

Three decades of research in micropropagation and *in vitro* conservation in Albania:

Now entering a new era with cryopreservation

EFIGJENI KONGJIKA¹, VALBONA SOTA^{1,2}

¹Research Center of Biotechnology and Genetics, ASA

²Department of Biotechnology, Faculty of Natural Sciences, University of Tirana

**CRYOCONNECT: KICK-OFF MEETING
TIRANA, ALBANIA
25 JUNE, 2025**

Micropropagation and *In Vitro* Conservation in Albania



Operating Laboratories *in Plant In Vitro Biotechnology*



Plant Tissue Culture Laboratory
Department of Biotechnology,
Faculty of Natural Sciences, University of Tirana

Plant Tissue Culture Laboratory,
Research Center of Biotechnology and Genetics,
Academy of Sciences of Albania

Plant Tissue Culture Laboratory,
Agricultural Technology Transfer Center, Vlora
Ministry of Agriculture and Rural Development

PLANT TISSUE AND CELL CULTURE LABORATORY, INSTITUTE OF BIOLOGICAL RESEARCH (IBR), ACADEMY OF SCIENCES OF ALBANIA (1993 – 2008) – HEADED BY ACAD. EFIGJENI KONGJIK

The first step (1993) was prompted by a specialization in the Laboratory of Plant Biology of the University "Pierre et Marie Currie" in Paris.

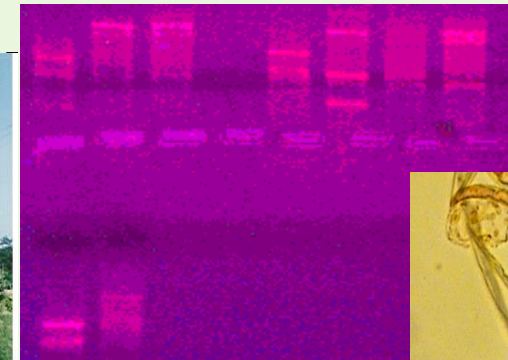


The research and technical group (IBR, ASA)

Micropropagation

Meristem culture

Embryo culture



***In vitro* genetic collections**

SOME NATIONAL, REGIONAL AND EUROPEAN SCIENTIFIC PROJECTS – IBR, ASA

The aim of the projects of IBR, AS:

Preservation and use of autochthonous floristic assets, especially those with unique value and economic interest in common structures to avoid genetic erosion



Malus domestica



Pyrus communis



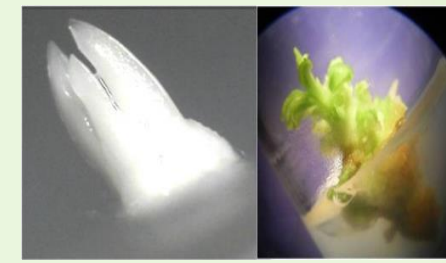
Prunus domestica



Prunus avium



Juglans regia



Prunus vebbii



Gjeçe



Vjeshtore



Tropojane



Belicë e kuqe



Frutmadh Nepravishta



Pocem

- "Use of new biotechnological methods for micropropagation, production of free-diseases plants, genetic improvement, preservation and enrichment of germplasm of some cultivated plants and spontaneous species with importance for conservation of autochthon biodiversity" 1999 - 2001 (National Program for Research and Development "Biotechnology and Biodiversity“;
- "Study Center for the protection and conservation of botanical species of Mediterranean area, with annex Botanical Garden", (1999-2001), European Community Programme INTERREG II Italy-Albania, Italian partners: University of Lecce and Bari, CIHEAM, Bari;
- "Use of biotechnological method of micropropagation and "in vivo" propagation for the production of rootstocks of stone fruit trees", Protocol of Scientific and Technological Cooperation Italy – Albania, (2002-2004). Experimental Institute of Fruit Trees, Rome, Italy.

SOME NATIONAL, REGIONAL AND EUROPEAN SCIENTIFIC PROJECTS – IBR, ASA

The aim: Use and preservation of some under utilization fruit trees species in Albania

Today, these species are the focus of research due to:

- re-interest in traditional local foods and "forgotten" nutritional sources;
- the concept of "terroir" also related to cultural heritage;
- the potential of these products with nutritional and medicinal value.

From an economic point of view, this source of germplasm should be discovered, evaluated, conserved and used, especially the forms with a high production level and for the quality of Mediterranean dietary ingredients and with therapeutic value.

In addition, these species are of great interest for the development of biological horticulture and eco-tourism in mountainous and hilly areas, so necessary in the conditions of the progress of eco-tourism.

