Implementation of the encapsulation-dehydration cryopreservation method for the cassava core collection

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Introduction

The encapsulation-dehydration technique allows the direct placing of cassava meristematic tissues into liquid nitrogen, thus avoiding the use of expensive equipment and opening the possibility for large-scale, long–term conservation at low cost (Escobar *et al.*, 2000). We are testing reproducibility between replications and conservation time in liquid nitrogen as steps to determine logistical aspects in the management of an *in vitro* gene bank.

Methodology

We applied to an MTA agreement to obtain a full copy of the Core collection. Then these materials were propagated on 4E medium (Roca, 1984) until enough shoots were obtained for cryopreservation experiments. The number of sixty (at least) to 100 shoots per clone is reached before starting an experiment. Ten clones are considered per experiment per day, and 2 days per week with cryopreservation activities. Escobar *et al.* reported on the methodology in 2001.

Results

The initial response with tissue frozen for 1 hour (control) should be monitored in order to discover the feasibility of keeping tissues in liquid nitrogen for long periods. We estimated that 30% should be the Minimum Shoot Recovery Rate (MSRR) (Escobar et al., 2001) as a minimum value for including and maintaining a clone under liquid nitrogen conditions. In some cases when MSRR is lower, it could be associated with the use of suboptimal tissue (Escobar et al., 2000).

MSRR helped us design a lower response group for planning future experiments and decide whether to maintain the clone under conservation in liquid nitrogen or whether it needs cryo conditions. Normally, clones with a poor MSRR in classical methods have a worse response and are considered in a recalcitrant group.

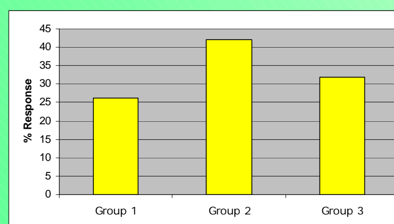
Three response groups were determine during our research: Group No. 1, clones with high shoot recovery after freezing up to 70%; Group No. 2, shoot recovery between 30% and 70%; and Group No. 3, lowest recovery rate, below 30% (MSRR). To date we have cryopreserved more than 55% of the core collection. Of the clones tested, 68% comply with MSRR requirements (Figure 1-a).

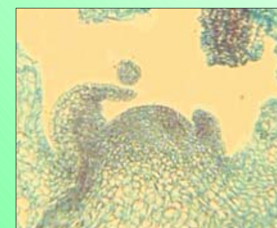
To test the consistency of the methodology across different times of permanence under liquid nitrogen, we compared two clones (MCol 22 and MPer436) from Group No. 1 with MVen 90 from Group No. 3. In all cases we observed that material frozen up to 12 months showed up to 80% recovery. The control was consistent its behavior (Table 1). Despite the huge amount of labor involved in each experiment, it is better to discard those clones that do not reach the MSRR during the initial measurement. To consider one clone for putting in the pipeline requires manpower for 4-5 propagation cycles during 3-4 months before starting cryopreservation experiments.

Table 1: Response of three Cassava clones after four different conservation times

Clone	MCol 22		MPer 436		MVen 90	
% response	Viability	Shoot	Viability	Shoot	Viability	Shoot
Conservation time						
1 hour (control)	88.1	88.1	100	87.5	88.8	88.8
1 month	100	100	91.65	78.75	95	95
6 month	96	96	100	83	95	95
12 month	100	100	96	92	92	80
Fisrt Report	95	85.45	94.1	79.7	76.7	50

Figure 1-A: Distribution of response of 348 cassava from the core collection (1B) Histology of frozen shoot.





Recovered shoots were observed under light-microscopy and observed in all cases regrown after freezing from initial explants (Figure 1-B); adventitious formation was not observed. In the last CBN Meeting, our group presented some advances in FEC cryopreservation. Based on that work, we initiated collaborative efforts with Danfort Center to test the reproducibility of this technique with nine African cultivars.

Conclusions

Three hundred and forty-eight cassava clones (55.3% of the core collection) were tested with encapsulation dehydration. Based on project results over time, we built a group of 10 clones that, independent of conditions or adjustments, never show better response after freezing. As an alternative method, we are considering work with vitrification procedures.

The CBN meeting allowed the implementation of collaborative research on cryopreservation topics.

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