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Cover photograph. Canopy of closely planted trees on a commerical cocoa estate in east Trinidad.

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## **Evaluation of cocoa germplasm for resistance to Witches' Broom disease**

R. Umaharan, J-M. Thévenin and S. Surujdeo-Maharaj

### Introduction

At present a total of 630 accessions have been grafted from the ICG, T for the Witches' Broom screening project, which is now in its third year. Mass screening and evaluation for resistance to WB, have been completed for nine grafting series, A to I, comprising a total of 227 accessions.

In this report, results of another four completed inoculations, representing series F, G, H and I (114 accessions) will be presented. The previous annual report (Umaharan *et al.*, 2000) outlined screening results of the first five batches of grafted accessions, Series A to E.

### Materials and Method

### **Inoculation**

Inoculation was carried out using the modified automated spray system in which the basidiospore (bs) suspension was pumped through air/liquid atomising spray nozzles at a pressure of 7 psi.

Plants were pruned two weeks prior to inoculation. At flushing, new shoots were tagged and each plant inoculated with 3.5 mL of inoculum at a concentration of 150,000 bs/mL. A concentration of 175, 000 bs/mL, applied at 3.0 mL per plant, was used for inoculation of plants in series H.

All series of plants inoculated were incubated in the dark for 24 hours, at 25-27°C and high relative humidity.

### Symptom Assessment

Symptom observation was carried out at fortnightly intervals over a four month period, beginning two weeks after inoculation. Observations were made on each plant for all swellings, brooms and necrosis which developed after shoots were inoculated.

Percentage infection was found by pooling all symptoms per accession (brooms, swellings and necrosis) and calculated from the total number of shoots inoculated.

### **Results and Discussion**

Results for resistant and susceptible controls, which were used repeatedly from series to series, were pooled for F, G, H and I and presented in Table 1. Analysis of mean percentage infection for each control yielded very variable results with controls showing generally high SE values. This indicates that there was much variation within each control accession, in terms of percentage infection, from series to series.

Table 1. Susceptible and Resistant controls common for series F, G, H & I.

| Clone  | Resistance | No. of Shoots | Mean<br>(%) | Standard<br>error |
|--------|------------|---------------|-------------|-------------------|
| ICS 84 | S          | 49            | 29.3        | 13.1              |
| NA 45  | S          | 67            | 19.8        | 7.8               |
| ICS 60 | S          | 57            | 19.4        | 3.5               |
| UF 11  | S          | 74            | 13.1        | 6.3               |
| IMC 6  | R          | 60            | 19.8        | 14.1              |
| IMC 67 | R          | 43            | 7.7         | 4.3               |
| IMC 3  | R          | 38            | 13.1        | 5.2               |
| SCA 6  | R          | 82            | 25.1        | 3.1               |

### Series F

A total of 100 plants were inoculated in series F, representing 36 clones, including 10 controls. Infection ranged from 0 to 70%.

### Series G

A total of 196 plants were evaluated for symptoms, representing 37 accessions, including 9 controls. Infection ranged from 0 to 60%.

### Series H

A total of 129 plants were evaluated for symptoms, representing 34 accessions including 6 controls. Infection ranged from 0 to 67%.

### Series I

One hundred and forty six plants were evaluated for symptoms out of a total of 196 inoculated. The remaining 50 plants showed no signs of infection. These plants represent 38 accessions, including 6 controls. Infection ranged from 0 to 100%.

A total of 114 accessions were evaluated, i.e. an average of about 29 per series. After analysis of the results, accessions were divided into five categories based on percentage infection i.e. 0-10%, 11-20%, 21-40%, 41-60% and 61-100%, with accessions falling into the 0-10% category being highly resistant and those in the 61-100% category, being highly susceptible. Figure 1 shows the frequency distribution of accessions according to the five categories of percentage infection.

Figure 1. Frequency distribution of accessions according to percentage infection.

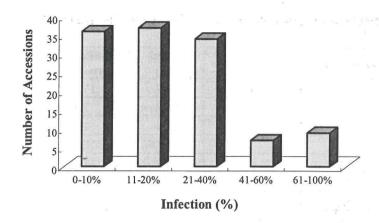


Table 4. List of 24 accessions selected as promising for confirmation of resistance to Witches' Broom disease.

| Accession        | Infection (%) |
|------------------|---------------|
| AMAZ 3/2 [CHA]   | 17.7          |
| AMAZ 6 [CHA]     | 8.8           |
| B 9/10-25 [POU]  | 16.0          |
| CL 10/15         | 17.2          |
| CRU 101          | 18.6          |
| CRU 72           | 20.0          |
| EET 272 [ECU]    | 15.0          |
| ICS 25           | 0.0           |
| IMC 14           | 3.7           |
| IMC 16           | 6.7           |
| IMC 38           | 10.3          |
| IMC 45           | 12.0          |
| IMC 55           | 0.0           |
| IMC 57           | 7.1           |
| IMC 58           | 5.0           |
| IMC 76           | 10.0          |
| LP 4/24 [POU]    | 10.5          |
| LV 28 [POU]      | 15.2          |
| NA 137           | 4.6           |
| NA 159           | 4.0           |
| PA 303           | 0.0           |
| PA 71 [PER]      | 0.0           |
| POUND 16/B [POU] | 15.8          |
| UF 667           | 20.0          |

The results indicated that the distribution of accessions were skewed to the left, i.e. the majority showed less than 40% infection. This implied that there was a large pool of germplasm with moderate to high levels of resistance to WB. This would be in keeping with the history of many of the accessions in the ICG, T, which were collected based on their apparent resistance to WB at their centre of origin.

However, because of the highly variable results for the controls, it could also indicate that a large number of escapes occur when this method of inoculation is used. Escapes could result from non-uniform targeting of inoculum and/or the rapid evaporation of the inoculum after spraying. In addition, it also indicates that percentage infection as a parameter for determination of resistance or susceptibility may not be reliable, when using this inoculation method.

Out of those accessions showing less than 20% infection, a further selection, based on average number of shoots inoculated (at least four shoots per plant), number of replicates per accession (at least 3 plants per accession) and type of symptom observed (swellings which do not develop into brooms or become necrotic), was carried out. This yielded 24 accessions that may be promising for further resistance studies (Table 4).

### **Future work**

Grafting and mass screening of accessions will be continued with the aim of screening as many of the accessions at the ICG, T as possible within the 5-year period of the project.

To overcome the problem of possible escapes, and also to give some confirmation or verification of the results obtained for mass screening via the automated spray method, another inoculation method will be used to confirm resistance on a subset of accessions from the mass screening which appears promising. In addition, analyses based on severity of symptoms will be undertaken.

### Reference

Umaharan, R., Thévenin, J-M., and Maharaj. S. (2000) Evaluation of cocoa germplasm for resistance to Witches' Broom disease. Pages 30-33 in: Annual Report 2000. St Augustine, Trinidad: Cocoa Research Unit, University of the West Indies.