Effectiveness of Thermotherapy and Cryotherapy in Eliminating Viruses from *Ullucus tuberosus* Caldas

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Introduction

Ullucus tuberosus Caldas (Pictures 1 & 2) known as ulluco, melloco or papa lisa, is the second most widely cultivated Andean root and tuber crop (ARTC) in the Andean region of South America. It is crucial for regional food security and holds significant importance in the area (Hermann 1997, Campos et al. 2018). The International Potato Center (CIP) Genebank currently preserves 432 ulluco accessions. Given the vast morphological and genetic diversity of these accessions, preserving and ensuring their accessibility is a key focus of the Genebank's efforts (Manrique et al. 2017).

Ulluco is a clonally propagated crop and is conserved and distributed as *in vitro* plantlets. To provide global access to plant genetic resources for research, education, and breeding, all accessions must be tested and confirmed virus-free before distribution (International Potato Center, n.d.). Therefore, identifying the most effective virus elimination therapy at CIP is essential.

This study assessed the effectiveness of two methods, thermotherapy with meristem cultivation and cryotherapy, in cleaning eight ulluco accessions in vitro plant material. The main objective was to obtain at least one virus-free line (biological repetition) per accession, which can then be multiplied and preserved in the Genebank for future distribution.

Picture 1: Tuber diversity of *Ullucus tuberosus* Caldas

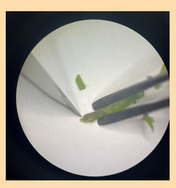
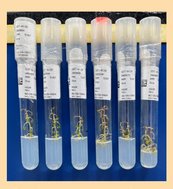
Picture 2: An *Ullucus tuberosus* Caldas plant

Materials & Methods

Eight accessions of *Ullucus tuberosus* Caldas *in vitro* plant material were used for the study. First, virus detection before therapy was performed through High Throughput Sequencing (HTS) of small RNA libraries, using VirusDetect Windows (VDW) as the bioinformatics tool.

Afterward, each accession was sub-cultivated into six lines (each line being a biological clone of the original plant). Three lines underwent thermotherapy and meristem culture, while the other three underwent cryotherapy. Virus detection after therapy was performed using HTS and analyzed with the VDW bioinformatics tool.

Thermotherapy with meristem cultivation: (Mori K. 1971, Golmirzaie, Panta, and Toledo 1999)

1 Thermotherapy 32-37ºC for a month → 2  Meristem isolation (0.2 - 0.35 mm) → 3  Cultivation & multiplication → 4 Si-RNA library preparation (a)

Cryotherapy (According to cryopreservation: Vollmer et al. 2021)

1 Excision of shoot tips (0.8-1.2mm) → 2 Freezing in liquid nitrogen → 3 Thawing of shoot tips → 4  Cultivation & multiplication → 5 Si-RNA library preparation*

*preparation of Si-RNA library according to Chen et al. 2012

Results & Discussion

Prior to virus elimination therapy, we identified nine different virus genera across the eight accessions, with potexvirus being the most prevalent and caulimovirus and tepovirus being the least common (Fig. 1). Notably, after cryotherapy, a mitovirus was detected more frequently than before therapy. Additionally, thermotherapy combined with meristem culture led to the detection of a tymovirus, which was not previously observed (Fig. 1). These findings indicate a need for further investigation.

