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Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago

Tel. +1 868 662 8788
+1 868 645 3232-7 Ext. 2115
Fax +1 868 662 8788
E-mail cru@cablenett.net

Cover photograph. Newly established introductions planted at the University of the West Indies, Campus 8 field.

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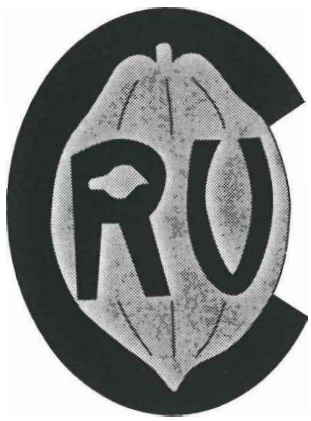

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Evaluation of cocoa germplasm for resistance to Witches' Broom disease

R. Umaharan, J-M Thévenin, A. Holder and J. Bhola

Introduction

The second phase of the WCF Witches' Broom disease screening project began in July 2003, and the project is being extended on an annual basis. In phase two of the project, revised targets were set in line with levels of financing and technical limitations encountered in phase one, (see Umaharan *et al.*, 2004). The new targets are to complete mass screening of 60 new accessions and to confirm the resistance of 30 accessions per year. Emphasis is on the confirmation and quantification of the level of resistance of clones found to be putatively resistant during preliminary mass screening.

The most salient conclusions drawn from the first five years of the screening programme (phase one) were that considerable variation of resistance to WB disease exists within the ICG,T and therefore with careful screening for the disease it was possible to identify numerous clones showing some level of resistance. Also inferred from previous observations was the need to use additional criteria for assessing resistance. Hence in addition to percentage infection, symptom severity based on the broom diameter and incubation period were used to provide a quantitative measure of resistance. The results also suggested that a two-tiered screening system was needed. Therefore the protocol for WB screening now involves initial mass screening and selection of promising clones using a spray inoculation system, followed by the confirmation and quantification of resistance of the selected clones using the agar droplet technique (Surujdeo-Maharaj *et al.*, 2003). This report gives a summary of the guidelines now adopted for evaluation of the results of WB resistance screening at CRU.

Material and Methods

Inoculation

Spray - mass screening

Inoculation of clones for mass screening is being carried out manually using a Preval Sprayer (Precision Valve Corp., NY, U.S.A), which delivers a fine spray. When spraying manually, inoculum is delivered in a downward direction from the top of the plants, in a regular and even movement.

Approximately 1 mL of inoculum at a concentration of 350,000 basidiospores/mL is applied to each plant. After inoculation, plants are left undisturbed for two and a half days in the dark at 25°C and high relative humidity to facilitate germination of basidiospores and host penetration by germ tubes. Relative humidity and temperature are monitored with a data logger throughout the incubation period.

Agar droplet - verification

Screening of clones for confirmation of resistance to WB is carried out using the agar droplet technique (Surujdeo-Maharaj *et al.*, 2003), where a single 30µL drop of basidiospore suspension

in 0.3% agar is delivered to the growing point of shoots via a micropipette. The inoculum concentration is 350,000 basidiospores/mL and incubation conditions are the same as for spray inoculation.

Material to be inoculated for confirmation of resistance is either propagated clonally by micro-grafting or grown as seedlings from open pollinated pods. For clonal propagation, young, green budwood is collected and grafted onto six-week-old seedlings. Inoculations are carried out when the grafts are at least six months old. Fifteen replicates of each clone are grafted, out of which ten plants per clone are inoculated.

For seedlings, 30 plants per clone are pruned at 5 weeks old and inoculated at 8 weeks.

Analysis

Selection of promising clones is based on more than one measure of resistance, since percentage infection alone commonly shows inconsistencies. Complimentary studies (Surujdeo-Maharaj, *et al.,* 2003) have shown that variables such as incubation period and broom-base diameter were found to be highly correlated with resistance.

Symptoms are assessed in two ways:

1. By calculating the percentage of shoots showing symptoms out of the total number of shoots inoculated and
2. By quantitatively assessing two variables to indicate symptom severity; broom-base diameter and number of days from inoculation to the first appearance of symptom (incubation period).