- **“Improvement and conservation of some under utilization fruit trees species in Albania”, (2005-2007), Italian Coordinator: Experimental Institute for Fruit Trees, Rome, Italy;**
- **“Biodiversity Evaluation in molecular level, characterization of abiotic stress tolerance, phytosanitary control, „ex situ“ and „in situ“ conservation of some under-utilization spontaneous fruit tree species, with special status and with economic and environmental values, 2007 – 2009, (NPRD "Biotech & Environment");**



Punica granatum



Devedishe



Myrtus communis



Mersina Qenam



Morus alba



Mani Karkavec



Zyziphus jujuba



Hide vendi

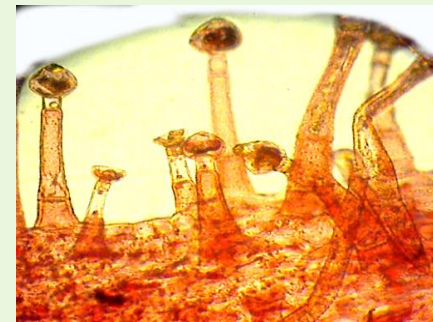
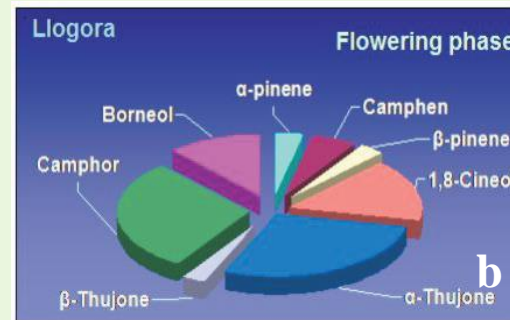
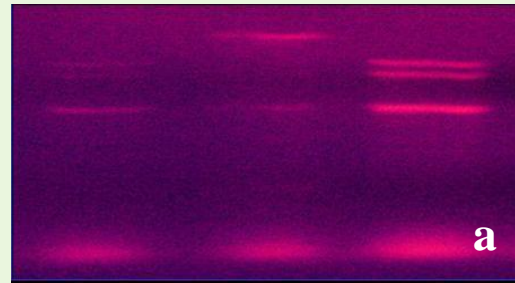
SOME NATIONAL, REGIONAL AND EUROPEAN SCIENTIFIC PROJECTS – IBR, ASA

The aim: Use and preservation of some plant genetic resources of national interest (aromatic-medicinal species)

- "Potential evaluation, identification of biodiversity in molecular level, propagation *in vivo* and *in vitro* with aim the cultivation of chosen chemotypes of some aromatic-medicinal plant species in poor soils of Albania", 2003-2005, (National Program for Research and Development "Biotechnology and Biodiversity,
- Project "CERATONIA – (CConservation of EEndemic RARities and TTransborder OOrganism for NNature of IItaly and AAlbania) "Progetto esecutivo e piano di gestione dell'Organismo Intergovernativo Mediterraneo per la ridiffusione in ambito mediterraneo di specie endemiche terrestri ed acquatiche", (2006-2008), Programma di Iniziativa Comunitaria PIC INTERREG III A Italia – Albania, Asse II – Misura 2.1 – Azione 3 (Universita degli Studi di Salento e di Bari.



Collection of 54
Albanian aromatic-
medicinal populations
(sage, oregano),
Valenzano, Bari
University)



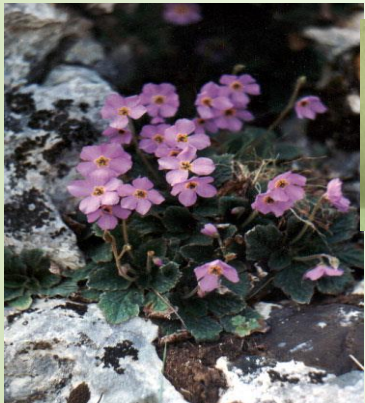
(Labs of IBR, ASA)

a – SSSR, molecular markers; b- essential oils, chromatography; c- trichome variation, histology; d –micropropagation; e – *in vitro* collection

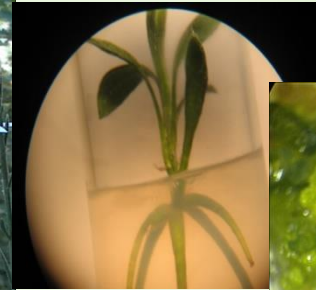
SOME NATIONAL, REGIONAL AND EUROPEAN SCIENTIFIC PROJECTS – IBR, ASA

The aim: Use and preservation of some endemic, rare and threatened species)

