Survival is insufficient, a cryopreservation case study on cassava

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Introduction

Cassava is a tuberous root crop and the **staple food** for nearly a billion people. Its **resistance to drought** and its ability to grow on poor soils makes it a **very versatile crop** and a good crop to combat food insecurity in the tropics.

Genebanks play an important role in this system since they **store the material** and **provide farmers access** to a huge variety of cultivars. This can be done in many ways: field banks, in vitro collections, etc. But the best way to **store** material safely for **the long term** is **cryopreservation.** Such a storage method is however, not optimized yet for cassava.

Materials and methods

A variety of methods is available to cryopreserve plant material, but the most applied one to conserve meristems of clonally propagated plants is the **droplet vitrification protocol.** This method was originally developed for bananas (Panis et al., 2005) but is now also used in other crops such as potato and yam. In this method, **meristems** are **excised** and **treated** with different **cryoprotective solutions** before they are **stored in liquid nitrogen**.

Among other parameters, **concentrations** and application time of the cryoprotective solutions need to be optimized for each plant and tissue. In our experimental setup **different parameters** such as the cultivar, loading solution, meristem position and preculture **were varied** (see Figure 1). The survival of the meristems was measured 1 month after cryopreservation while the regeneration was measured one month later.

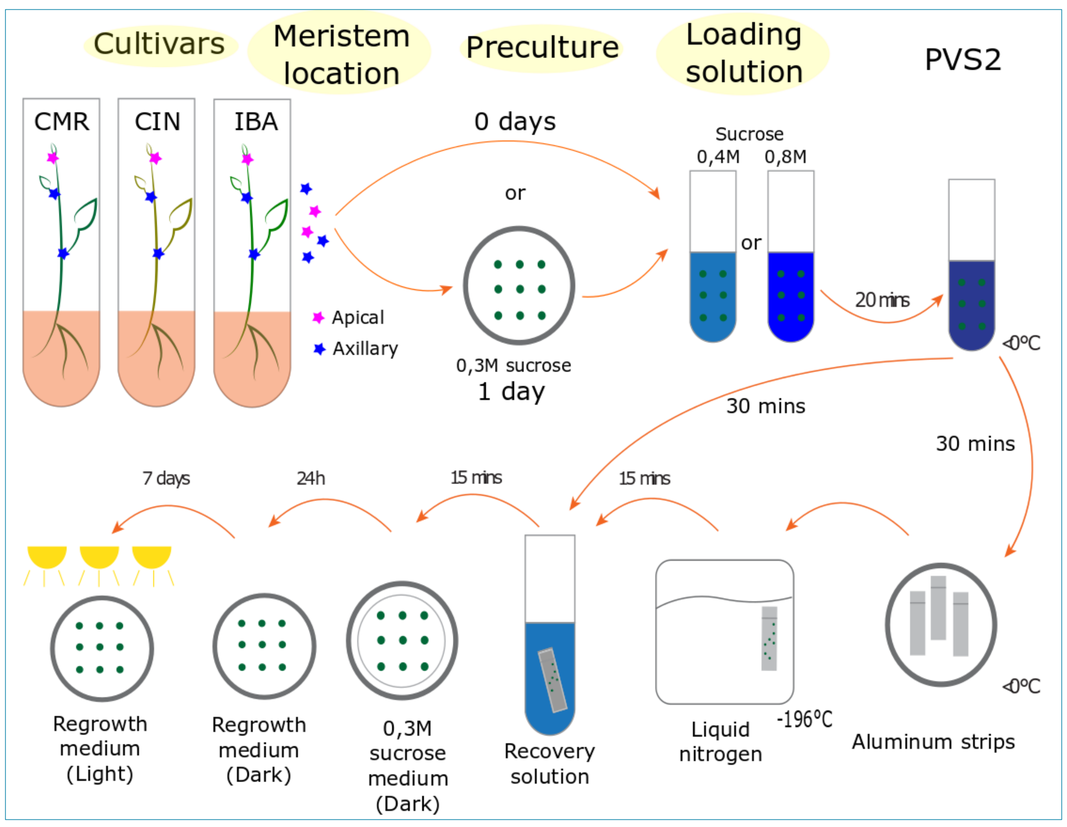

Figure 1: The droplet vitrification procedure that was used to cryopreserve the cassava plant material. The parameters that were varied, are highlighted in yellow.

