12	64.5
	MUL98-33	-0.05	-0.04	0.07	0.06	52.7
	BAB98-31	0.08	0.11*	0.25**	0.30**	66.2
Burdekin	PIO98-3132	-0.33**	-0.02	-0.01	0.17*	57.3
	PIO98-3334	0.64**	0.63**	0.53**	0.57**	64.0
Central	MAR98-3132	-0.11**	0.10**	0.15**	0.05	67.0
	RAC98-3132	-0.24**	-0.01	-0.04	0.15*	59.8
	RAC98-35	-0.04	0.07	0.11	-0.08	73.3
Southern	BIN98-31	-0.34**	0	0.20**	0.02	78.0
	MQN97-39	-0.54**	-0.06	0.12	0	56.3
	MQN98-31	-0.50**	-0.11*	0.33**	0.03	69.2
	MQN98-32	-0.47**	-0.04	0.05	0.07	61.2
<i>2000 harvest</i>						
Herbert	VIC99-3132	0.06	0.21**	0.32**	0.15*	26.3
	VIC99-33	-0.38**	0.23*	0.42**	0.05	60.0
	VIC99-34	-0.07	0.26**	0.12	-0.09	84.6
	VIC99-3639	-0.08*	0.22**	0.04	0.05	83.3
Burdekin	PIO99-3132	-0.22**	0.12	0.37	-0.19	44.8
	PIO99-3334	-0.34**	-0.03	-0.15	0.11	52.6
Central	RAC99-31	-0.18**	0.17**	0.04	0.08	58.7
	RAC99-3234	-0.18**	0.15**	0.17*	0.16*	68.2
	RAC99-33	-0.30**	0.21**	0.17*	0.08	57.8
Southern	BIN99-31	-0.48**	0.07	0.21**	0.11	65.3
	MQN99-31	-0.40**	-0.09	-0.04	0.15	76.5
	MQN99-32	-0.57**	0.01	0.26	0.34*	55.0
	MQN99-39	-0.30**	-0.16*	0.22**	0.17*	84.2

* $P < 0.05$; ** $P < 0.01$

^A The percentage of clones in common in the top 10% based on spatial analysis and the current method used by BSES plant breeders

3.1.4 Discussion

The spatial analysis techniques applied to the 28 sugarcane trials showed the presence of two dominant factors that would have been difficult to identify by the classical method of analysis. Firstly, there were large competitive effects for TCH, particularly in southern Queensland. The competition present in southern Queensland was consistently greater than in any other breeding program. Although the trials in this breeding program are mostly irrigated, it was very much supplementary and was used strategically to avoid the production of huge unmanageable crops. Compared with the other breeding programs, southern Queensland is the driest. The stressed environment could certainly explain the larger competition effects.

For CCS, extraneous and global trends were present in many trials but there was only one trial which had significant competition effects. These results are consistent with the findings of Jackson and McRae (2001) who found less competition for CCS than for cane yield.

Given the presence of significant extraneous and global trends in many trials for TCH and CCS, it was not surprising that there were large differences in the selections based on the top 10% for the spatial and current method. Agreement between the two methods ranged from approximately 26 to 85%. In trials TUL98-32 and VIC99-3132, agreement was particularly low and this was due to the presence of highly significant global trend effects for TCH and CCS ($P < 0.01$). In contrast to this were southern Queensland trials, where agreement between the methods was often good. These trials had high competitive effects and few global or extraneous trends. It is important to remember that, although competition was identified, it was not taken into account in the modelling process. If accounted for, then it is expected that agreement between the two methods in southern Queensland would be lower.

3.2 Joint modelling of spatial variability and interplot competition

3.2.1 Methodology

Besag and Kempton (1986) presented two approaches for estimating interplot competition in field trials. The first model (phenotypic interference) is a simultaneous autoregressive approach, with competition assumed to be directly related to yields of neighbouring plots. This has been shown to be applicable to root crops such as sugar beet (Kempton 1982; Durban *et al.* 2001), potatoes (Connolly *et al.* 1993) and swedes (Bradshaw 1989). In the second model (genotypic interference), competition effects are associated with varietal characteristics such as height, tillering ability, date to maturity or canopy size (Kempton 1984b; Talbot *et al.* 1995). In the latter, the cause of interference is associated with the average genotypic response of nearest-neighbouring genotypes rather than the phenotypic response.

