Cryopreservation of Plant Genetic Resources Under Ultra-Cold Conditions and Establishment of a National Cryobank

Erdinç OGUR, PhD

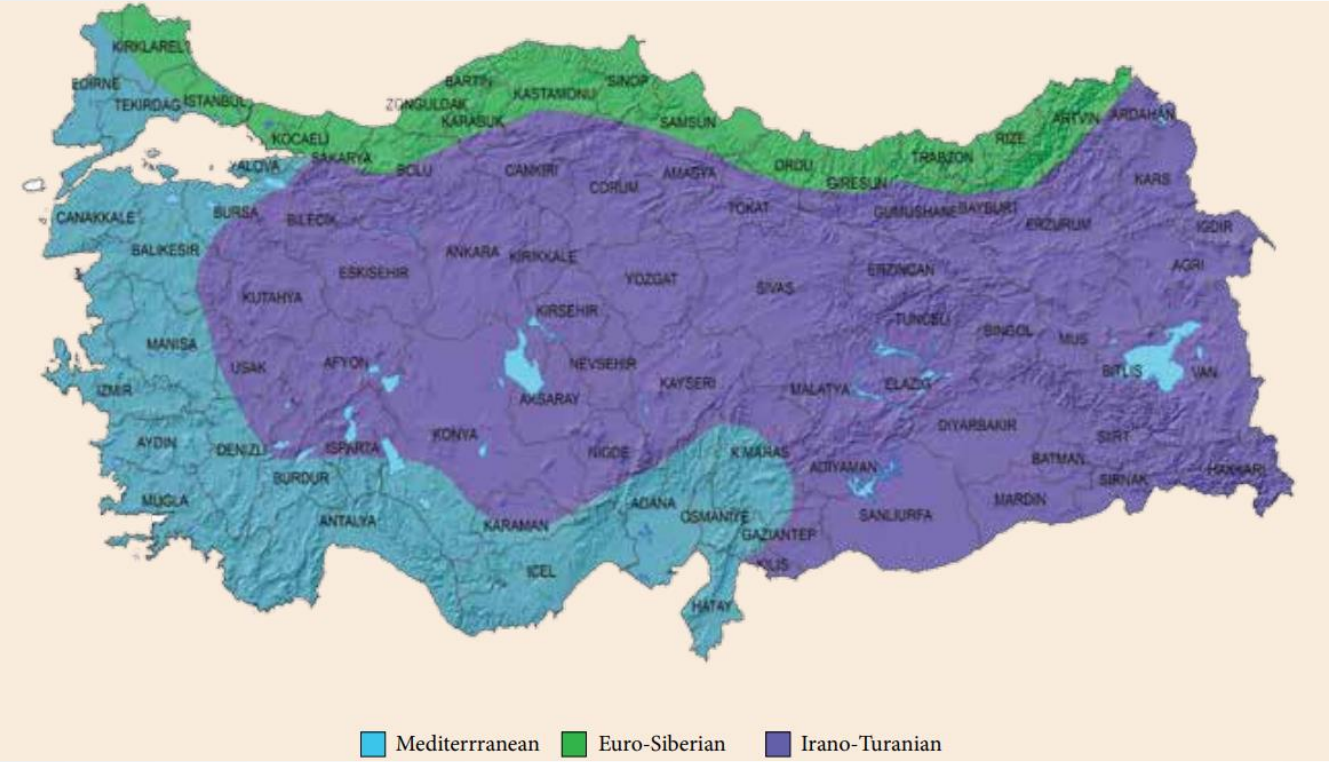
erdinc.ogur@tarimorman.gov.tr

Aegean Agricultural Research Institute Department of

Biodiversity and Genetic Resources

Menemen- IZMIR

2023



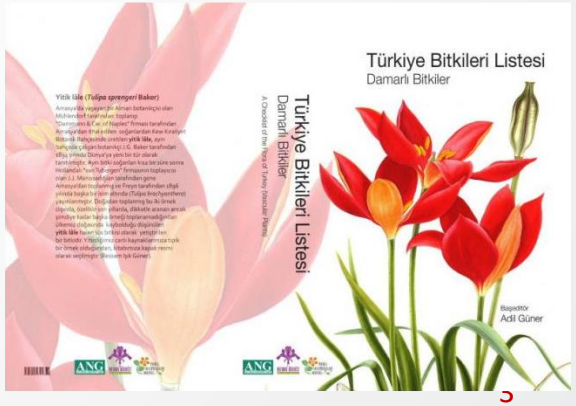
Turkey has a very rich diversity in terms of plant genetic resources.

Particularly, preserving the diversity of plant genetic resources of the cultivated species is extremely important for the sustainability of plant production.

FLORA OF TÜRKİYE

Endemic
Non-endemic

- 167 family
- 1321 genus
- 10036 species
- **11707**↑ vascular plant taxon (include subspecies, variety, hybrid)
  - 3649 (% 31,82) endemic



EX	CR	EN	VU	CD
10	83	392	584	272

LC	NT	DD	NE
468	203	207	2

In Turkey, 2221 plant species are threatened.

Conservation Strategies

Ex situ

- Seed Gene Bank
- Field Gene Bank
- (Pollen Gene Bank, DNA Bank etc.)

In situ

- Wild species and culture plants in their own growing environment
- Conservation of local varieties under farmer conditions

Biotechnological methods

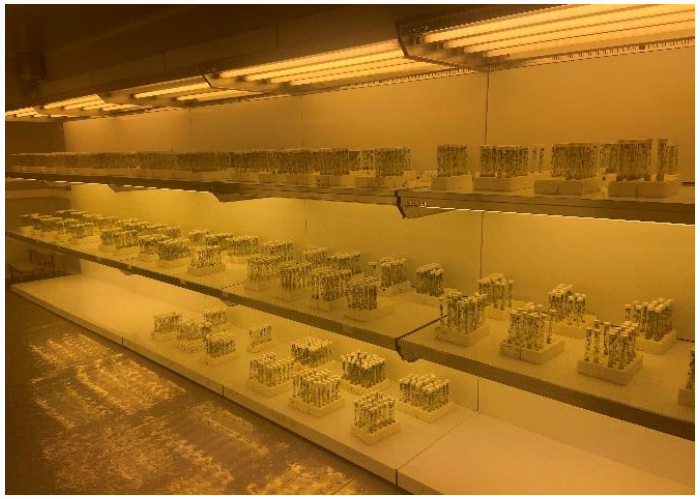
- In vitro
- Cryopreservation

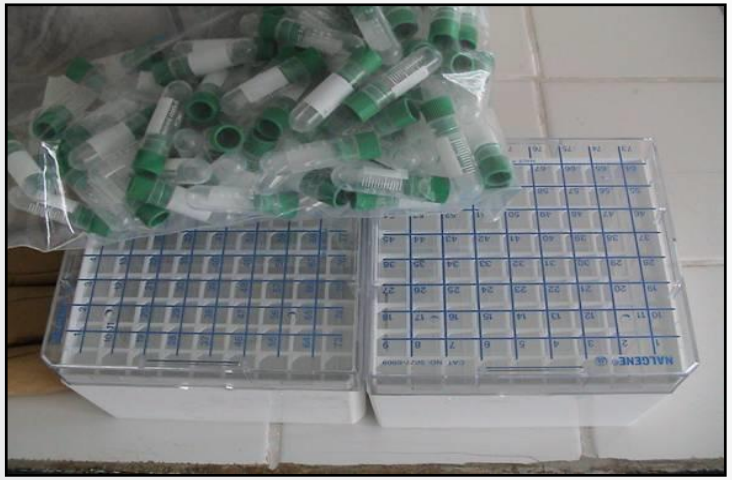
Aim of Project

While the aim is to preserve all genetic resources of our country in a cryobank, priority is given to;

