BUREAU OF SUGAR EXPERIMENT STATIONS

QUEENSLAND, AUSTRALIA

FINAL REPORT
SRDC PROJECT BS27S
INHERITANCE OF RESISTANCE
TO PACHYMETRA ROOT ROT

by

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SD94002

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1. SUMMARY

Genetical resistance to root pathogens is a sensible strategy for disease control in a perennial crop such as sugarcane. Pachymetra root rot is a serious disease in Queensland and can cause losses of 30-40% in susceptible varieties. An understanding of the mode of inheritance of resistance is important in developing breeding strategies.

This study estimated narrow (h^2) and broad sense (g^2) heritabilities and the efficiency of selection for resistance to Pachymetra root rot. Estimates of h^2 ranged from 0.218 to 0.635 from two different types of analyses. The true value of h^2 probably lies in the upper range of these estimates, i.e. approximately 0.50. The estimates of g^2 were all high and ranged from 0.870 to 0.926. From these results it was concluded that seedling populations from parental clones selected for high Pachymetra root rot resistance will have improved resistance. Currently, only crosses which have a mid-parent resistance rating for this disease of less than 6.5, on a scale where 0 is immune and 9 is highly susceptible, are made for northern Queensland. In this study, restricting crosses to mid-parent ratings of less than 6.5 gave only 2% fewer highly susceptible progeny than did unselected crosses. Restricting the crosses to mid-parent ratings of less than 6.0 gave 11% fewer highly susceptible progeny than did unselected crosses. These results suggest that the current restriction of crosses be reduced to those with mid-parent ratings less than 6.0. This will improve the efficiency of the breeding program significantly.

Large root systems have been shown to improve the persistence of perennial pastures and to be correlated with resistance to displacement of plants from the soil. In this study, root number was found to be highly heritable, which suggests that breeding for larger root systems would be successful. This may have broader implications for improvement of ratoon performance and prevention of stool tipping.

Assessment of the design of glasshouse experiments for rating varieties for resistance to Pachymetra root rot was possible from the results of the project. Variation between multiple pots of a clone within a replicate was important. There was also a significant trials x clones interaction. The results suggested that clones be tested in three experiments to ensure accurate ratings.

The progeny and parent clones from this project have been maintained for possible use in gene mapping studies.

2. BACKGROUND

The yield of sugarcane in all areas of Queensland has tended to reach a plateau over the last 15-20 years, even though genetically superior varieties were released.

Methyl bromide fumigation experiments conducted in the 1988/89 season have shown that increases in yield of 30-40% can be obtained in all regions of Queensland. These trials confirmed that sugarcane growing on land which has grown cane for many years is yielding well below its potential, and that the limiting factors are biological and soil borne.

During the 1980s, the phenomenon known as stool tipping caused serious processing

problems in all northern sugar mills. Stool tipping in some cases also reduces the yield of ratoons by leaving gaps in the fields, and in severe cases can lead to premature ploughout of fields. Stool tipping is a complex problem but inadequate root anchorage is a major contributing factor.

Research into poor root syndrome identified the root rot fungus *Pachymetra chaunorhiza* (Croft and Magarey, 1989) and a number of pathogenic *Pythium* species (Croft and Magarey, 1984) in Queensland soils. These fungi are present in many fields affected by yield decline and stool tipping and may be contributing to these problems. Nematodes are known to cause severe root damage in some areas of Queensland (Bull, 1981) and also may be involved in yield decline and stool tipping. Pachymetra root rot is a severe pathogen and can cause losses of 30-40% in susceptible varieties (Magarey, 1993).

Soil fumigation and solarisation give effective control of Pachymetra root rot but are uneconomic for broad area application (Croft *et al*., 1984; Reghenzani, 1987). None of the fungicides tested so far gives effective control of Pachymetra root rot at non-phytotoxic rates (Croft *et al*., 1984).

A glasshouse technique for screening varieties for resistance to Pachymetra root rot has been developed (Croft, 1989) and commercial and parental clones resistant to the disease have been identified. The glasshouse screening technique is highly correlated with field reaction of varieties (Croft, 1989; Magarey, 1993).

No previous studies of the inheritance of resistance to Pachymetra root rot in sugarcane have been conducted.

3. PROJECT OBJECTIVES

- ∃ Determine the mode of inheritance of Pachymetra root rot resistance.
- ∃ Develop a strategy for breeding for Pachymetra root rot resistance.

4. INTRODUCTION

Pachymetra root rot is a major disease of sugarcane in Australia and is an important component of yield decline of sugarcane. The fungus *Pachymetra chaunorhiza* Croft and Dick attacks the primary roots of the sugarcane plant and yield losses of up to 40% have been recorded in susceptible varieties. Pachymetra root rot occurs in all sugarcane regions of Queensland but has not been recorded outside of Australia. During the late 1970s, when a susceptible variety was widely grown in the Babinda mill area, losses from the disease were estimated at greater than $5 M annually in that mill area alone.

