Molecular Marker-Assisted Breeding for Resistance to the Cassava Mosaic Disease in Latin American Cassava Gene pools.

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INTRODUCTION

Molecular marker-assisted selection (MAS) for CMD resistance at CIAT is both a pre-emptive measure, should in case the disease is accidentally introduced in Latin America, and a dynamic measure, to enable a true evaluation of the value of CIAT improved germplasm in India and Africa. MAS using the single dominant gene CMD2 from the Nigerian land race TME3 as source has completed its first year at CIAT. A total of 2315 seeds were harvested from more than 3000 controlled crosses between CMD resistant parents introduced from IITA and elite parents of the 5 cassava gene pools by agro-ecology or backcross derivatives of *M. esculenta* sub spp *flabellifolia* resistant to the green mite. More than 1,100 genotypes were germinated as embryo axes and multiplied for molecular analysis with the SCAR marker RME1 and the SSR marker NS158 closely linked to CMD2 (Fig.1). Establishment of breeding populations in vitro is to aid shipment to collaborators in Africa and India. CMD resistant genotypes, as revealed by MAS, will be sent to the green house for hardening and also shipped to partners in India and Africa. We describe here results of the first year of MAS at CIAT.

MATERIALS AND METHODS

Sexual seeds of crosses between CMD resistant parents and elite parents of cassava gene pools or backcross derivatives of *M. esculenta* sub spp *flabellifolia* resistant to the green mite, were germinated from embryo axes. DNA was extracted using a miniprep adaptation of the Dellaporta method (1983) from leaves of *in-vitro* plants. Isolated DNA was stored to -20°C for more than 2 months without any degradation and can be used to do 100 PCR reactions. To harvest leaves, do DNA extraction and process a 96 wells plate 9 hours are required. To make PCR and run and stain acrylamide gels required another 8 hours (Fig.2). The entire process for one plate takes 17 hours.

SSR marker (NS 158) and SCAR RME 1 were used to implement the MAS scheme in more than 20 cassava families. SSR marker analysis was on a polyacrilamide gel (19:1 acrilamide : bisacrilamide) and silver staining. The SCAR marker was scored on ethidium bromide stained agarose gel (1.5%). The raw marker data was entered in a microsoft excel table including information about parents, phenotypic evaluations, PCR amplification, etc.). The gel images were added at the end of the table for easy diagnosis of the genotype of each individual.

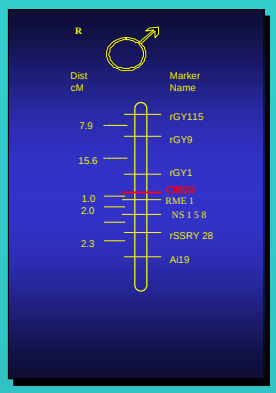

Fig. 1. Linkage group showing the CMD2 gene and marker associated with it

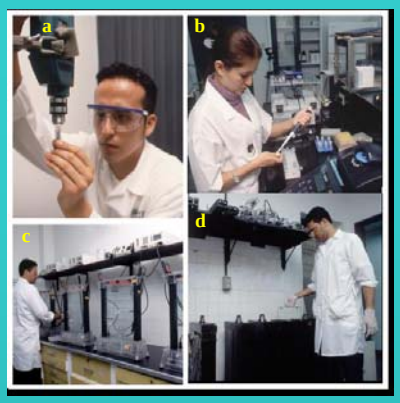

Fig. 2. Different steps in the MAS process:
a) Mini-extraction of DNA from leaves *of in-vitro* plants
b) PCR Amplification,
c) Acrylamide gel electrophoresis for SSR marker,
d) Silver staining for visualization of SSR marker alleles

RESULTS AND DISCUSSION

More than 1,100 plants representing a breeding population for CMD resistance at CIAT were established from embryo axes and analyzed using the NS158 marker and the SCAR marker RME1 associated with CMD resistance. An example of the worksheet to handle the MAS data is shown in Table 4. The versatility of Excel spreadsheets makes it the appropiate sofware to handle the diverse information generated by MAS. The excel table can be used to select very quickly individuals to be advanced in the breeding programs.

The cassava molecular marker lab can process 192 genotypes in 2 days or 480 genotypes per week or over 24,000 samples in a year. The current costs of a single SSR marker data point analysis for cassava at CIAT is U$ 0,30, processing 24,000 amples in a year requires a budget of U$ 7,200. One of the several advantages of MAS is the time and costs saved in eliminating a significant proportion of breeding populations that do not have the desired traits at a very early stage. These savings get bigger when several traits are evaluated simultaneously.