- ✓ **Plants with germination problems in their seeds,**
- ✓ **Plants with recalcitrant seeds,**
- ✓ **Plants that are difficult to preserve vegetatively,**
- ✓ **Endemic or threatened plant species have priority for preservation in a cryobank**

INFRASTRUCTURE ESTABLISHMENT





- Liquid nitrogen tank
- Sterile cabinets
- Material preservation tank
- Other equipment (cryotubes, gloves, goggles, liquid nitrogen refill pump, and glass materials)
- Chemicals

MATERIAL

***Mentha x piperita* L.** (Mint)

***Allium sativum* L.** (Garlic)

Thymus cilicicus Boiss. & Bal.

Origanum sipyleum L.

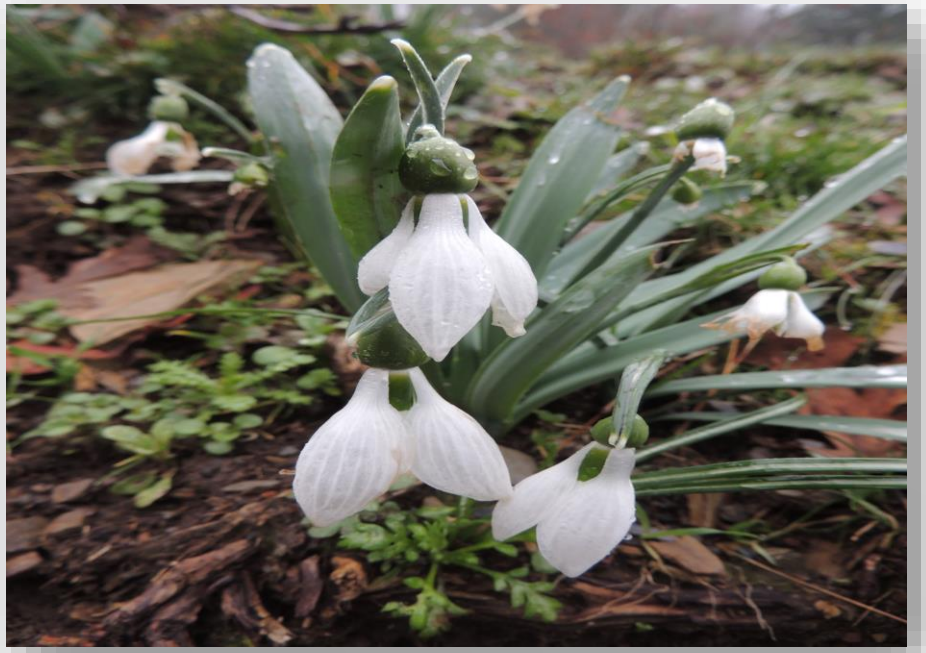
Salvia smyrnaea Boiss.

Prunus cerasifera Ehrh

Sideritis tmolea P.H.Davis

There is no existing propagation or cryo study.

Galanthus elwesii Hook.f.



METHOD

Sterilization of the material taken under field conditions and transferring it to in vitro culture.

↓

Application of the cryopreservation techniques

↓

Regeneration studies of dissolved samples

↓

Rooting and acclimatization to external conditions

↓

Measurements and observations

1- Sterilization of material collected from field conditions and in vitro culture initiation

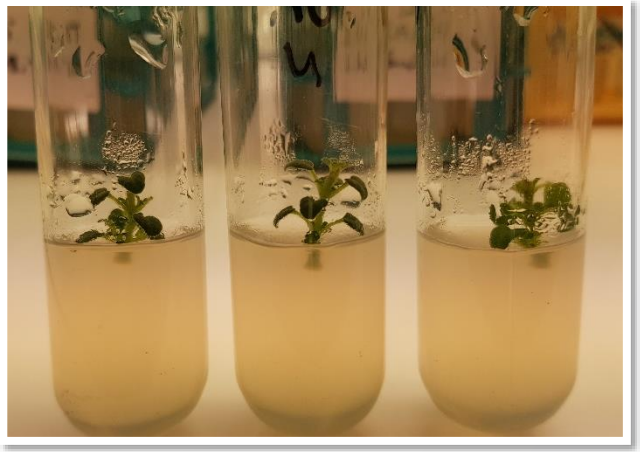
- **Surface Sterilization**
  - Rinse with running tap water for 20 minutes
  - In 70% (v/v) ethanol
  - % 20-40 hypochlorite (5% NaOCl) containing 1-2 drops of Tween-20
  - Rinse with sterile distilled water for 3 times, 5 minutes each
- **Trasfer to in vitro culture medium**
  - Shoot tips and nodal segments will be cultured on MS medium

In vitro Stock Cultures

***Mentha x piperita* L.**

Allium sativum L.

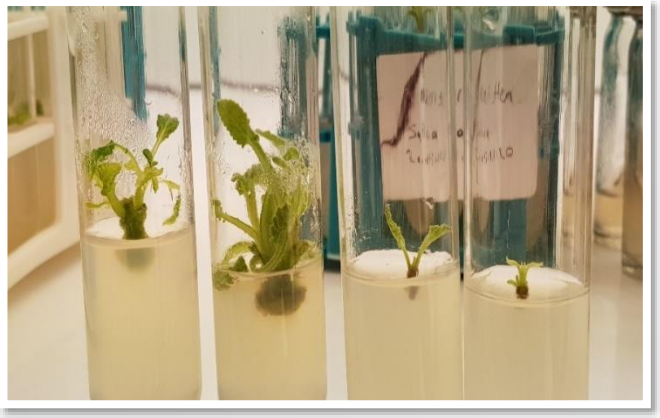
Origanum siypleum L.