- *In vitro* cultivation and germplasm preservation of rare and spontaneous Albanian plant species”, “Friedrich-Schiller” University of Jena, Institute of General Botany, Germany (DAAD), 1997;
- “Propagation, sanitation, germoplasm conservation and genetic improvement of some spontaneous threatened species by biotechnological methods of *in vitro* culture and conventional methods”, 2005 – 2007, (Academy of Sciences);
- “Conservation and investigation of the biodiversity of species from the Gesneriaceae family (*Harberlea* sp., *Ramonda* sp.) occurring in different habitats on the Balkans by establishing an *in vitro* gene bank”, Program of National Science Fund of Bulgaria, Department of Plant Physiology and Molecular Biology, University of Plovdiv, 2008 – 2012.



Ramonda serbica Pany.



Ramonda serbica, Albania, Kosovo

Forsythia europaea, Albania, Kosovo

Aster albanicus subsp. *paparistoi*, Albania

PhD – Prof. As. Bekim Gashi «Biomorphological, physiological characterization bio and *ex situ* conservation of germplasm of "resurrection" species of *Ramonda* genus in Kosovo and Macedonia»

These species can be used as ornamental plants and propagated by *in vitro* culture. The extreme drought tolerance and the geographic isolation of *Ramonda* genus make this species the excellent candidates for isolation of genes involved in the mechanism of drought resistance.

During the 2008 reform in science, the Department of Biotechnology, FNS, UT was established, by incorporating all infrastructure and human resources within its composition.

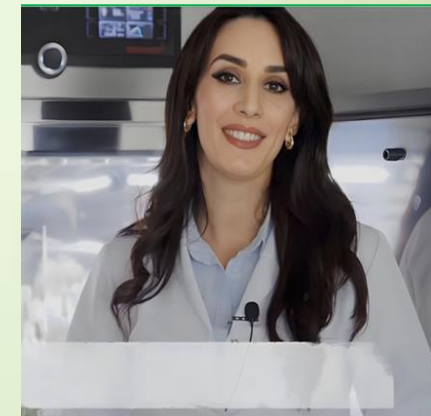
The infrastructure that has undergone improvements in recent years (Project "Enrichment of the *in vitro* Cultures Laboratory, in the service of mass and quality production of fruit trees, required by farmers", within the Program “Scientific Research Infrastructure for 2021”, National Agency for Scientific Research and Innovation).

Department of Biotechnology, FNS, UT

4 study programs:

1. Bachelor in Biotechnology
2. Scientific Master on Molecular and Industrial Biotechnology
3. Professional Master on Environmental Biotechnology
4. PhD on Biotechnology

Ongoing collaboration with
the Academy of Sciences of Albania



BILATERAL SCIENTIFIC PROJECTS - ASA; DEP. OF BIOTECH, UT; ATTC, VLORA

“Improvement in the large-scale micropropagation of some economically-important fruit species by the innovative technology of liquid culture in temporary immersion system, (2018 – 2019), Agreement for Scientific Cooperation between Italy –Albania, (CNR – MES), CNR-IVALSA (IBE) and Academy of Sciences of Albania, Coordinators: M. Lambardi, E. Kongjika



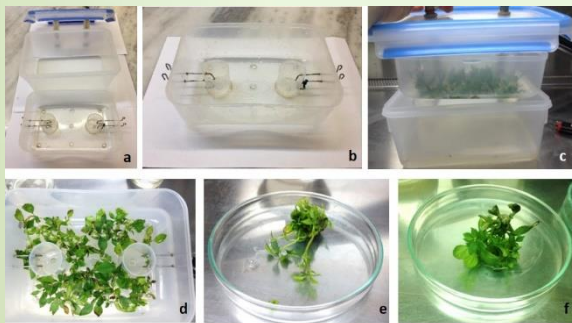
**Albanian-Italian team,
ASA Conference**



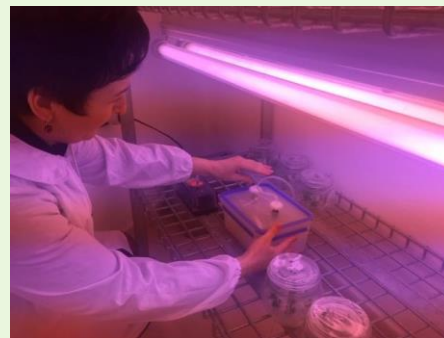
**Azienda Agricola “Meristema”, Cascine di Buti (PI),
Prof. M. Lambardi, Dr. C. Benelli**