Results

Observation after 1 month

After one month, the survival rate of the meristems was measured. These data were analyzed with ANOVA to determine which parameters had an influence on the survival rate (table 1).

Table 1: ANOVA test to check if the parameters influence the survival rate of cassava meristems after cryopreservation

Source	LogWorth	PValue
Accession	18,963	**0,00000**
preCulture	5,531	**0,00000**
Meristem	2,587	**0,00259**
Accession*Meristem*Loading	2,160	**0,00692**
Accession*Meristem*Loading*preCulture	1,546	**0,02844**
Loading	1,107	0,07822
Accession*Loading*preCulture	1,103	0,07881
Accession*Loading	0,888	0,12947
Accession*Meristem	0,880	0,13193
Accession*preCulture	0,771	0,16940
Accession*Meristem*preCulture	0,544	0,28608
Meristem*Loading	0,306	0,49433
Meristem*preCulture	0,222	0,59969
Loading*preCulture	0,130	0,74080
Meristem*Loading*preCulture	0,005	0,98744

All parameters but the loading solution had a significant influence on the survival of the meristems**.** The **best method** when considered over all cultivars combined was "**no preculture**" and "**axillary meristems**" which gave **survival of up to 90%.**

Observations after 2 months

After two months, **most** of the meristems **turned brown** (figure 2) and stopped growing. **Less than 5%** of the meristems that survived, turned into **rooted and growing plantlets**.

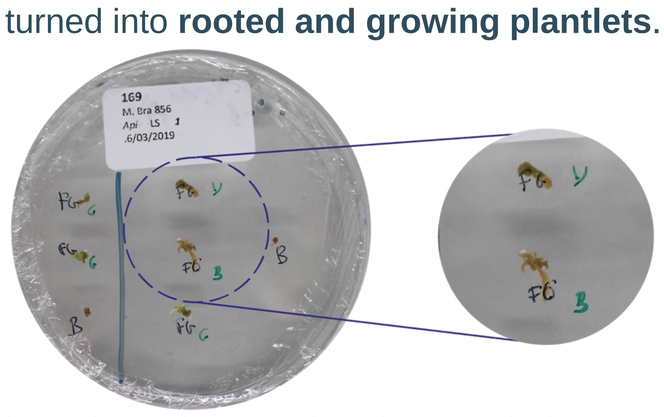

Figure 2: Browning meristems that stopped growing, 1 month after cryopreservation

Conclusion

Although most of the meristems, that are subjected to liquid nitrogen, survive the treatment, almost none of them grew into normal rooted plantlets. A high plant regeneration rate, however, (>50%) is a requirement to establish a cryopreserved collection. Since both controls and cryopreserved meristems show the same kind of browning (see also figure 2), we assume that the cryopreservation itself is not the problem. Therefore future research will focus on optimizing the regeneration conditions. To stop the browning after one month by formulating new regeneration media.

References

Panis, B., Piette, B., and Swennen, R. (2005). Droplet vitrification of apical meristems: a cryopreservation protocol applicable to all Musaceae. Plant Sci. 168, 45-55

Acknowledgments

We gratefully acknowledge the Gene Bank CGIAR Research Programme and the CGIAR Research Programme on Roots, Tubers and Bananas (RTB) and the Directorate-General for Development, Belgium (DGD) for financial support of the project ‘Safeguarding vegetatively-propagated crop diversity to nourish people now and in the future’. This project was funded by DGD (Directorate-general Development Cooperation and Humanitarian Aid, Belgium).

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