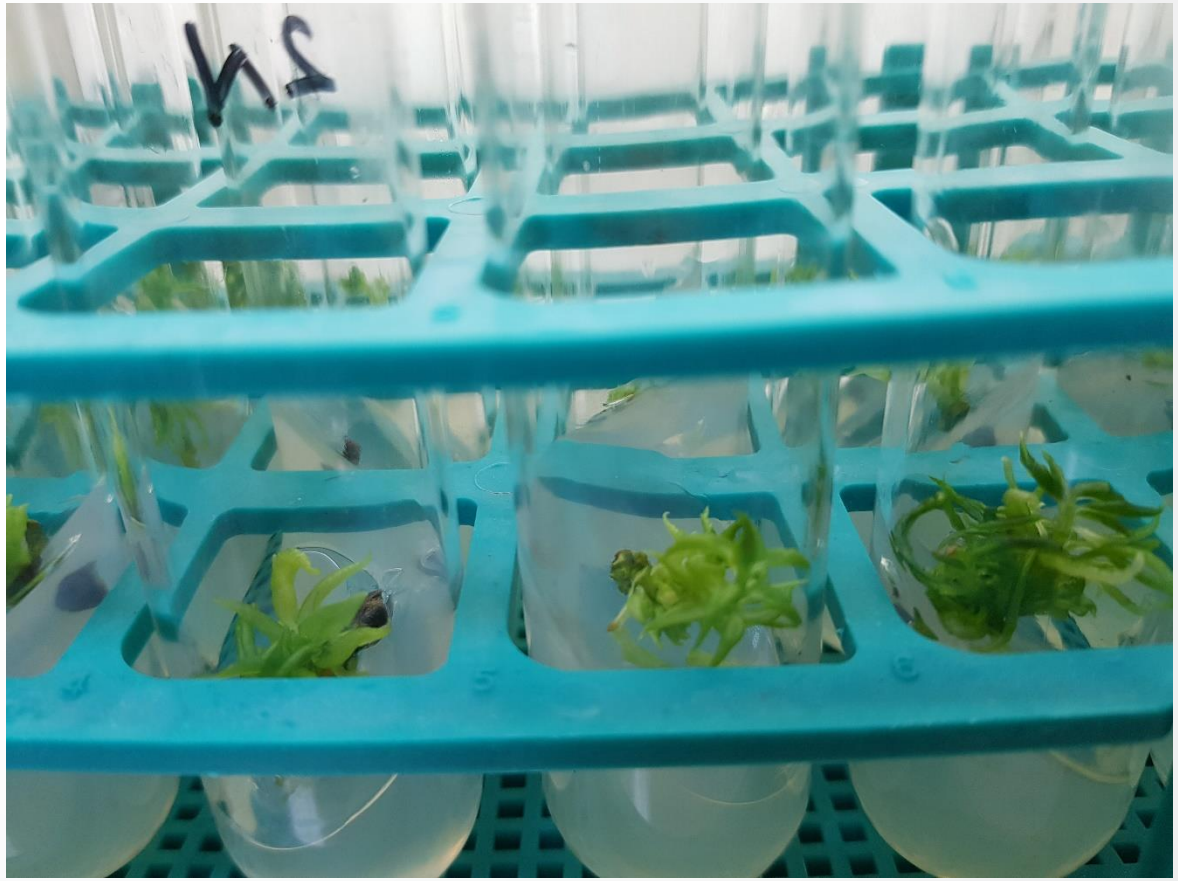
Thymus cilicicus Boiss. & Balansa

TAGEM
AR-GE & İNOVASYON

Salvia smyrnaea Boiss.



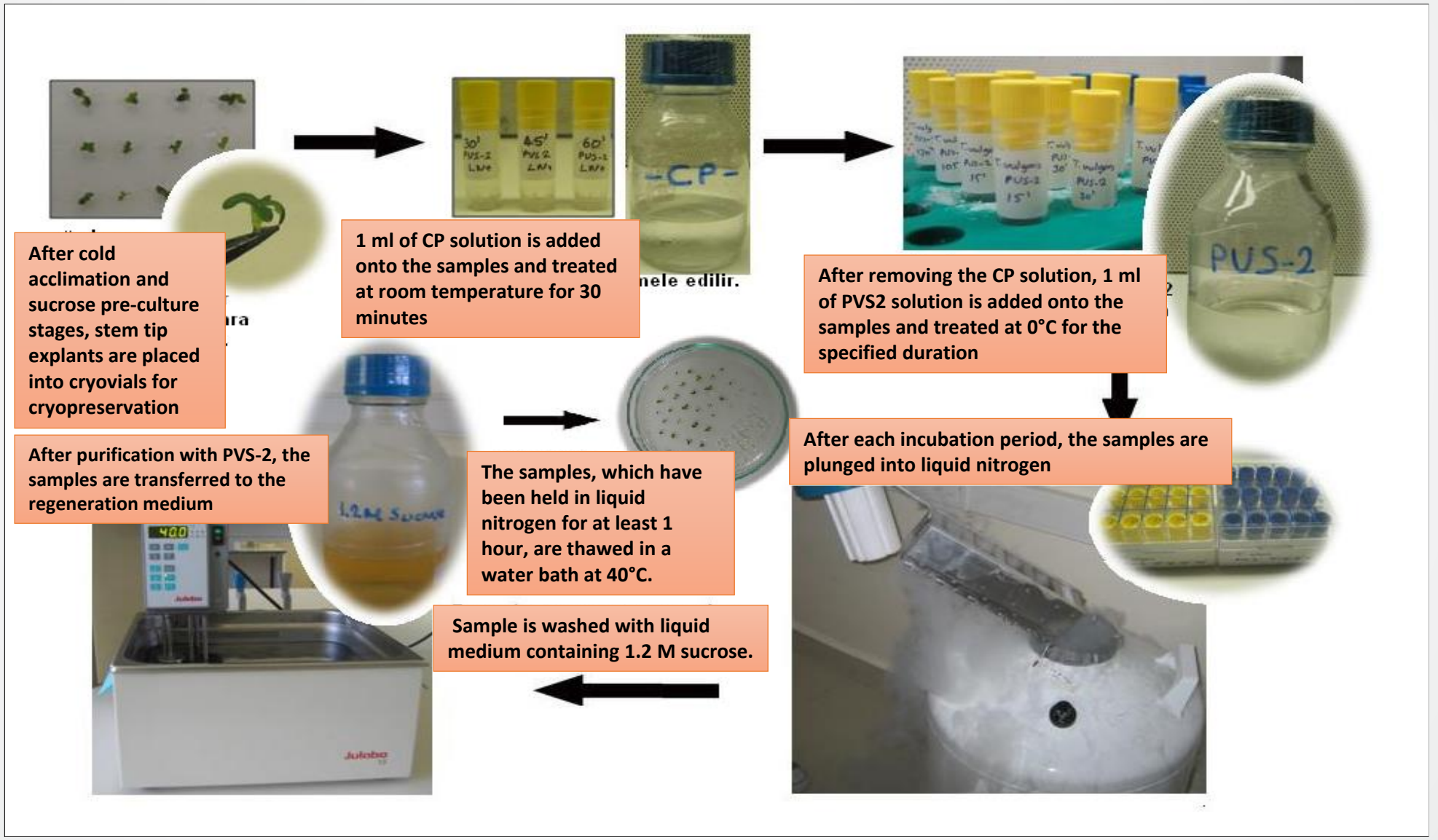
Prunus cerasifera Ehrh.