**Round table in CNR, Florence
about TIS technologies**



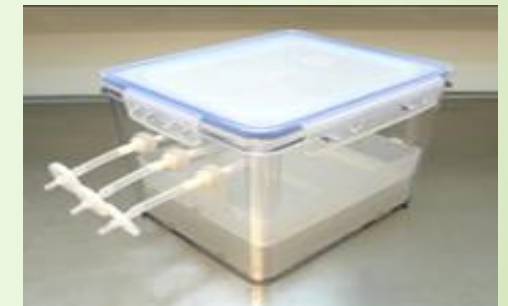
Bioreactor ElecTIS, DB, FNS, UT



ElecTIS, ATTC, Vlora,



Bioreactor SETIS, DB, FNS, UT



Bioreactor PlantForm, DB, FNS, UT

These innovative methods adopting practices to reduce the unit cost of micropropagule, at the same time, guaranteeing a very high level of plant quality, will be used in our projects related to the nursery business for the micropropagation of autochthonous fruit tree species.

The new Scientific Research Center "Biotechnology & Genetics" recently established near the Academy of Sciences (Decision of the Presidency of the Academy of Sciences No. 20, dated 28.02.2020) aims to:

- (i) coordinate scientific research in the field of biotechnology and genetics;
- (ii) highlighting the current state of research in Albania and the region at the scientific, logistical and intellectual level;
- (iii) establishing cooperation with research and scientific centers for joint projects, publications, conferences.

The Unit (2020) transformed in Center (2024) is considered as a concrete option for the promotion of challenges for a new model of economic development focuses on:

- 1. The organization and coordination of the Plant Biotechnology Network** with institutions inside and outside the country and with business to establish a contemporary scientific basis for the certification system of fruit tree saplings with national importance, and as one of the legal requirements of the European market, especially now when Albania is at the beginning of the EU membership process;
- 2. Establishment of Biotechnology and Plant Molecular Biology Laboratories** near the Center for two main directions:
 - the establishment of the Cryobank “Academy”, the first in Albania for the conservation of the genetic material of native fruit tree species;
 - the setting up of the Laboratory of *in vitro* cell cultures, the elicitation and use of bioreactors for production of secondary metabolites of Albanian aromatic-medicinal plants of economic importance.



Ongoing collaboration with the Academy of Sciences of Albania



Current projects

Proposed by Biotechnology & Genetics Research Unit at the Academy of Sciences:

"Production and certification of autochthonous cultivars of plum and apple with unique and competitive values in the market (VITROCERT)".

Financed by ASA, 2023 – 2026

The Plant Biotechnology Network composed by:

- the Section of Natural and Technical Sciences, ASA;
- the Department of Biotechnology, FNS, UT;
- the Institute of Plant Genetic Resources, AUT;
- the Agricultural Technology Transfer Center, Vlora;
- and the Private Nursery Rabeta;





Ongoing collaboration with the Academy of Sciences of Albania



Current projects

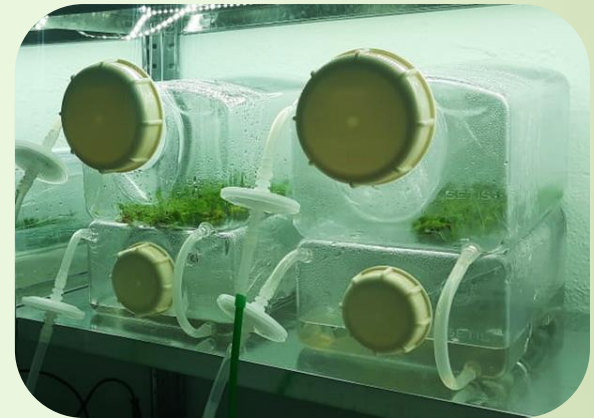
Coordinated by The Academy of Sciences entitled:

"Use of in vitro biotechnological methods and molecular biology in an attempt to obtain planting material of the "base" category of autochthonous plum cultivars"

Financed by NASRI, MoE, 2022 – 2024

The Plant Biotechnology Network composed by:

- the Section of Natural and Technical Sciences, ASA;
- the Dep. of Biotechnology, FNS, UT
- the Institute of Plant Genetic Resources, AUT;
- the Agricultural Technology Transfer Center, Vlora;
- and the Private Nursery Korani;