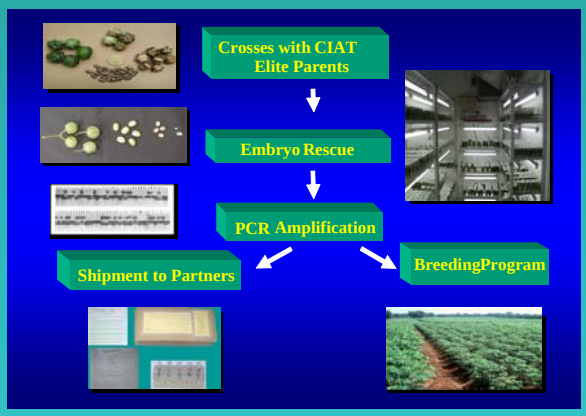

Fig 3. Scheme for Molecular Assisted Selection (MAS) for cassava Improvement

FORMATO EVALUACIÓN PARA MAS.				EVALUACIÓN:	CMD	PLACA No9			
ITEM	CODIGO	MADRE	PADRE	NÚMERO DE FRASCOS CC	EVALUACIÓN SSRY 158	EVALUACIÓN SCAR RME 1	POSO No.	PARA CAMPO/ENVIO	CÓDIGO TEJIDO
1	CR52A-32	C-243	SM1219-9	2	-	S	1		998
2	CR52A-37	C-243	SM1219-9	2	R	R	5	YES	1003
3	CR52A-38	C-243	SM1219-9	2	R	S	6	NO	1004
4	cr52a-39	C-243	SM1219-9	2	R	R	7	SI	1005
5	CR52A-40	C-243	SM1219-9	2	R	R	8	SI	1006
6	CR52A-41	C-243	SM1219-9	2	R	R	9	SI	1007
7	CR52A-43	C-243	SM1219-9	2	R	R	11	SI	1009
8	CR-52B-1	SM1219-9	C-243	2	'	S	12	NO	1010
9	CR53-2	C-243	MCOL 2206	2	R	S	13	NO	1012
10	CR53-3	C-243	MCOL 2206	2	R	R	14	SI	1013
11	CR53-4	C-243	MCOL 2206	2	S	R	15	NO	1014
12	CR54A-1	C-243	MTAI 8	2	S	R	16	NO	1015
13	CR54A-2	C-243	MTAI 8	2	R	S	17	NO	1016
14	CR54A-3	C-243	MTAI 8	2	R	R	18	SI	1017
15	CR54A-5	C-243	MTAI 8	2	R	R	20	SI	1019
16	CR54A-7	C-243	MTAI 8	2	R	R	22	SI	1021
17	CR54B-1	MTAI 8	C-243	2	S	S	23	NO	1022
18	CR54B-3	MTAI 8	C-243	2	S	S	24	NO	1024
19	CR54B-9	MTAI 8	C-243	2	S	S	25	NO	1030
20	CR54B-17	MTAI 8	C-243	2	S	S	29	NO	1038
21	CR54B-31	MTAI 8	C-243	2	R	R	30	SI	1052
22	CR54B-32	MTAI 8	C-243	2	R	R	31	SI	1053
23	CR54B-38	MTAI 8	C-243	2	R	S	32	NO	1059
24	CR54B-40	MTAI 8	C-243	2	R	S	33	NO	1061

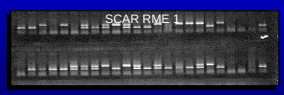

Table 4. The cassava MAS data management system in Microsoft excel, the spreadsheet shows part of a 96-well plate molecular markers (NS158 and SCAR RME 1) analysis, and permits easy access to molecular, phenotypics, diagnostic and other types of data.

CONCLUSIONS AND ONGOING WORK

MAS for CMD resistance breeding have been initiated at CIAT. More than 1,100 genotypes have been processed last year and it is expected that three times that number will be processed this year as the entire system from crosses to embryo rescue to molecular analysis becomes more efficient.

Future perspectives include development of a 96-well method for grinding leaf tissue and DNA isolation to eliminate the need for time-consuming transfers from eppendorf tubes to 96-well plates. Another objective is to obtain new molecular markers (PCR-based) associated with genes controlling traits of agronomic importance.

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ACKNOWLEDGEMENTS

The Rockefeller Foundation provided funds for this project, we thank the cassava productivity (IP3) project for generating the crosses, and the agrobiodiversity project (SB2) for assistance with molecular analysis.

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