INTRODUCTION

Molecular marker-assisted selection (MAS) for CMD resistance at CIAT is both a prerequisite 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 TME2 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 sp. 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 polyacrylamide gel (19:1 acrylamide : bisacrylamide) 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.

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 appropriate software 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 in 4 days. This includes 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.

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.

REFERENCES


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