There are many desirable features which are considered to be essential for modelling competition and spatial variability in sugarcane data. Firstly, the model must allow for the presence of competition at a phenotypic or genotypic level. Following on from Gilmour *et al.* (1997), the joint model must be able to accommodate global trend and extraneous

variation by including additional design factors based on row and column co-ordinates. The joint model should also be able to model the spatial dependence in one or both directions before or after accounting for competition. Lastly, the model may be a conditional or simultaneous approach. Full details of the joint modelling approach can be found in Chapter 5 of Appendix 1.

The joint modelling approach was applied to TCH data from section 3.1.2. For many of the trials in North Queensland, only selected plots were harvested. This resulted in a large number of missing values and, hence, these trials were omitted from the joint modelling analyses. The modelling procedure is a sequential approach and commenced by modelling the local trend and then revising this model using diagnostic tools. Up to 15 models per data set were examined. For all datasets BLUP (best linear unbiased predictor) estimates for TCH from the joint modelling and classical approaches (i.e. analysed as an augmented, randomised complete block or incomplete block designs) were obtained. The percentage of clones in common in the top 10% from the two methods was compared.

Additionally, for two of the datasets, MQN97-39 and RAC98-3132, the series of models applied to the data are described in detail in Chapter 10 of Appendix 1 and the results are summarised in this report. For MQN97-39 and RAC98-3132, analyses were undertaken on TCH and CCS. After completion of the analyses, a calculated value for TSH was obtained by applying the formula $(TCH \cdot CCS) / 100$ to the predicted values for each clone. This spatially adjusted TSH was then compared with the relative TSH used by BSES plant breeders in their NMG calculations. The percentage of clones in common in the top 10% from the two methods was compared.

Analyses were performed using ASReml (Gilmour *et al.* 2001), S-PLUS (Mathsoft 1999), and Sann (Butler *et al.* 2000).

3.2.2 Results and discussion

To determine the effect of simultaneously adjusting for spatial variability and interplot competition the percentages of superior varieties in common in the top 10% for the joint model and classical approaches were compared (Table 3). Agreement between the two approaches varied from approximately 38% to 90%. Hence, for some trials there would be large differences in the selections to be advanced to final assessment trials.

Table 3 Percentage of clones in common in the top 10% based on joint modelling of spatial variability and interplot competition and the classical approach for TCH from 23 sugarcane datasets.

Trial code	% of clones in common^A
<i>1999 harvest</i>	
MUL98-33	89.3
PIO98-3132	74.7
PIO98-3334	44.0
MAR98-3132	75.5
RAC98-3132	67.9
RAC98-35	75.6
BIN98-31	79.8
MQN97-39	68.8
MQN98-31	60.8
MQN98-32	38.1
<i>2000 harvest</i>	
VIC99-3132	84.2
VIC99-33	60.0
VIC99-34	84.6
VIC99-3639	89.6
PIO99-3132	60.5
PIO99-3334	52.6
RAC99-31	80.0
RAC99-3234	85.9
RAC99-33	76.6
BIN99-31	71.0
MQN99-31	83.9
MQN99-32	75.0
MQN99-39	73.7

^A The percentage of clones in common in the top 10% based on spatial analysis and the current method used by BSES plant breeders

Closer examination of RAC98-3132 and MQN97-39 were undertaken and for these trials the BLUPs for TSH from the joint model and classical approaches were plotted against each other and the correlations given in Figures 1 and 2. On the figures, the cut-offs for the top 10% for the classical and joint modelling approaches are indicated by the dotted lines and it is the varieties in the top left and bottom right quadrants that are of most interest. The varieties in the top left-hand quadrant would not have been selected by the classical approach but not the joint modelling approach, and vice versa for varieties in the bottom right-hand quadrant.