Clonal resistance is an environmentally acceptable and economically viable means of controlling Pachymetra root rot, and has been adopted by BSES as the primary control strategy. An understanding of the inheritance of Pachymetra root rot resistance is important to allow the development of logical breeding strategies for this disease. Resistant varieties

are now widely grown in areas affected by the root rot.

5. MATERIALS AND METHODS

5.1 Crosses

Bi-parental crosses for this project were made at the Meringa Sugar Experiment Station during the 1989 cross-pollination season. Pollen contamination of these crosses was minimised. The object was to produce a progeny set conforming to a Design I mating (Comstock and Robinson, 1948). Pachymetra root rot resistance ratings were not considered when selecting parental clones for the crosses. Availability of panicles dictated the clones used, and the combinations in which they were mated. The Design I consisted of seven male-fertile clones (62C476, 74C29, CP57-526, CP57-603, 66N2008, 76N1772, 59S55) each mated to a set of different female (= female fertile, limited male fertility) clones. The female sets mated to these males were of five, five, six, six, six, six and six clones, respectively. The Design I was unbalanced. Twelve seedlings per cross were used in each trial.

5.2 Inoculum

Pachymetra chaunorhiza was isolated from diseased sugarcane roots by techniques described previously (Croft and Magarey, 1984). Inoculum was grown for 3-4 weeks on cornmeal agar in aluminium trays fitted with clear plastic autoclavable lids, and enclosed within clear autoclavable plastic bags. Inoculum was prepared by blending fungal cultures in water for 30 s in a blender (Instablend, GEC, USA). Oospore concentration was determined using a nematode-counting chamber (Hawksley and Sons, England).

5.3 Pot trial procedure

Single-eye cuttings were germinated in 7.5 cm diameter plastic pots containing a 1 sand: 1 peat mix. Uniform plants were transferred to terracotta pots containing 1.4 kg of inoculated potting mix, after 2 to 3 weeks. Inoculum was added to the potting mix to give a concentration of 3×10^4 oospores/kg in Experiment 1, and 1×10^5 in Experiments 2 to 5. The pots were placed into two air-conditioned, temperature-controlled benches, which were an advanced design of a concept developed earlier by Reghenzani (1984). The pots rested in terracotta saucers which were filled with water three times daily by a drip irrigation system.

Each bench was 1 m high x 1.35 m wide x 14 m long, and was constructed from 75 mm steel-polystyrene-steel cold room panel. The benches were covered with 12 mm plywood with holes cut to exactly fit the terra cotta pots. The union between ply and pot was sealed with a rubber sewage pipe seal. Temperature was controlled by a ducted air-conditioner. The desired temperature of the pots' contents was $28 \pm 2^{o}C$. Pot and ambient temperatures were checked with copper-constantan thermocouples wired to a Kaye Digistrip II data logger (Leeds and Northrup, PA).

The plants were grown for 6-8 weeks in a greenhouse, after which the potting mixture was washed from the roots. Each plant was scored for the number of primary shoot roots > 25 mm and the number of these roots infected with *Pachymetra*.

5.4 Experimental design

Each trial contained four replicates of 197 pots. Pots in each replicate were occupied by the following material:

10 standards x 3 pots	=	30
47 parental clones	=	47
3 progeny from each of 40 crosses	=	120

Most of the standard clones have been used in this role since *Pachymetra* research began, and their ratings were based on many trials. The clones, and their Pachymetra root rot resistance ratings at the commencement of this research, were - Q114 (1.0), Q78 (1.3), 58N829 (2.3), Q120 (3.4), Q117 (4.0), Q113 (4.5), Q96 (5.5), Q132 (6.0), Q90 (8.3), and Q83 (9.0). Ratings are on the ISSCT scale 0 = immune and 9 = highly susceptible (Hutchinson, 1967). Five such trials were repeated temporally during the period from April 1990 to October 1991 for exposure to differing environmental conditions.

5.5 Statistical analyses

Data were available from three genetic samples included in each trial (standard clones, parental clones, and bi-parental progenies) and for two directly scored characters - number of rotted roots (NRR) and number of roots (NR). Percent rotted roots (PRR) was computed as PRR = 100 x NRR/NR. All analyses were performed with MSTAT C Version 1.41 (Michigan State University, MI). Results from analyses for NR and PRR only are presented and discussed in this report. Analyses are discussed under headings of genetic samples.

5.5.1 Standard clones

Data were available from three pots of each standard clone in each replicate. Reliability of the individual and combined trials was assessed by regressing PRR data for the standard clones, as the dependent variable, against their long-term Pachymetra root rot resistance ratings, as the independent variable.