Ongoing collaboration with the Academy of Sciences of Albania



Current projects

Joint Research Project between CNR-IBE / Istituto per la BioEconomia and Section of Natural and Technical Sciences, Academy of Sciences of Albania:

"Safeguard of Albanian autochthonous fruit germplasm by synthetic seed technology and advanced conservation at ultra-low temperature (cryopreservation, -196°C)"

Funded by NASRI/MoES, 2022 – 2024

The Plant Biotechnology Network composed by:

- the Section of Natural and Technical Sciences, ASA;
- the Department of Biotechnology, FNS, UT;
- the Institute of Plant Genetic Resources, AUT;
- the Agricultural Technology Transfer Center, Vlora;





Ongoing collaboration with the Academy of Sciences of Albania

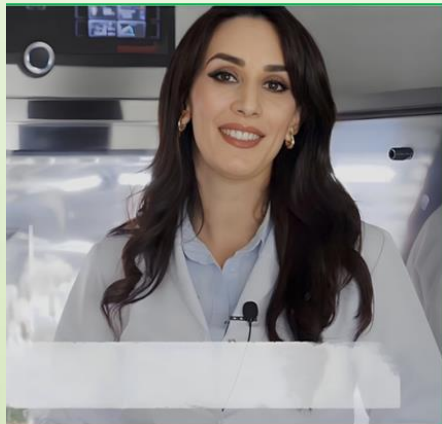


Current projects

Proposed by Biotechnology & Genetics Research Unit at the Academy of Sciences:

"The establishment of the First Cryobank in Albania for preservation of Albanian autochthonous fruit tree species (CRYOFRUIT)"

Financed by the Academy of Sciences of Albania, 2023 – 2027



Yllka Lala:
PhD thesis

The Plant Biotechnology Network composed by:

- the Section of Natural and Technical Sciences, ASA;
- the Department of Biotechnology, FNS, UT;
- the Institute of Plant Genetic Resources, AUT;
- the Agricultural Technology Transfer Center, Vlora;
- Private Nurseries

Micropropagation: Prunus domestica L. var. “Tropojane

CKX 19 – 1-(2-(2-Hydroxyethyl)phenyl)-3-(3-(trifluoromethoxy)phenyl)urea

CKX 20 – 1-(3-Chloro-5-(trifluoromethoxy)phenyl)-3-(2-(2-hydroxyethyl)phenyl)urea

CKX 52 – 1-(3,5-Dichlorophenyl)-3-(3-(trifluoromethoxy)phenyl)urea

CKX 77 – 2-(3-(3,5-Dichlorophenyl)ureido)-4-methoxybenzamide

CKX inhibitors

- 2 TIS bioreactors
- 4 basal media/each
- 4 CKX /each
- 3 immersion periods
- 3 ventilation periods
- 4 CKX inhibitors

Brunilda Cuko
PhD thesis



1. Micropropagation of plant material

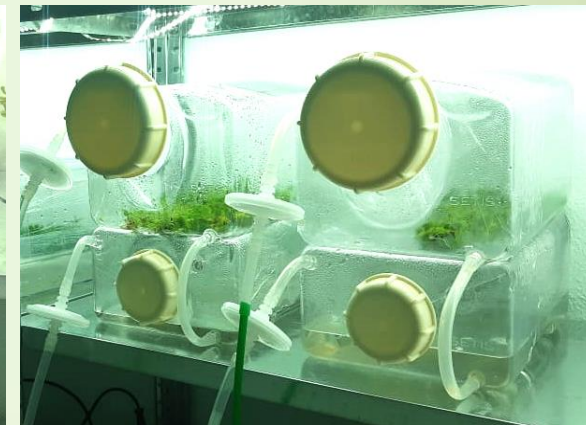
The object of these projects is preservation of the following autochthonous fruit species with nutritional and environmental values:

- *Prunus domestica* L. (plum) var. “Tropojane” and var. “Vlonjate”;
- *Punica granatum* L. (pomegranate) var. “Devedishe”
- *Vaccinium myrtillis* L. (blueberry) var. “Kukësi”.