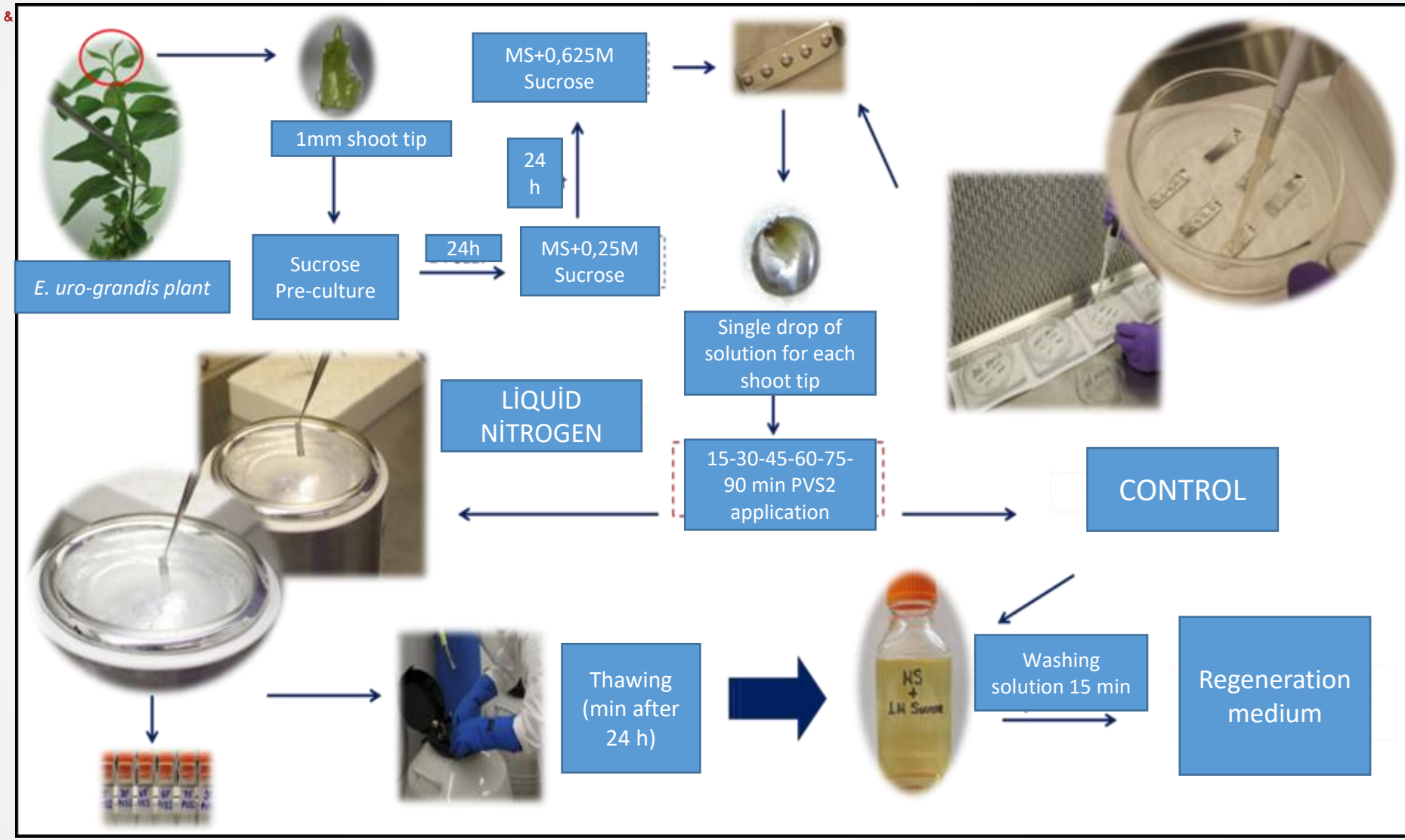
Cryopreservation Techniques

- Vitrification
- Droplet-vitrification
- Encapsulation-vitrification
- Cryoplate
  - V-cryoplate
  - D-cryoplate
- Cryo mesh technique
- Vacuum infiltration vitrification (VIV)

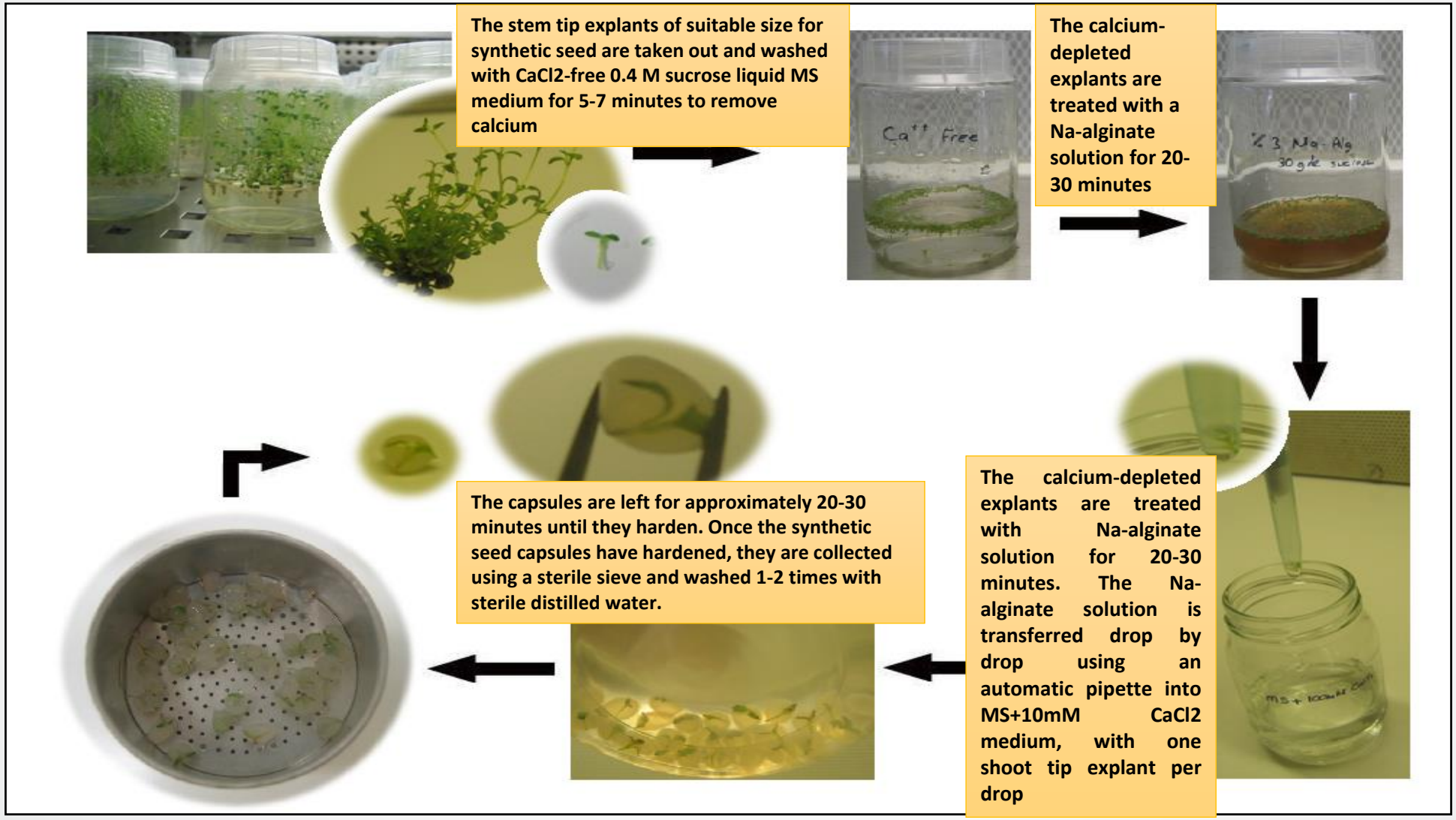
Vitrification



Droplet Vitrification



Encapsulation-Vitrification



The Application of Cryopreservation Techniques

❑ +4 °C Pre-conditioning

Plants grown in vitro will be placed in a dark environment at +4 °C for 1-2 weeks (Özüdoğru ve ark., 2012).