Estimates of total genetic variance, and phenotypic variance on a replicated pot basis, were derived from the expectation of mean squares for a clonal trial (Burton and de Vane, 1953). Standard errors were derived for these using a generalisation of the approach of Anderson and Bancroft (1952). Broad sense heritability, or degree of genetic determination, on a replicated pot basis, was calculated from the variance estimates as:

$$g^2 = \hat{\sigma}^2_G / (\hat{\sigma}^2_G + \hat{\sigma}^2_E / r).$$

5.5.2 Parental clones

Data were available from a single pot of each parental clone in each replicate. Estimates of genetic and phenotypic variance and broad sense heritability on a replicated pot basis were performed as for the standard clones.

5.5.3 Design I

Data for single trials were analysed by AOV that had random, main effects of replicates (df = 3), and crosses (df = 39). The latter was partitioned into effects due to males (df = 6), and females/males (df = 33). These analyses were conducted on an individual plant basis. There were no complications about variance ratio tests. Expectations of mean squares, and translation to components of genetic variance, were as detailed by Hallauer and Miranda (1981) for a Design I mating. The number of females within males (f_i) was unequal. A weighted value of f_o appearing in the coefficient for $\hat{\sigma}^2_m$ in the expectation of mean squares was computed (Snedecor and Cochran, 1967**,** p 290). Narrow (h^2) and broad (g^2) sense heritabilities were computed on a replicated pot basis, using $\hat{\sigma}^2_P = \hat{\sigma}^2_A + \hat{\sigma}^2_D + \hat{\sigma}^2_E /r$.

The expectations of mean squares for the combined analyses of variance of the Design I over trials follow the example provided by Hallauer and Miranda (1981, p 79), but sets/environments did not exist. All main effects were regarded as random. The analyses again were conducted on an individual plant basis. F-tests and estimation of components of variance could be made directly for all sources of variation except the main effects of trials and males. Satterthwaite's approximation (Snedecor and Cochran, 1967, p 368-369) was used for these. Estimates of narrow (h^2) and broad (g^2) sense heritabilities were computed on individual plant (h^2_{sp} and g^2_{sp}), replicated pot over trials (h^2 and g^2), and unbiased by genotype x environment (h^2_{ub} and g^2_{ub}) bases. For example,

$$h^2_{sp} = \hat{\sigma}^2_A /[\hat{\sigma}^2_w + \hat{\sigma}^2_E + \hat{\sigma}^2_{f/m} + \hat{\sigma}^2_m]$$

$$h^2 = \hat{\sigma}^2_A /[\hat{\sigma}^2_G + \hat{\sigma}^2_E /tr]$$

$$h^2_{ub} = \hat{\sigma}^2_A /[\hat{\sigma}^2_E /tr + 4\hat{\sigma}^2_{Tf/m} /tf_o + 4\hat{\sigma}^2_{f/m} /t + 4\hat{\sigma}^2_{Tm} /t + \hat{\sigma}^2_m]$$

Variance components used in these equations are defined as shown below:

The variance due to crosses ($\hat{\sigma}^2_C$) in the Design I was partitioned as follows:

$\hat{\sigma}^2_{f/m}$ = variance due to females within males,

σ^2_m = variance due to males.

Variance due to the trials x crosses interaction (σ^2_{TC}) was partitioned as follows:

$\sigma^2_{Tf/m}$ = variance due to trials x females/males,

σ^2_{Tm} = variance due to trials x males.

Additionally,

σ^2_A = additive genetic variance,

σ^2_G = total genetic variance,

σ^2_E = error variance,

σ^2_w = within family variance, and

f_o, t, r = weighted number of females within males, number of trials, and number of replicates, respectively.

5.5.4 Progeny mid-parent regression

An additional estimate of narrow sense heritability (h^2) for each character was obtained by regressing the mean cross performance (Y) determined in individual trials, and the combined analysis over trials, against the mean mid-parent performance determined in the respective trials. The slope of the regression line (b) gave a direct estimate of h^2, while the standard error of the slope provided an estimate of the precision of the estimate. The significance of the regression in accounting for the variation between the progeny and mid-parent values was determined by t-test.

5.5.5 Seedling clone analyses

An AOV was conducted for seedling clones over trials for both root characters. Trials were regarded as replicates and clones as treatments. A broad sense heritability (g^2) on a replicated pot basis was calculated (Burton and de Vane, 1953). Using expectations of mean squares for a combined clonal analysis, AOV and analyses of co-variance allowed routine calculation of genetic, phenotypic, and environmental correlations between the root traits.

5.6 Breeding strategies

From the regression equations for PRR versus the long-term rating for the standard clones in each individual trial and in the combined analysis, ratings were assigned to parents and progeny using the ISSCT scale. Values were rounded to the nearest whole number as is the standard procedure in the BSES Plant Improvement Program.