Selection of putatively resistant clones from mass screening

Clones are identified as putatively resistant from mass screening based on the following criteria:

Percentage infection

1. Those with at least three replicates per accession inoculated with at least four shoots per replicate and
2. Those for which the total percentage of symptoms is less than 20% or,
3. Those showing absence of brooms after 16 weeks of observation in the greenhouse.

Further analysis of those clones which produced symptoms is undertaken and clones are selected according to the following criteria.

Broom development and broom-base diameter

1. At least three replicates per accession inoculated
2. At least four shoots per replicate plant inoculated
3. No brooms developed
4. Have a broom-base diameter less than the most resistant control or,
5. Have a broom-base diameter of less than 6mm (most brooms seem to show this size as the minimum base diameter)

Broom-base diameter is taken at the time of maximum broom development.

Results for symptom severity (incubation period and broom-base diameter) are evaluated by ANOVA using the general linear model and the Tukey-Kramer Multiple comparison test

(MINITAB Release 14 or NCSS 2001 software).

Criteria for selecting and ranking clones - confirmation screening

Clones are confirmed as resistant based on the following:
1. At least three replicates per accession inoculated
2. Having a broom-base diameter less than the most resistant control or,
3. Having a broom-base diameter of less than 6mm
4. No brooms
5. Delayed onset of the first symptom.

For the Tukey-Kramer Multiple comparison test:
- Significantly different from susceptible control and/or statistically similar to the resistant control for both incubation period and broom-base diameter (or no brooms).
- No brooms developing from shoots with swellings.
- Significantly different from susceptible control and/or statistically similar to the resistant control for incubation period alone.
- Significantly different from susceptible control and/or statistically similar to the resistant control for broom-base diameter alone.

Clones are then ranked according to the level of resistance, with those clones which show good performance for both incubation period and broom-base diameter/ no broom being designated as the most resistant.

Table 1. Accession groups which contain clones selected for further confirmation of WB resistance and clones which have been confirmed.

Accession group	No. of clones	No. confirmed	Accession group	No. of clones	No. confirmed
AM [POU]	4		LX	1	
AMAZ [CHA]	2	1	LZ	2	
B [POU]	11		MATINA	1	
C [TRI]	1		MO	1	
CL	4	1	MOQ	8	
CLM	1		NA	6	2
CRU	7	2	POUND [POU]	5	1
DOM	3		PA [PER]	12	5
E [ECU]	1		PLAYA ALTA [VEN]	1	
EET [ECU]	6	1	REDAMEL	1	
GS	2		SCA	2	1
GU	1		SJ [POU]	3	
ICS	16	2	SLA	2	
IMC	16	5	SLC	2	
JA [POU]	1		SP [VEN]	1	
LCT EEN	4		SPEC	1	
LP [POU]	8	2	UF	4	
LV [POU]	3				

Overall results

Having developed and fine-tuned the above criteria for screening over the last seven years it is now possible to routinely evaluate the results of WB screening with a good level of accuracy and consistency.

Since the commencement of WB screening in July, 1998, over 777 accessions have been spray-inoculated and screened for WB resistance in the greenhouse, and a total of 145 promising clones have been selected for further confirmation of resistance. These clones belong to thirty five (35) accession groups (Table 1) represented in the ICG, T so cover a good range of the genetic diversity available in the genebank,

Promising clones were selected by considering both percentage infection and the development of brooms (either no brooms or those with a small base diameter). Thirty eight (38) of these clones have been inoculated by the agar droplet method to confirm and quantify their resistance, with a total of 23 clones (Table 1) confirmed with resistance to WB, under the experimental conditions at CRU.

References

Surujdeo-Maharaj, S., Umaharan, P., Butler, D.R. and Sreenivasan, T.N. (2003) An optimized screening method for identifying levels of resistance *to Crinipellis perniciosa* in cacao (*Theobroma cacao* L.). *Plant Pathology* **52**: 464-475.

Umaharan, R., Thévenin, J-M., Holder, A., and Bhola, J. (2004) Evaluation of cocoa germplasm for resistance to Witches' Broom disease under controlled conditions. Pages 37-41 in: *Annual Report 2003*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.