❑ Shoot tip isolation

Meristems of 0.5-1 mm will be isolated from the shoot tips of plants that have been cold acclimated for 1-2 weeks.

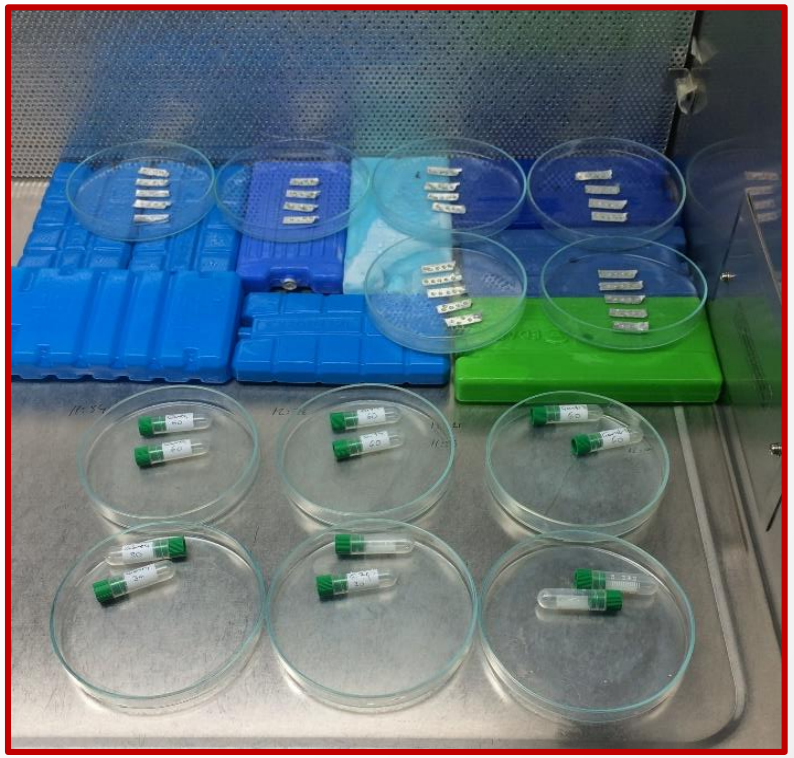
❑ Sucrose pre-culture

The meristems will be incubated in MS semi-solid medium containing 0.4 M sucrose for 24 hours (Özüdoğru ve Kaya, 2012).

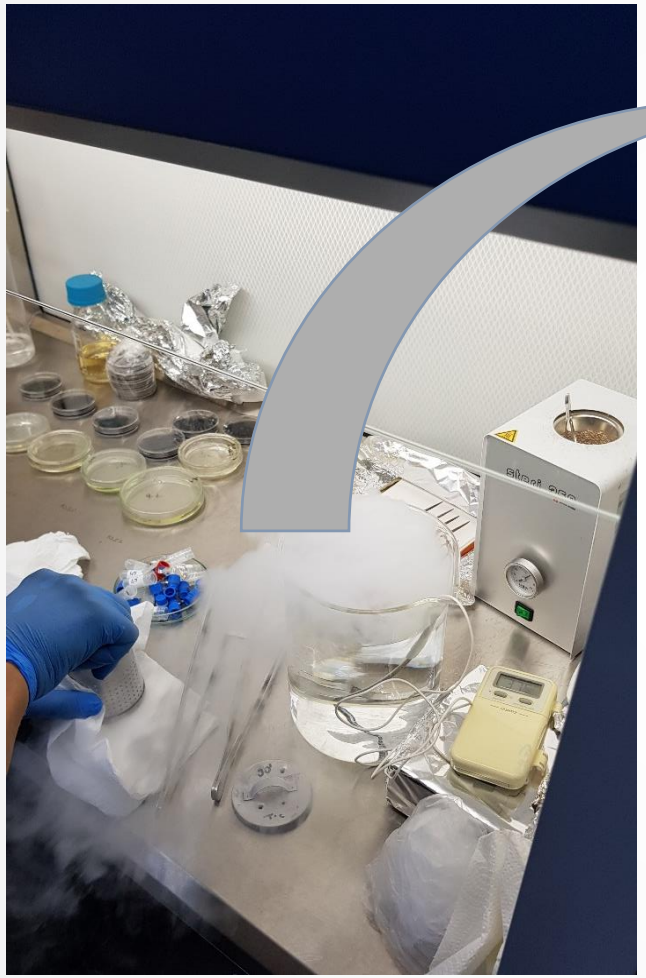
Application of cryoprotectant solutions

In the literature, it has been reported that one of the important parameters affecting the success of the method is the treatment time with cryoprotectant solution.