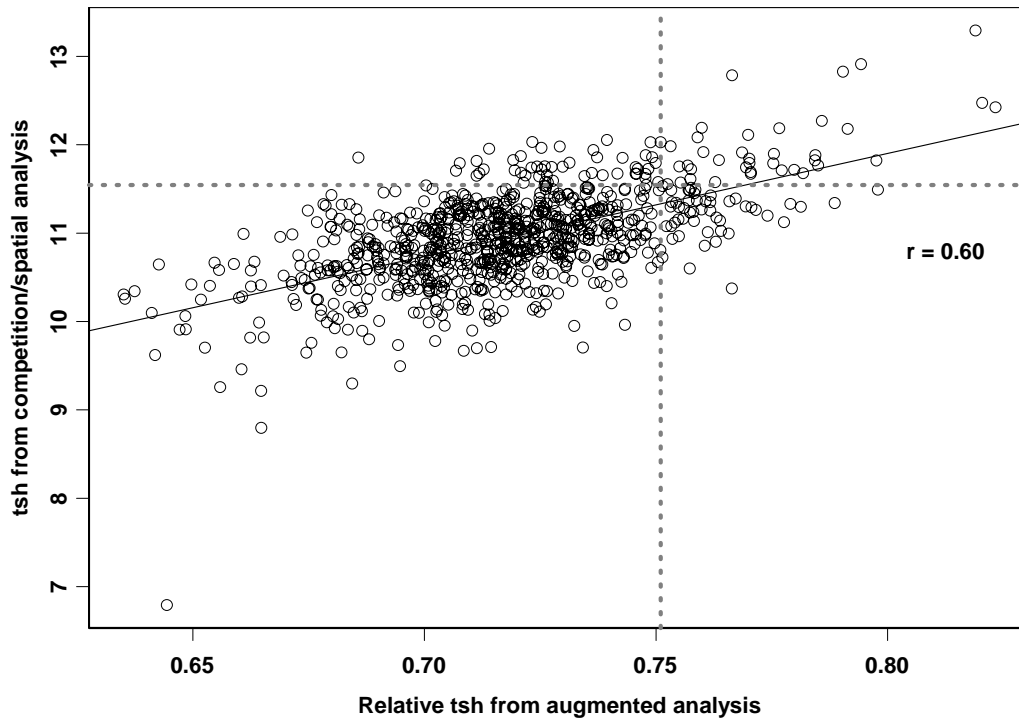


Figure 1 BLUPs from an augmented design versus BLUPs from joint modelling of competition and spatial variability for tonnes sugar per hectare from RAC98-3132

The cut-offs for the top 10% for the augmented and joint modelling approaches are indicated by the dotted line.