The efficiency of the breeding strategy for Pachymetra root rot currently used by BSES was assessed. Before the results of this project were available, BSES policy was not to make crosses for northern Queensland which have mid-parent Pachymetra root rot resistance

ratings of greater than six. The distributions of progeny with different resistance ratings were compared for all crosses and for groupings of crosses with varying mid-parent ratings. The progeny and parent ratings from the combined analysis were used.

5.7 Selection efficiency

The effectiveness of selection within the parent clones in each of the five individual trials was estimated by comparing the number of clones common to the combined analysis within each of the resistance categories. The parent clones were selected since they were included as one pot in each of four replicates in each trial, which is comparable to the standard BSES procedure of testing clones as one pot in five replicates. The BSES policy on discarding clones for susceptibility before this project was to discard clones rated 8 or 9 in northern Queensland unless they had exceptional yield. Clones with high yield would be tested in a second trial and discarded if their high susceptibility was confirmed. Clones with a Pachymetra root rot resistance rating of 7 would only be considered for commercial release in northern Queensland if they had significantly better yield than current commercials in a range of environments throughout the region.

6. RESULTS

6.1 Standard clones

Comparison of the ratings given to the standard clones in a disease trial with their long-term disease ratings is used to determine the efficacy of the trial. Regression and correlation analyses for the five individual trials, as well as the combined trial data, revealed that most trials were satisfactory. Correlation coefficients ranged from 0.63 to 0.90.

Combined AOV over all trials were conducted for the two characters in each of the three genetic samples included in each trial. These revealed many highly significant main effects and interactions (Table 1). For the standard clones, all terms except error for PRR were highly significant. For both characters, there were highly significant differences among trials for this group of 10 standard clones, as well as highly significant differences among individual clones over trials.

Estimates of variance components for trials and clones were about equal for NR. The estimate of the variance component for clones for PRR exceeded that for trials. Standard errors attached to the estimates of the trials variance component showed these were estimated with less precision than the clones component. The trials x clones components were estimated with acceptable precision, but were smaller than the trials and clones component estimates for all three characters (Table 2).

Estimates of degree of genetic determination for the two characters from the combined AOV revealed all were highly determined genetically. The highest estimate of 0.926 was for PRR (Table 2).

6.2 Parental clones

All main effects and interaction terms in the combined AOV over trials for the three characters measured on the parental clones were highly significant (Table 1). As for the standard clones, estimates of the variance components for trials were estimated with less precision than those for the variance components for clones (Table 2). Again, estimates for the clones and trials x clones components for the three characters were precise. All trials x clones estimates were substantially smaller than the respective estimates for trials and clones. The g^2 estimates for NR and PRR were high (0.870 and 0.886) and differed only marginally from those obtained from the standard clones (Table 2).

6.3 Design I analysis

With two exceptions (NR, Trial 3; PRR, Trial 1), estimates of additive genetic variance were much smaller than corresponding estimates for dominance genetic variance (Table 3). Estimates of additive and dominance genetic variance were made with mixed precision. No estimates of additive genetic variance were more than twice their standard error. The estimates of dominance genetic variance for NR for Trial 4 was the only estimate more than twice its standard error (Table 3). In the Design I mating, there is no `clean' estimate available for dominance genetic variance (Hallaeur and Miranda, 1981). The standard error of the estimate usually is large because of the complexity of the function for estimating dominance genetic variance.

Estimates of total genetic and phenotypic variance for all characters were made with good precision (Table 3). Estimates of narrow sense heritability (h^2) were low for NR (0.076 - 0.328) and PRR (0.068 - 0.359). Estimates of broad sense heritability (g^2) were moderate to high for NR (0.684 - 0.809 and PRR (0.635 - 0.703).

The main effects of trials and crosses in the combined AOV of the Design I over trials were highly significant for both characters (Table 1). The partition of crosses into effects due to males and females/males revealed the former was significant for NR only. The latter was highly significant for both characters (Table 1). The trials x crosses interaction was highly significant for NR. The partitioning of the interaction term into trials x males and trials x females/males revealed a significant effect for the former, and highly significant effects for the latter for NR (Table 1).

Estimates of the trials variance component for NR and PRR, from the combined AOV over trials, were greater than the respective variance components for crosses. Neither was greater than twice the standard error of the respective estimate. The crosses components were estimated with acceptable precision. Estimates of the variance components due to males were small relative to the estimates of the components due to females/males. Consequently, estimates of dominance genetic variance were considerably larger than those for additive genetic variance (Table 4). As for the individual trials, the standard errors for the estimates of additive genetic variance were high. Dominance genetic variance was estimated with acceptable precision for both characters.

The estimate of the trials x crosses variance component for NR was small relative to the

respective estimates for trials or crosses (Table 4). The estimates for trials x males for NR was small relative to the estimate for trials x females/males. These estimates of interaction components over trials were of little consequence relative to the main effects (Table 4).