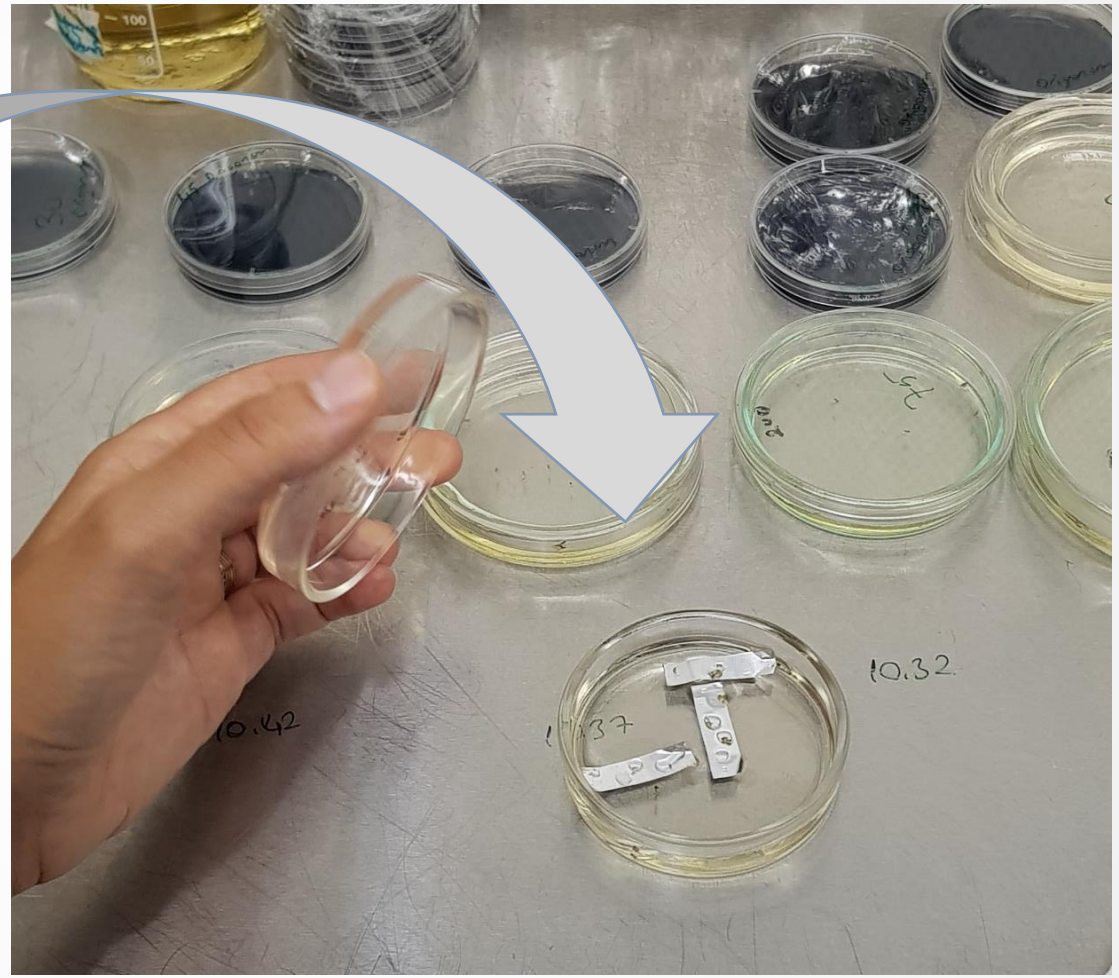
Therefore, in this study, the treatment time with cryoprotectant solution called **PVS2** (%30 glycerol, %15 ethylene glycol, %15 dimethyl sulfoxide, 0.4 M sucrose) was investigated in the range of **15-120** minutes.



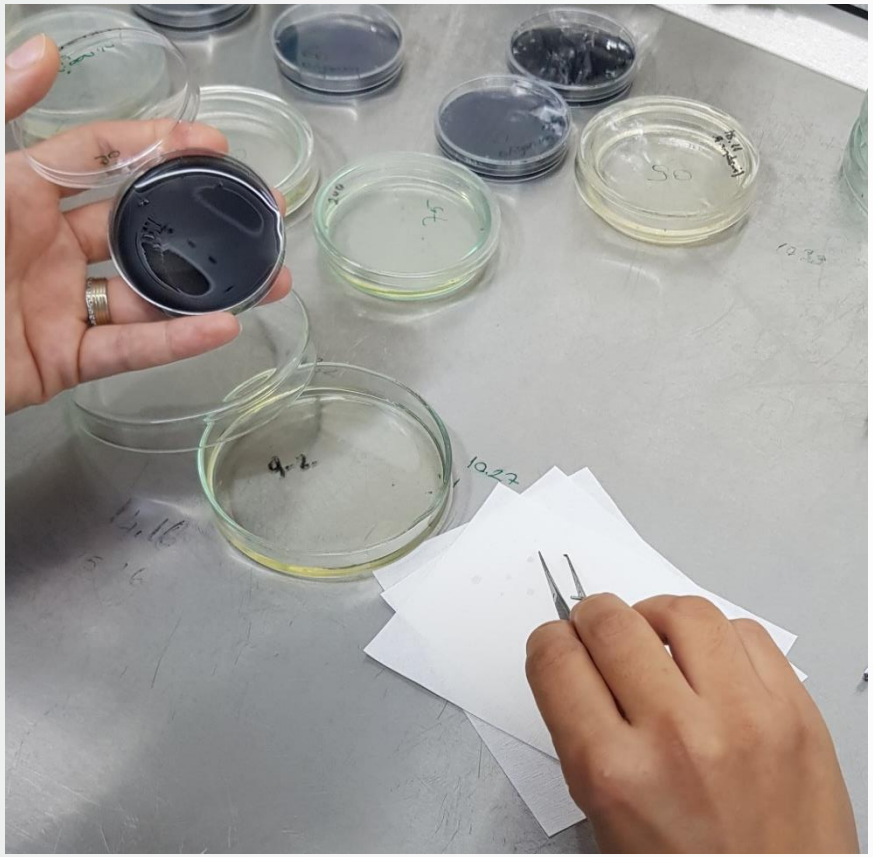
3-Regeneration studies of the thawed samples

