Implementation of a cryopreservation system to establish a duplicate of the cassava core collection

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INTRODUCTION

Cryopreservation is considered an alternative for the establishment of germplasm collection duplicates for long-term storage. This methodology needs to be synchronized with other in-vitro conservation techniques, so they serve as back ups for ex-situ germplasm conservation. The Encapsulation-dehydration technique has been shown to be the optimal method to preserve most of the cassava core collection (approx. 640 clones). Based on their after-freezing response, cassava germplasm has been classified within three groups of response, in which 65% of the core collection has shown up to 30% recovery, in the form of shoot formation after freezing. In the current cryopreserved germplasm we maintain at least 4 tubes per clone in liquid nitrogen. Morphological and molecular monitoring of recovered clones shows no evidence of change compared to no-frozen controls. The logistical aspects for handling a cryopreserved bank, such as the number of beads and tubes per clone, and their position in the tank, are well established. A database has been developed allowing quick identification and location in the tank. Today we focus our attention in improving the response of more recalcitrant clones (35%) to achieve success with the entire collection.

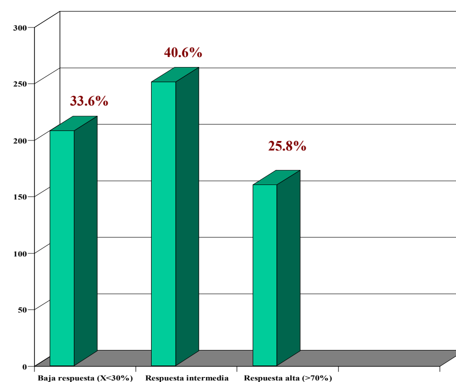

Figure 1: Tissue's response after cryopreservation using the core-collection located at CIAT as a model using the Encapsulation-dehydration method. Total material cryopreserved = 619

MATERIALS AND METHODOLOGY

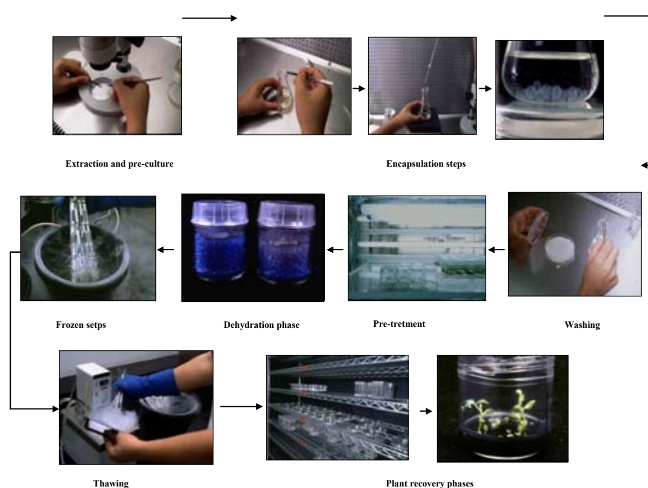

Figure 2:Screen of the CryoGen programm developed for the management of a pilot cryopreserved cassava-collection. Programm developed by Ants web.

RESULTS AND DISCUSSION

1. We consider as a minimum-value of tissue's response a 30% as plant recovery after freeze phase. Based on response of the cassava core collection, we could consider that this methodology could be apply to most of the genotypes (66.4%) (Figure 1).

2. It was established a procedure for handled the cryo-collection that include: (a) 60-100 shoots/clone distributed in 6-10 vials (b) each vial with 10 beads (c) youngest tissue's coming grown on 4E medium (Roca 1984), (D) pre-treatment on a medium supplemented with kinetin during a short-period before encapsulation it.

3. To make an inventory and monitoring of the stored-material it was developed a database written in MySQL. It allows to locate each of tubes/clone stored and make their annotation. This database combine morphological data, isozyme (α, β- esterase), location data with cryopreservation response's across conservation time on liquid nitrogen

4. The analysis of a morphological patterns, isozyme profile (α, β- esterase) and a molecular fingerprinting (using AFLP's methodology) it have been developed to determine the genetic stability in a sample after freeze it. Non-significant differences were observed between the in-vitro and frozen materials.

Figure 3: (A) Comparison between a morphology roots of a frozen and an in-vitro plants (B) electrophoretic pattern obtained by isozyme α-β-esterase (each clone compares 2 lines: in vitro-frozen).

PERSPECTIVES

(1)Implement our cryo-protocol with the entire collection to determine the clonal effect, (2) Establish a duplicate of the collection (2) To establish a protocol to handled low responding materials