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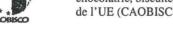
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Evaluation of cocoa germplasm for resistance to Witches' Broom disease

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Introduction

This report covers the second year of the ACRI funded Witches' Broom disease (WB) screening project. We have been able to attain favourable and fairly stable experimental conditions, leading to the successful inoculation of grafted accessions obtained from the ICG,T. However, considerable preparatory work was required before we could successfully adapt this inoculation method to screen grafted clones from the ICG,T. An account of all the modifications made to the inoculation system to adapt it for use at CRU and results from preliminary experiments conducted to standardise experimental conditions, was given in the previous report (Umaharan, et al., 2000).

In this report an account is given of the progress of germplasm screening for resistance to WB. To date nine grafting series, A to I, have been inoculated. The results of five series of accessions, representing series A, B, C, D and E, for which inoculation and symptom assessment are complete, will be discussed here.

Materials and Method

Series A, B, and C represent clones which were inoculated using a hand-held mister, while Series D and E, represent the first and second series of inoculations carried out with the modified automated atomising spray system. Each series of inoculations, with the exception of series D, consisted of at least 30 top-grafted accessions and at least six controls (three resistant and three susceptible, Table 1). Series D consisted of 10 accessions, and at least 15 plants per accession, all normally used as controls.

Based on the results obtained from the preliminary experiments (Umaharan *et al.*, 2000), it was possible to refine and standardise experimental conditions and inoculum concentration for mass screening. A 250,000 bs mL⁻¹ inoculum concentration was shown to be sufficient to induce disease in inoculated plants and allowed a distinct separation between resistant and susceptible accessions. Therefore this inoculum concentration was used for mass screening. Five plants per accession were pruned, new shoots tagged and each plant inoculated with 2 mL of inoculum at a concentration of 250,000 bs mL⁻¹.

After inoculation, plants were incubated for 24 hours at 25-27°C and high humidity. Symptoms were observed fortnightly over a four-month period.

These activities have been previously discussed in detail (Umaharan et. al., 2000).

Results

Assessments of five out of nine series of inoculated accessions were completed in the year 2000. Pooled results of 9 clones commonly used as susceptible and resistant controls, from the 5 inoculation series are presented in Table 1. Percentage infection was found by calculating the

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percentage of diseased shoots (shoots with brooms, swelling and/or necrosis) out of the total number of shoots inoculated. Percentage infection ranged from 1.3 - 67.5%. Resistant clones tended to cluster towards the lower end of this symptom range (1.3 - 8.6%), with M 8, a susceptible control, producing the most symptoms and IMC 67, a resistant control, the least.

Table 1. Pooled results of 9 accessions from 5 series of inoculations, used as susceptible and resistant controls in screening for Witches' Broom disease.

Clone	% infection	Resistance
M 8	67.5	S
ICS 1	52.0	S
AMELONADO	40.4	S
ICS 60	46.9	S
ICS 95	14.8	S
IMC 3	2.7	R
IMC 67	1.3	R
IMC 6	5.7	R
SCA 6	8.6	R

Good correlation was found among the five series (Table 2), except between series A and C, indicating that experimental conditions have been sufficiently constant from series to series for the reaction of most clones to be consistent from series to series.

Table 2. Results of a correlation analysis of the control clones for five completed inoculation series.

	Series A	Series B	Series C	Series D	Series E
Series A	0+1				
Series B	0.68	1			
Series C	0.26	0.71	1		
Series D	0.44	0.73	0.87	1	
Series E	0.81	0.81	0.63	0.87	1

The symptoms from 132 accessions were assessed and 119 accessions belonging to 21 populations (populations which were represented by at least 2 accessions each, were chosen) were divided into 4 groups based on % infection (Figure 1). The majority of clones fell into the 0-10% infection group (36), followed by an almost even distribution of clones in the other three groups.

The mean results for eight populations, with at least five clones in each are presented in Table 3 with clones from the remaining populations grouped together as "others". The LP and IMC populations had the largest percentage of clones which fell into the low (0-10%) and moderate (10-25%) infection range, with no accessions in the higher infection ranges. However the majority of populations contained a good distribution of accessions, which produced infection, ranging from low to high.

Figure 1. The distribution of clones for resistance to Witches' Broom disease according to percentage (%) infection observed.

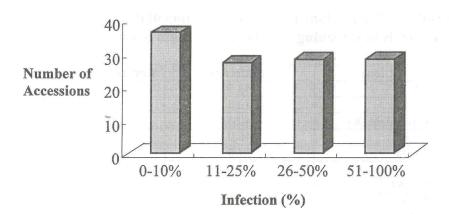


Table 3. Percentage of clones per population showing different percentage infection.

Population	0-10%	10-25%	25-50%	50-100%
AM	33.3	33.3	33.3	0
В	41.2	23.5	17.7	17.7
CL	0	60.0	0	40.0
ICS	9.1	27.3	45.5	18.2
IMC	37.5	12.5	0	0
LP	77.8	22.2	0	0
NA	30.8	23.1	0	46.2
PA	30.8	15.4	30.8	23.1
OTHERS	22.8	14.3	31.4	31.4

Discussion and conclusion

Good correlations obtained between controls for all the series indicated that experimental conditions were sufficiently constant from series to series for repeatable results. Also the results from inoculations using the hand-held mister and those from the automated spray system were comparable from one series to the next. Clear distinctions were also seen between resistant and susceptible controls, when symptoms were evaluated as percentage infection. Using the automated spray system, it was possible to efficiently screen large numbers of clones at the same time.

Results from the mass screening have yielded interesting results. For example, clones belonging to the LP population seem to show the least amount of symptoms, with the majority having only 0-10% infection. While it is too early to substantiate, a tentative conclusion may be that clones belonging to this population are potential sources of resistance to WB. In addition, a good distribution of percentage infection, ranging from low to high, was found for most of the

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populations. All those accessions with low infection may also be potential sources of resistance to WB. Future screening is expected to yield additional interesting clones.

In conclusion, mass screening for WB has so far been successful in consistently discriminating between resistant and susceptible controls and initial results indicate that a significant number of clones in the ICG,T may be potential sources of resistance to WB.

Reference

Umaharan, R., Thévenin, J-M., Surujdeo-Maharaj, S., and Latchman, B. (2000) Evaluation of cacao germplasm for resistance to Witches' Broom Disease: Preliminary screening experiments. Pages 34-37 in: *Annual Report 1999*. St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.