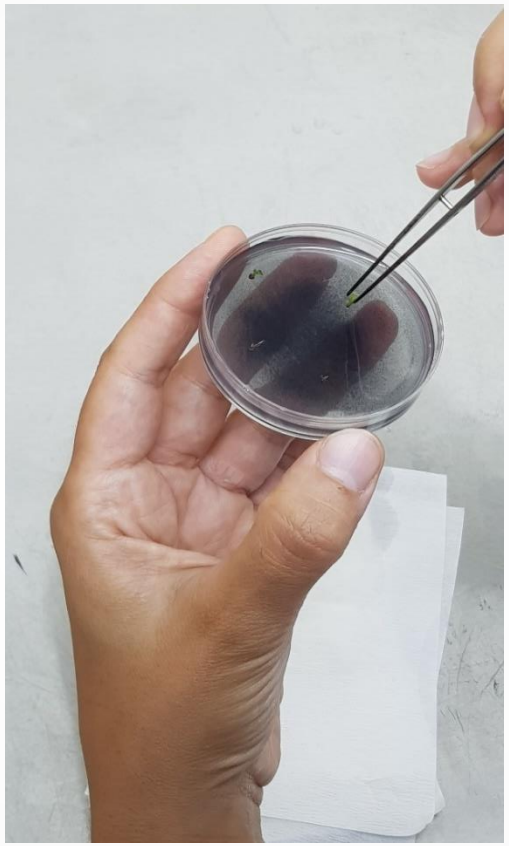


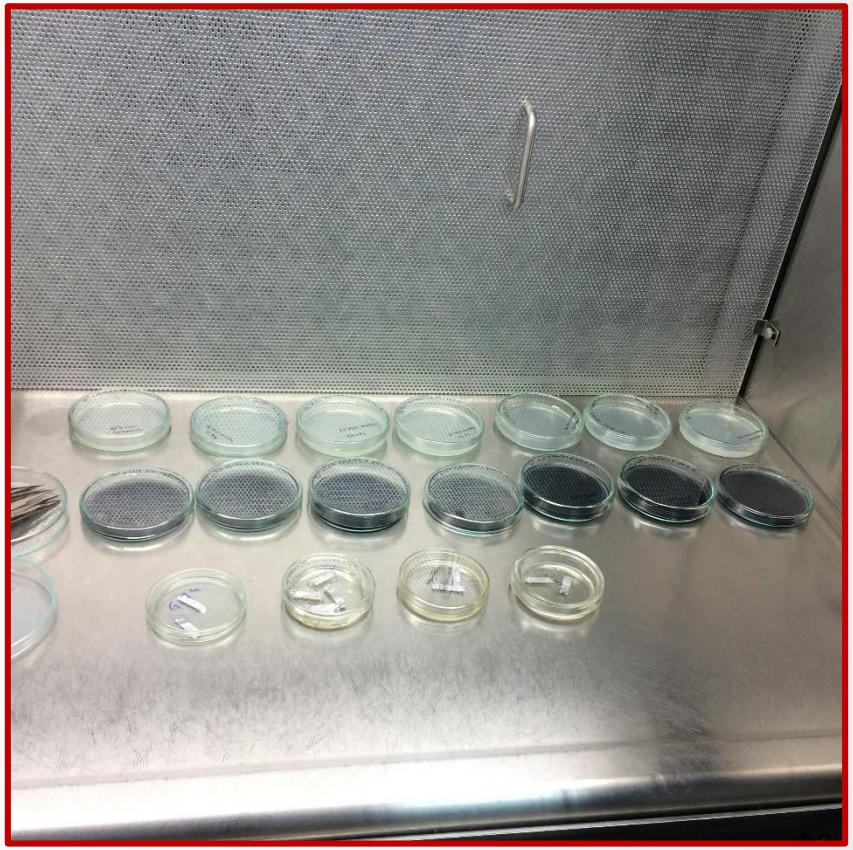
In a water bath at 40°C



1.2 M sucrose solution

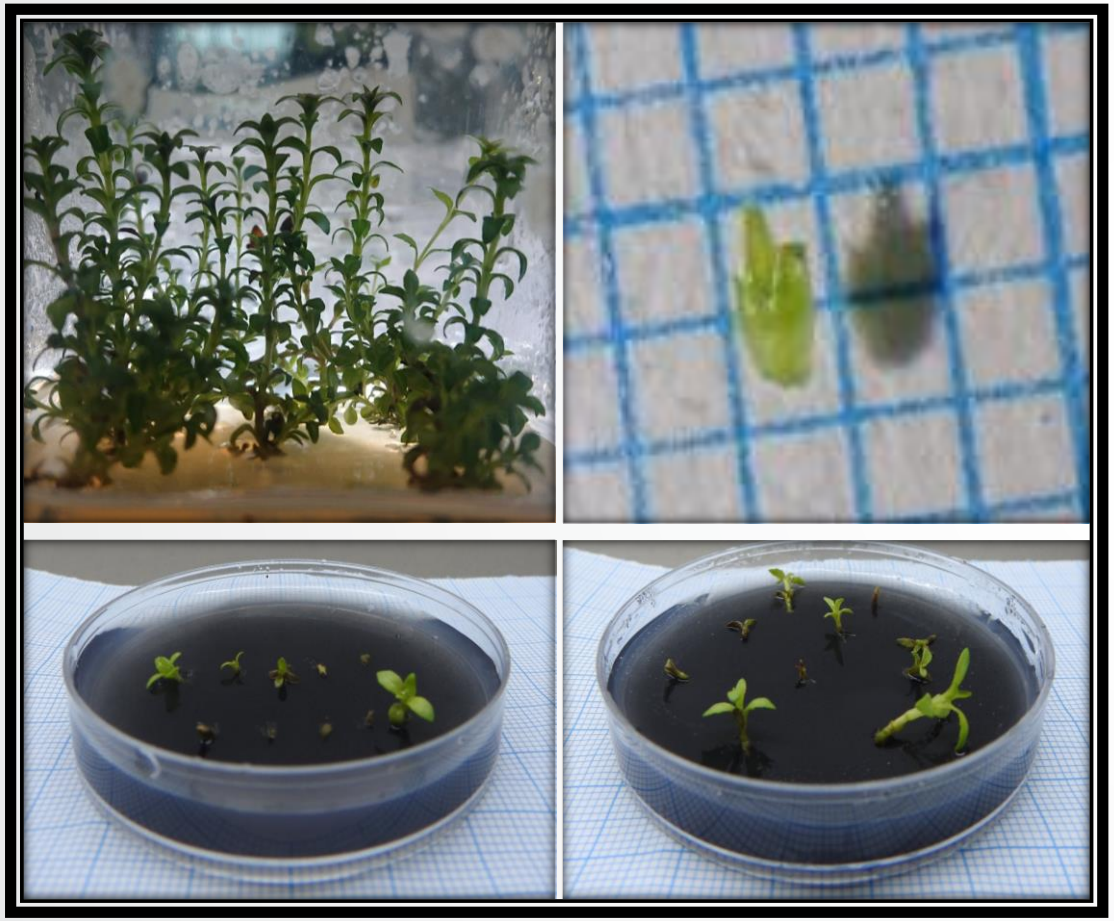


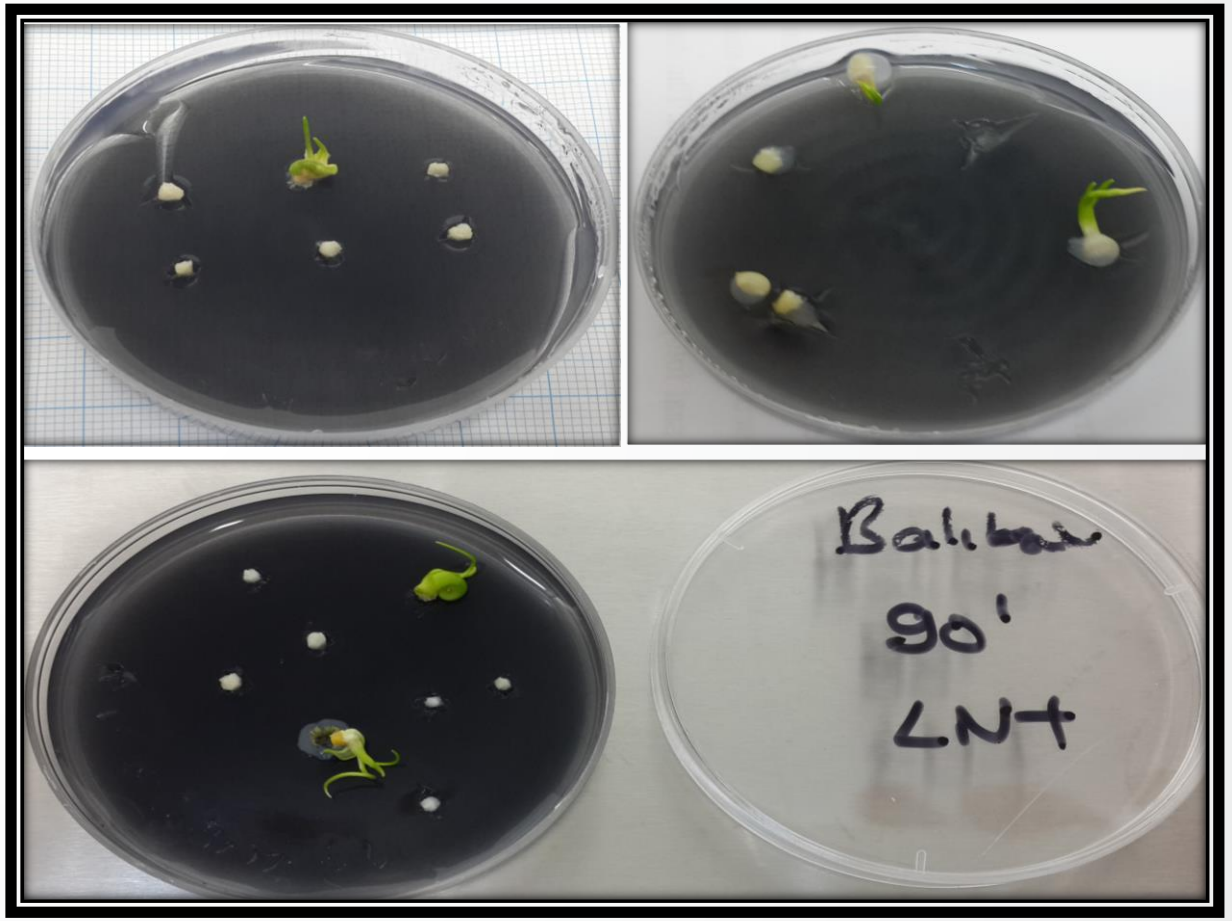




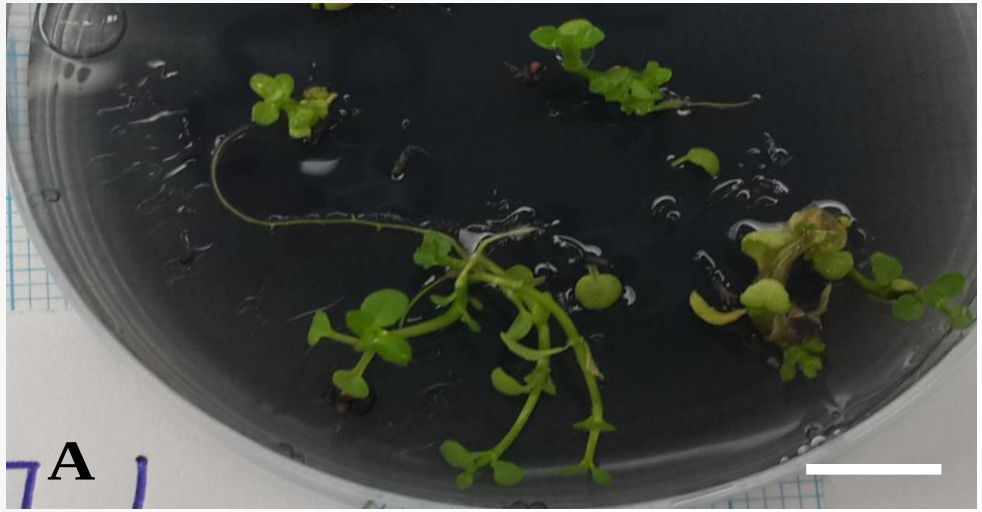
The explants transferred to the regeneration medium will be placed in a growth chamber and left to develop in the light after 48 hours of darkness

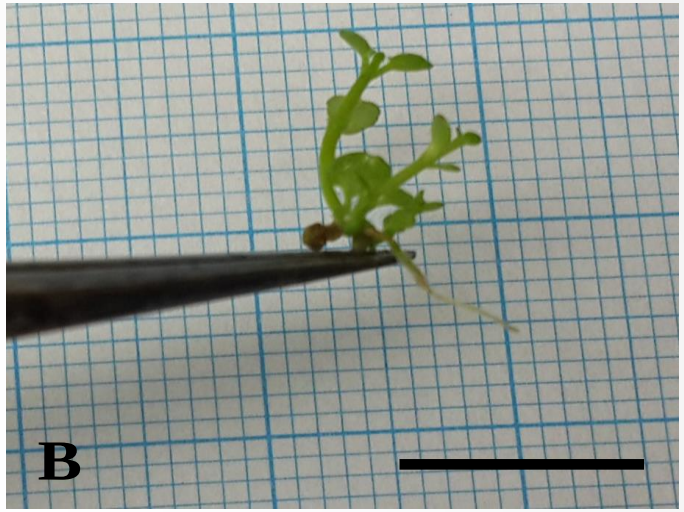
Regeneration results





4-Rooting and acclimatization to external condition





5- Measurements and Observations

During in vitro production studies;

- In vitro shoot formation rate (%)
- Number of in vitro shoots
- In vitro rooting rate (%)
- Adaptation rate of external environment (%)

After Cryopreservation;

-Viability (%)

-Regenaration success rate (%)

- Adaptation rate of external environment (%)

Cryopreservation protocols;

- Mint (*Mentha x piperita*) 3 population
- Garlic (*Allium sativum* L.) 2 population
- *Thymus cilicicus*
- *Origanum sipyleum*

Mentha sp.

Allium sativum L.

Thymus cilicicus Boiss.

Origanum sipyleum L.

Result

Material	Method/Application	Succes rate
Mentha sp. (Gömeç local variety)	**Droplet - Vitrification**	**%50**
Mentha sp. (Çandarlı local variety)	**Droplet - Vitrification**	**%80**
Mentha sp. (Genotype 74 local variety)	**Droplet - Vitrification**	**%60**