These estimates were brought together in estimates of total genetic variance and phenotypic variance, which showed good precision (Table 4). Estimates of narrow sense (h^2) heritabilities on a single plant basis were 0.089 for PRR and 0.130 for NR. Estimates for broad sense (g^2) heritabilities on a single plant basis were moderate, ranging from 0.375 to 0.424. On a replicated pot basis, h^2 estimates were low, ranging from 0.218 for PRR to 0.286 for NR (Table 4). Estimates of g^2 on a replicated pot basis were high for both characters (Table 4). Estimates for h^2 and g^2 unbiased by trials x crosses interaction were not computed because of the number of zero or non-significant interactions involving the main effect of trials.

6.4 Progeny mid-parent regression

Regression accounted for a highly significant portion of the variation between progeny and mid-parent performance for both NR and PRR in the combined analyses. Estimates from the combined AOV were 0.781 and 0.635 for NR and PRR, respectively. These moderate estimates contrasted markedly with the low h^2 estimates obtained from the Design I variance component estimates (Table 4). Estimates of genotypic, phenotypic and environmental correlations between NR and PRR were all very low (0.09 - 0.08).

6.5 Breeding strategy

The efficiency of eliminating highly susceptible progeny and increasing the proportion of resistant progeny, by restricting crosses to those below certain mid-parent rating limits, was examined (Table 5). Crosses with mid-parent ratings of less than 6.0 gave 11% fewer progeny with ratings of 8 or 9, and 10% more progeny with ratings $\geq$3.0, than did unselected crosses. Relaxing the restriction on crosses to those with mid-parent ratings less than 6.5 increased the percentage of highly susceptible progeny by 9% and reduced the percentage resistant progeny by 6%. Restricting crosses to those with mid-parent ratings less than 5.5 and 5.0 did not decrease the percentage of highly susceptible progeny although there were slight increases in the percentages of resistant progeny (1 and 3% respectively). These shifts in the percentages of highly susceptible and resistant progeny were at the expense of restricting the number of crosses which could be selected. Restricting crosses to those with mid-parent ratings of less than 6.0 reduced the number of crosses to 47.5% of the total (Table 5). Figure 1 compares the distribution of progeny within different rating categories for all crosses:- crosses with mid-parent ratings of $<$6.0 and $\geq$6.0 (Figure 1A) and crosses with mid-parent ratings of $<$6.5 and $\geq$6.5. The distribution of resistance ratings for all crosses was skewed towards the susceptible range.

6.6 Selection efficiency

The effectiveness of selection of clones in a trial depends on the accuracy of the rating and its repeatability. Effective selection will enable susceptible clones to be discarded without discarding potentially acceptable clones. The results for the parent clones from this project confirmed that it would be inadvisable to discard highly susceptible clones on the results of one trial alone. Trials 1, 2, 3, 4 and 5 would have discarded 1 (2%), 10 (21%), 5 (11%), 2 (4%) and 6 (13%) clones respectively that would not have been discarded by the combined analysis (Table 6). When considering the results of resistance trials, the degree of confidence in the trial is assessed by examining the correlation coefficient. The trials with the higher levels of inappropriate discarding of clones were those with lower correlation coefficients. Trials 2 and 5 had the highest mean PRR in both parent and progeny and gave poorer discrimination between clones.

7. DISCUSSION

Genetical studies are difficult to conduct on the field reaction of plant populations to soil-borne organisms affecting production by direct or indirect effects on root systems. Spatial and temporal heterogeneity of pathogen distribution is often a major consideration. Any evaluation of root systems for pathological, physiological or agronomic studies is daunting because of resource requirements. Potential experimental errors arising from measurements, independent of pathogen heterogeneity, can be significant. Consequently, such studies are uncommon.

In this research, we tried to reduce many of these problems by using sugarcane plants grown in pots filled with *Pachymetra*-inoculated potting medium, and maintained in temperature-controlled benches. The advanced bench design used in this project was considerably better than earlier designs, and allowed trials to be conducted throughout most of the year with acceptable temperature control.

Improvement of sugarcane seedling populations for a desired trait, such as Pachymetra root rot resistance, is achieved by crossing parental clones selected for desirable, high expression of the trait. Progress depends on the narrow sense heritability (h^2) for the trait. Estimates of h^2 from the Design I analysis were low for NR (number of roots) and PRR (% rotted roots), and this was true for estimates from individual trials and combined analyses over trials. These estimates contrast markedly with those obtained from progeny mid-parent regression analyses.

Assumptions invoked for analysis of a genetic design have been considered by Kempthorne (1957) and Comstock and Robinson (1964). The assumptions involved in the derivation of expectations of mean squares are unlikely to have been violated seriously in this study. Assumptions involved in derivation of the genetical interpretations of variance components - regular diploid behaviour at meiosis, no correlation of genotypes at separate loci, no multiple alleles and no epistasis - are unlikely to be satisfied.