2 plant species

1 plant species

1 plant species



2. Cryopreservation trials

1. Cryogenic storage using encapsulation – dehydration method (30, 60, 90 min)::

- Cryogenic test at -196°C for 1 hour, and regeneration at 25°C ;

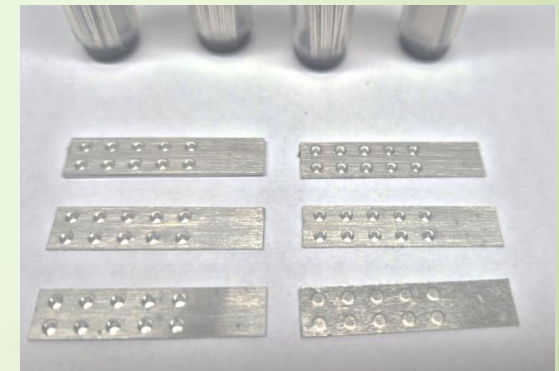
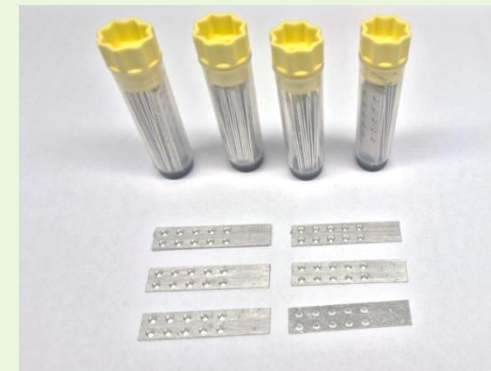
2. Storage using vitrification method (30, 60, 90 min):

- Cryogenic test at -196°C for 1 hour, and regeneration at 25°C ;

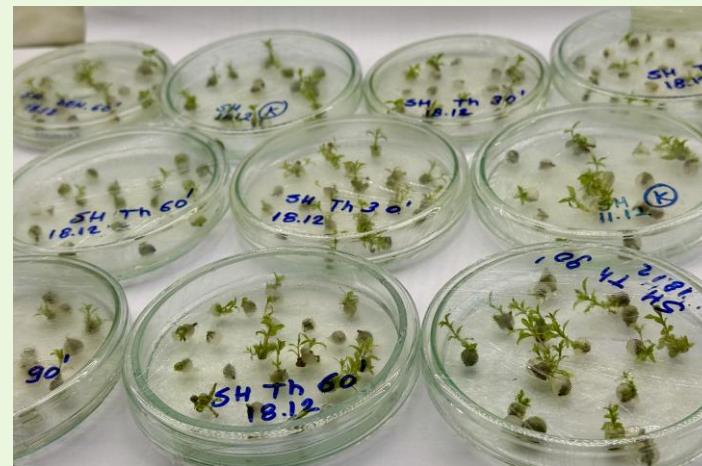
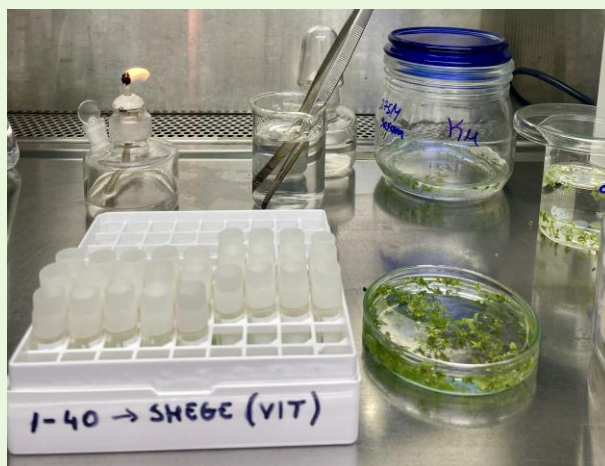
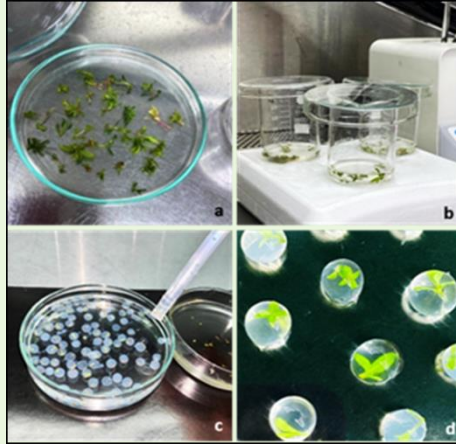
3. Storage using encapsulation - vitrification method (2 procedures of dehydration - chemical and physical one - 30, 60, 90 min)

- Cryogenic treatment at -196°C for 1 hour, and regeneration at 25°C ;

Early future - CRYOSTORAGE: Using cryoplates



2. Cryopreservation trials



Presented by:

Efigjeni KONGJICA (kongjikaef@yahoo.com)

Valbona SOTA (valbona.sota@fshn.edu.al)