***Allium sativum* (Garlic)**	**Vitrification**	**%78,5**
***Origanum sipyleum* L.**	**Droplet - Vitrification**	**% 80**
***Thymus cilicicus* Boiss. & Balansa**	**Droplet - Vitrification**	**% 72,5**

2023 Studies

The in vitro protocols of ***Prunus cerasifera*** and ***Salvia smyrnaea*** were established, and studies on developing cryopreservation protocols have been initiated.

Studies have been started on in vitro propagation protocols of ***Sideritis tmolea and Galanthus elwesii***

Our main limitations;

- Human resources
- Lack of training
- Budged

We suggest;

- Training and knowledge transfer
- Funding
- Networking

Publications

1- Ogur, E. Adanacioglu, N., Galatali, S., CeylanM., and Kaya E. 2023. CRYOPRESERVATION OF MENTHA PIPERITA L. GERMPLASM AND CONFIRMATION OF GENETIC STABILITY AFTER CRYO-STORAGE. Journal of Animal & Plant Sciences, 33(2): 2023, Page: 345-356 ISSN (print): 1018-7081; ISSN (online): 2309-8694

2- Oğur, E., Adanacıoğlu, N., Doğan, S., & Şenel, Ü., 2022. Long-Term Conservation of Two Garlic (*Allium sativum* L.) Local Varities of Turkey via Cryopreservation

3- Doğan, S., Oğur E., Adanacıoğlu, N., 2022. Cryopreservation of Well-Known Turkish Medicinal & Aromatic Plant: *Origanum sipyleum* L.

THANK YOU FOR YOUR ATTENTION

Don’t hesitate to write:

erdinc.ogur@tarimorman.gov.tr