The commercial sugarcane hybrids used in this study are of complex inter-specific,

polyploid origin. Although diploid behaviour is assumed, no simply inherited characters have been described. The assumption of no linkage, or the existence of linkage equilibrium, is probably invalid. However, it is unlikely that linkage will have a pronounced effect on the estimation of variance components relative to other possible sources of error (Cockerham, 1963). Additive genetic variance components estimated from a Design I were considered biased upwards by epistasis (Comstock, 1955). Evidence from other clonal crops (Morrow *et al.,* 1958; Watkins and Spangelo, 1968) suggests that the assumption of no epistasis in sugarcane is unrealistic.

Estimates of h^2 from progeny mid-parent relationships are sensitive to genotype x environment interaction (Casler, 1982). The h^2 values for PRR of 0.218 and 0.635 from the Design I and the progeny mid-parent analyses, respectively, are difficult to reconcile. Violation of the assumptions for the Design I analysis is the most likely explanation, given the genetic complexity of commercial clones. The true h^2 value probably lies in the upper range of the bounds set by these estimates, ie ~ 0.50. A significant improvement in the Pachymetra root rot resistance status of seedling populations produced from parental clones selected for Pachymetra root rot resistance will result.

Estimates for h^2 of 0.41 and 0.38 for plant and ratoon crops, respectively, were reported for resistance to sugarcane smut (Chao *et al*., 1990). The h^2 estimates for NR and PRR, from progeny mid-parent analyses conducted using means from the combined AOV over trials, were all higher than these values. The standard errors of our estimates, however, were higher.

The estimates of broad sense heritability in this study were extensive. They were gathered using three genetic samples over individual and combined trials. Estimates were determined on several bases in some cases. Estimates of g^2 for PRR on a replicated pot basis for standards and parent clones, and for Design I analysis for progeny, were 0.926, 0.870 and 0.916. These estimates of g^2 must be regarded as particularly robust, and show that exploitation of total genetic variation by selection among clones or families for Pachymetra root rot resistance would be successful. Single-plant selection within families would be less productive.

Estimates of g^2 for NR from combined analysis for standard clones, parent clones and progeny revealed that selection for this character would be as successful as selection for PRR. This finding may have broader agronomic implications. Root size and extensiveness have been shown to be related to survivability in harsh conditions in perennial alfalfa pastures (Saindon *et al*., 1991) and reduced lodging in corn (Jenison *et al*., 1981). Studies of the relationship between size of root systems and ratooning and reduction of stool tipping should be considered.

Consideration of the standard error of a mean for the standard clones assessed over multiple pots per replicate, replicates, and trials revealed two important points. Firstly, variation among multiple pots of a clone within a replicate was important, and this requires further research. Secondly, the trials x clones interactions were very important. Although this variance component was significantly smaller than the sampling component, it contributed substantially to the standard error of a clonal mean. A reduction from five to three trials

resulted in marginal inflation of the standard error, but reduction to a single trial resulted in significant inflation in the standard error.

The BSES breeding strategy of rejecting crosses for northern Queensland which have a mid-parent rating of greater than 6.5, was shown to be an inefficient means of reducing the proportion of unacceptably susceptible clones in the breeding program. Increasing the severity of the restriction so that crosses with mid-parent ratings greater than or equal to 6.0 are rejected, would significantly reduce the proportion of susceptible clones. Inclusion of an economically important character, that can be controlled economically by non-genetic means, in selection criteria addressed by a breeding program will restrict the potential for overall gain from breeding. However, environmentally and economically acceptable alternative strategies for control of root diseases are not readily available.

Discarding clones for Pachymetra root rot susceptibility should only be done after results are obtained from at least two and possibly three trials. The ratings for parent clones should also be made on 2-3 trials. Further research to reduce trials x clone interaction would result in significant resource economies.

Developing a strategy for breeding for Pachymetra root rot resistance was an objective of this project. The project showed that the interim strategy adopted by BSES should be changed. Restricting crosses to those with mid-parent ratings of less than 6.0, compared with the current limit of 6.5, should increase the efficiency of the breeding program by 9% and save BSES in the order of $200 000 annually. The more efficient use of BSES resources should provide more profitable varieties in the future.

8. DIFFICULTIES

One trial in this experiment was abandoned due to failure of the *Pachymetra* inoculum to cause sufficient disease. Loss in virulence of cultures in storage is a significant problem for research and all future studies should use only cultures recently isolated from plants. Improved storage techniques for reference cultures would assist future research.

9. RECOMMENDATIONS FOR FUTURE RESEARCH

The parents and progeny from this project have been maintained and may be suitable for future research into genetic mapping of sugarcane. The pot trial system for rating clones for Pachymetra root rot resistance is labour intensive, so a genetic probe for resistance genes would greatly increase the efficiency of breeding for resistance to this disease.