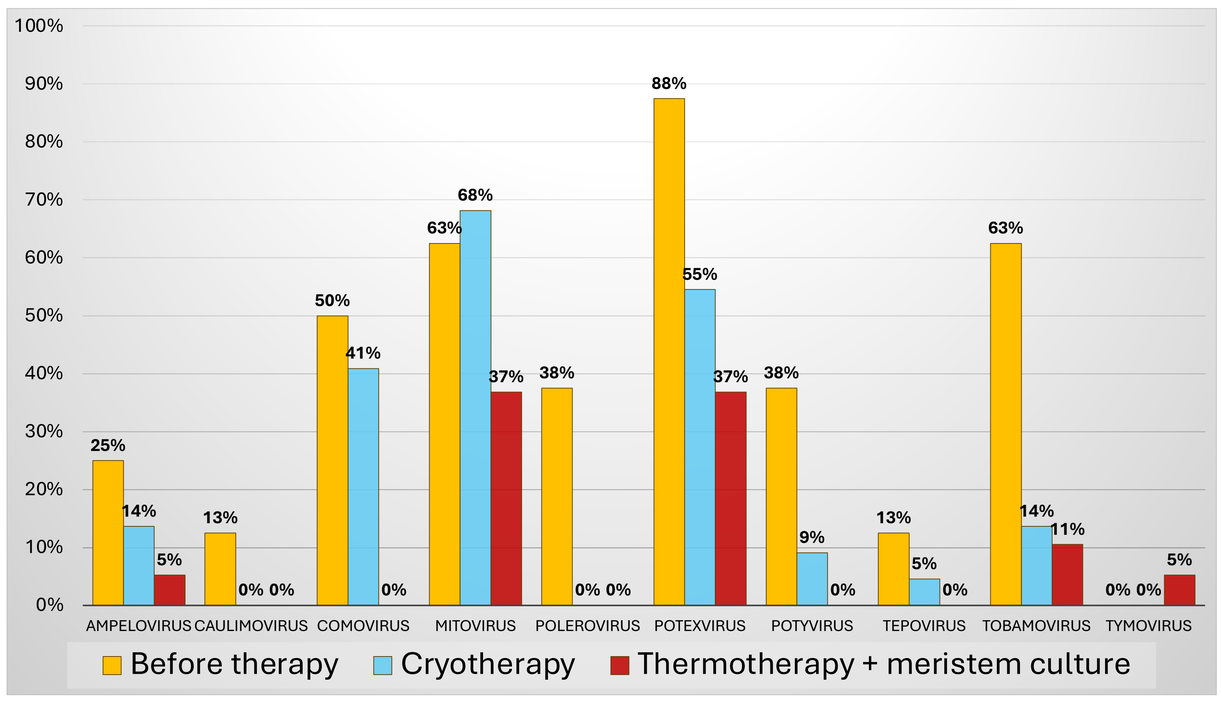


Figure 1: Number of virus-genera present in all the lines. Numbers were calculated with the sum of each genus present per line.

Genus	Before therapy	%	Cryotherapy	%	Thermotherapy + meristem culture	%
ampelovirus	2	25%	3	14%	1	5%
caulimovirus	1	13%	0	0%	0	0%
comovirus	4	50%	9	41%	0	0%
mitovirus	5	63%	15	68%	7	37%
polerovirus	3	38%	0	0%	0	0%
potexvirus	7	88%	12	55%	7	37%
potyvirus	3	38%	2	9%	0	0%
tepovirus	1	13%	1	5%	0	0%
tobamovirus	5	63%	3	14%	2	11%
tymovirus	0	0%	0	0%	1	5%
TOTAL Lines	8		22		19	

Table 1: Percentage of virus-genera present in the lines after one cycle of therapy.

	Number of accessions with at least one virus-free line	%
Thermotherapy + meristem culture	6	75%
Cryotherapy	2	25%

Table 2: Percentage of virus-genera present in the lines after one cycle of therapy.

Table 1 clearly illustrates the effectiveness of the two therapies. Thermotherapy combined with meristem culture eliminated five virus genera entirely and reduced the presence of one additional genus to 5% after a single treatment cycle. In contrast, cryotherapy completely eliminated only two virus genera and reduced the presence of one more genus to 5% (excluding tymovirus results, which require further investigation).

The primary objective was to obtain at least one virus-free line per accession. This was achieved for six accessions after one cycle of thermotherapy with meristem culture, representing a 75% effectiveness rate (Table 2). In comparison, only two accessions achieved a virus-free line after one cycle of cryotherapy, resulting in a 25% effectiveness rate (Table 2).

Conclusion

Thermotherapy combined with meristem culture has proven to be the most effective method for eliminating several virus genera from ulluco. Utilizing this therapy allows us to effectively clean ulluco plant material, advancing our progress toward global *in vitro* distribution of these plant genetic resources. This step enhances food security and supports the preservation of this valuable genetic resource.

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