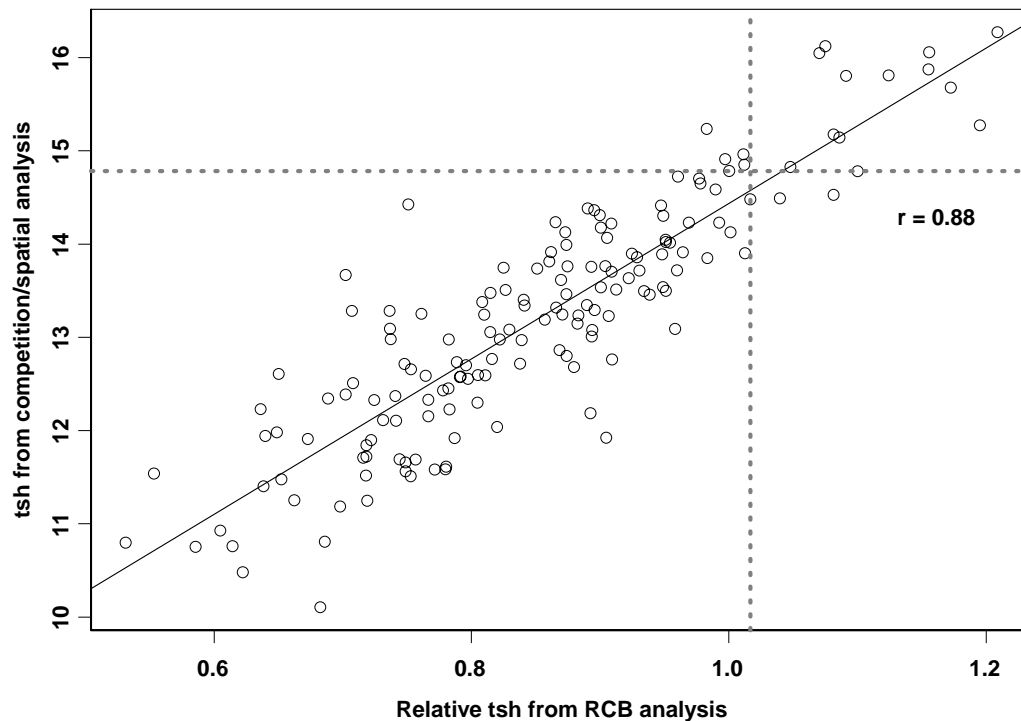


Figure 2 BLUPs from randomised complete block design versus BLUPs from joint modelling of competition and spatial variability for tonnes sugar per hectare from MQN97-39

The cut-offs for the top 10% for the randomised complete block and joint modelling approaches are indicated by the dotted line.

The two trials resulted in quite different correlations; 0.60 and 0.88 for RAC98-3132 and MQN97-39, respectively. Although the correlation for MQN97-39 was quite high, only 75% of the elite clones in the top 10% for TSH were in common. This 25% difference in clones to be planted in later generation trials may include selections which become far superior commercial cultivars. For RAC98-3132 only 43% of the elite clones were in common.

It was proposed early in the project that once both spatial variability and interplot competition were taken into account in the modelling process, then agreement between the joint model and classical approaches for southern Queensland trials should be poor. This was found not necessarily to be the case and, in fact, for the eight trials analysed only two were poorer. As the project developed it became apparent that large competitive effects could not just be associated with significant negative values of ρ_r as the covariance structure is complex. If trend is present in this direction, then this would give rise to positive values and competition would give rise to negative values with the end result that ρ_r could be approximately zero. The only method to determine if competition is present to apply the joint model and formally test if this is better than the simple model.

4 OUTPUTS

The major output from this project is a more efficient selection system for BSES plant breeders. Breeders now have an improved selection method for clones to be advanced from stage 2 to stage 3; this has been implemented within the BSES/CSIRO plant improvement program. Other outputs from the project are scientific papers and a PhD thesis.

5 OUTCOMES

As this project has resulted in an improved selection system, this is likely to result in increased genetic gain through the advancement of superior varieties to later stages.

6 FUTURE NEEDS AND RECOMMENDATIONS

To determine if the joint model enhances genetic gain, the select varieties from the joint model and standard approaches need to be planted into comparative trials. Five trials were planted in 2003 and the plant crop harvest results will be available in 2004.

When this project commenced, most selection programs were using an augmented design for stage 2 trials. Most sugarcane breeders now use partially replicated designs in which a subset, say 30%, of the test lines is replicated and these are arranged in a resolvable spatial design. The remaining unreplicated test lines are allocated at random to the remaining plots. Much work is currently being undertaken in cereals in Australia in optimising these designs. When the results become available these enhanced designs will be phased into use in stage 2 trials for sugarcane.

7 PUBLICATIONS ARISING FROM THE PROJECT

Stringer JK & Cullis BR. 2001. Application of spatial analysis techniques to adjust for fertility trends and identify interplot competition in early stage sugarcane selection trials. *Proceedings of the International Society of Sugar Cane Technologists* **24(2)**, 517-519. (Appendix 2).

Stringer JK & Cullis BR. 2002a. Application of spatial analysis techniques to adjust for fertility trends and identify interplot competition in early stage sugarcane selection trials. *Australian Journal of Agricultural Research* **53**, 911-918. (Appendix 3).

Stringer JK & Cullis BR. 2002b. Joint modelling of spatial variability and interplot competition. In: *12th Australian Plant Breeding Conference* (ed. JA McComb), pp. 614-619. Australian Plant Breeding Association Inc, Perth. (Appendix 4).

Papers on the joint modelling of spatial variability and inter-plot competition are planned for a statistics journal (theoretical aspects) and for a plant-breeding journal (applied aspects). These are based mainly on Chapter 5 and 6 in the PhD thesis.

8 ACKNOWLEDGEMENTS

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APPENDIX 1 – DRAFT PHD THESIS

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