Root number was found to be highly heritable. Further understanding of the importance of root systems in maintaining profitable ratoons and preventing stool tipping is recommended. If a strong link can be established and it is thought appropriate to breed for larger root systems, this project suggests that the breeding would be successful.

10. PUBLICATIONS

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11. ACKNOWLEDGMENTS

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Table 1

`F' statistics for the combined analyses of variance, over five Pachymetra root rot bench trials, of two root characters determined for three genetic samples of sugarcane

Genetic sample	Source of variation	Root character	
		No. roots (NR)	% rotted roots (PRR)
Standard clones	Trials	11.530**	6.686**
	Reps/trials	3.219**	3.420**
	Clones	7.583**	25.024**
	Trials x clones	5.763**	2.184**
	Error	1.195	1.230
Parental clones	Trials	25.959**	11.745**
	Reps/trials	4.964**	8.219**
	Clones	8.750**	7.680**
	Trials x clones	1.876**	1.377**
Crosses	Trials	33.938**	16.601**
	Reps/trials	5.618**	10.928**
	Crosses	10.496**	11.604**
	Males	2.356*	2.136
	Females/males	8.752**	9.277**
	Trials x crosses	1.319**	0.934
	Trials x males	1.569*	0.629
	Trials x females/males	1.273**	0.989
		0.862	1.047

*,** P . 0.05 and 0.01, respectively.

Table 2

Variance component estimates, and their standard errors, on a replicated plot basis, and estimates of broad sense heritability (g^2), on a replicated pot basis, from combined analyses of variance for two root characters

Genetic sample	Parameter/Statistic[1]	No. roots (NR)	% rotted roots (PRR)
Standards	$\hat{\sigma}^2_I$	40.861 . 25.593	83.018 . 54.957
	$\hat{\sigma}^2_C$	32.784 . 16.143	273.365 . 121.443
	$\hat{\sigma}^2_{IC}$	20.579 . 5.736	30.842 . 13.427
	$\hat{\sigma}^2_E$	51.850 . 6.265	312.638 . 37.774
	$\hat{\sigma}^2_s$	43.393 . 3.061	254.240 . 17.933
	$\hat{\sigma}^2_P$	39.493 . 16.104	295.165 . 121.422
	g^2	0.830	0.926
Parents	$\hat{\sigma}^2_I$	63.188 . 37.735	175.141 . 109.652
	$\hat{\sigma}^2_C$	50.583 . 11.677	146.882 . 34.546
	$\hat{\sigma}^2_{IC}$	15.237 . 3.511	30.116 . 12.182
	$\hat{\sigma}^2_E$	69.582 . 3.741	319.317 . 17.167
	$\hat{\sigma}^2_P$	57.110 . 11.657	168.871 . 34.471
	g^2	0.886	0.870

[1] $\hat{\sigma}^2_C$ = variance due to clones; $\hat{\sigma}^2_E$ = error variance; $\hat{\sigma}^2_P$ = phenotypic variance, on a replicated plot basis; $\hat{\sigma}^2_s$ = variance due to sub-sampling (multiple pots/replicate); $\hat{\sigma}^2_I$ = variance due to trials; $\hat{\sigma}^2_{IC}$ = variance due to interaction of trials x clones.

Table 3

Estimates of additive ($\hat{\sigma}^2_A$), dominance ($\hat{\sigma}^2_D$), total genetic ($\hat{\sigma}^2_G$) variance and their standard errors, estimates of narrow sense (h^2) and broad sense (g^2) heritabilities on a replicated pot basis, and genetic coefficient of variation (GCV), for two root characters determined from a Design I experiment of 40 biparental sugarcane crosses in five Pachymetra root rot bench trials

Parameter/ Statistic	Trial				
	1	2	3	4	5
	Number of roots (NR)				
$\hat{\sigma}^2_A$	24.737 . 26.107	33.234 . 32.216	26.788 . 21.622	8.791 . 16.189	54.325 . 45.477
$\hat{\sigma}^2_D$	87.992 . 48.826	79.363 . 57.085	28.996 . 33.162	81.346 . 38.325	81.236 . 71.971
$\hat{\sigma}^2_G$	112.729 . 35.528	112.597 . 40.600	55.784 . 21.669	90.137 . 29.918	135.561 . 48.056
$\hat{\sigma}^2_P$	139.390 . 35.450	154.962 . 40.430	81.550 . 21.555	116.098 . 29.827	184.022 . 47.864
h^2	0.177	0.214	0.328	0.076	0.295
g^2	0.809	0.727	0.684	0.776	0.737
GCV	25.6	20.0	18.6	29.2	23.9
	% rotted roots (PRR)				
$\hat{\sigma}^2_A$	127.920 . 100.062	49.059 . 71.181	13.671 . 28.374	24.380 . 58.072	43.927 . 66.639
$\hat{\sigma}^2_D$	108.270 . 150.221	265.235 . 157.139	114.864 . 69.505	284.408 . 146.536	233.807 . 150.095
$\hat{\sigma}^2_G$	236.191 . 96.985	314.294 . 121.158	128.535 . 54.707	308.788 . 115.937	277.734 . 116.468
	356.285 . 94.880	451.363 . 118.966	202.492 . 54.324	438.982 . 115.350	425.903 . 113.797
	0.359	0.109	0.068	0.056	0.103

$\hat{\sigma}_P^2$	0.663	0.696	0.635	0.703	0.652
h^2	31.8	28.6	43.1	38.5	27.9
g^2					
GCV					

Table 4

Estimates of variance components and genetic and environmental parameters from the Design I analysis of combined trials for two root characters

Parameter/Statistic[1]	No. roots (NR)	% rotted roots (PRR)
$\hat{\sigma}^2_I$	64.377 . 38.155	182.980 . 111.906
$\hat{\sigma}^2_C$	28.243 . 6.903	78.341 . 18.953
$\hat{\sigma}^2_m$	6.810 . 5.765	15.445 . 14.165
$\hat{\sigma}^2_{f/m}$	22.263 . 6.019	64.778 . 17.382
$\hat{\sigma}^2_{TC}$	3.813 . 1.774	0.0 . 4.754
$\hat{\sigma}^2_{Tm}$	0.583 . 0.913	0.0 . 1.471
$\hat{\sigma}^2_{Tf/m}$	4.253 . 1.788	0.0 . 5.309
$\hat{\sigma}^2_E$	121.264 . 7.078	474.617 . 27.704
$\hat{\sigma}^2_w$	140.615 . 4.968	453.247 . 16.015
$\hat{\sigma}^2_A$	27.240 . 23.060	61.780 . 56.660
$\hat{\sigma}^2_D$	61.809 . 29.263	197.333 . 84.061
$\hat{\sigma}^2_G$	89.052 . 24.076	259.112 . 69.528
$\hat{\sigma}^2_P$	95.814 . 24.075	282.844 . 69.542
h^2_{sp}	0.130	0.089
g^2_{sp}	0.424	0.375
h^2	0.286	0.218
g^2	0.936	0.916

[1] h^2_{sp} and g^2_{sp} = narrow and broad sense heritabilities on a single plant basis; h^2 and g^2 = narrow and broad sense heritabilities on a replicated pot over trials basis; $\hat{\sigma}^2_A$ = additive genetic variance;

$\hat{\sigma}^2_C$ = variance due to crosses; $\hat{\sigma}^2_D$ = dominance genetic variance; $\hat{\sigma}^2_E$ = error variance; $\hat{\sigma}^2_{Tm}$ = variance due to females within males; $\hat{\sigma}^2_G$ = total genetic variance; $\hat{\sigma}^2_m$ = variance due to males; $\hat{\sigma}^2_P$ = total phenotypic variance, on a replicated pot basis; $\hat{\sigma}^2_T$ = variance due to trials; $\hat{\sigma}^2_{TC}$ = variance due to interaction of trials x crosses; $\hat{\sigma}^2_{Tf/m}$ = variance due to trials x females/males; $\hat{\sigma}^2_{Tm}$ = variance due to trials x males; $\hat{\sigma}^2_w$ = within family variance.

Table 5

Effect of selecting crosses with varying mid-parent ratings on percent progeny rated as resistant (. 3) and highly susceptible (rating 8 and 9) and on the percentage of crosses rejected

Mid-parent rating of selected crosses	Progeny		% crosses rejected
	% rated . 3	% rated 8 and 9	
< 5.0	35	15	75.0
< 5.5	33	15	67.5
< 6.0	32	15	52.5
< 6.5	26	24	27.5
< 7.0	25	24	17.5
All	22	26	0

Table 6

Comparison of the number of parent clones in each rating category[1] in individual trials and the number of clones common with the combined analysis for all trials

Trial	Number of clones							
	Resistant Rating ≤ 3		Intermediate Rating > 3 ≤ 6		Susceptible Rating > 6		Highly susceptible Rating > 7	
	This trial	Also in combined analysis	This trial	Also in combined analysis	This trial	Also in combined analysis	This trial	Also in combined analysis
1	9	7	26	17	12	9	7	6
2	3	3	18	11	26	13	17	7
3	19	7	15	5	13	6	10	5
4	19	12	20	15	8	8	6	4
5	4	4	24	13	19	10	12	6
Combined	12		22		13		7	

1 Resistance ratings are based on ISSCT scale where 0 is immune and 9 is highly susceptible. Ratings were calculated using the regression for the standard clones for each individual trial.