

Micropropagation of Grapevine Rootstock Cultivar '1103-Paulsen'

COST CA17111 - Integrape 2022 - Fourth Annual
Meeting “Data integration for grapevine research in
the context of environmental transition” 14 - 16
March 2022 Lemesos, Cyprus

Gayane Melyan

Grape is one of the major and ancient fruit crops in Armenia. Vine-growing and winemaking in Armenia have a thousand-year history.



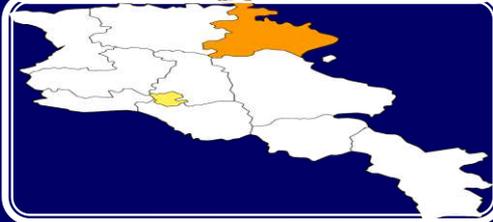
In Armenia Viticulture is concentrated in 5 agricultural zones and are about 17000 ha.



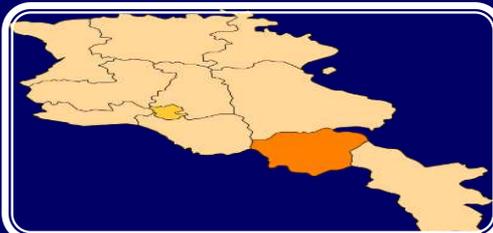
Ararat Valley (Ararat and Armavir regions)



Foothills of the Ararat Valley (Aragatsotn ı Kotayk regions)



North-Eastern Zone (Tavush and Lori)



Vayots Dzor (Vayots Dzor region)



Zangezur (Syunik region)

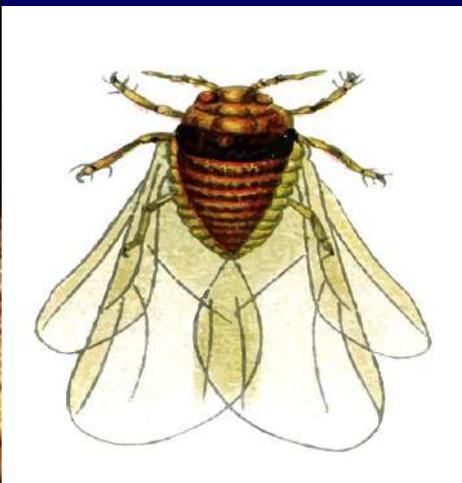
About 71% of the grapevine plantations are situated in Ararat and Armavir marzes (Regiones). In the Republic of Armenia about 90 % of vineyards are own rooted and about 10 % are grafted.

Grape is our national wealth, and the keeping of the grape growing traditions is very important. The most important precondition for further development of this branch is the pest and disease management of vineyards. However, currently viticulture in Armenia is facing a very serious problem.

Since 2009, the presence of phylloxera has been confirmed in the main viticultural zone of Armenia-Ararat Valley, and the infection, unfortunately has been expanding.

First time in Armenia (former Noyemberyan region) Phylloxera was noticed in 1926.

Grape phylloxera (*Daktulosphaira vitifoliae*) originated in North America, it is a microscopic aphid, which lives in the soil and attacks the roots of vines by sucking their saps, affecting their productivity, causing damage and ofthen death of vines (Grannet et al. 2001). Phylloxera spread almost all over the world, covering the main regions of viticulture.



Phylloxera may 5-6 annual generatios of thr root form.

(<http://vinograd.info/spravka/slovar/filloksera-vinogradnaya.html>).

Since 2017 the Ararat Valley is considered as phylloxera infested zone. The use of phylloxera - resistant rootstocks is the only real and effective way for control of that dangerous pest.

Presently, the use of phylloxera resistant rootstocks in Armenia is becoming increasingly important.

Grapevine is generally propagated by hardwood cuttings. But this method does not ensure the production of healthy plant material.

Several diseases especially those caused by viruses are transmitted to cuttings from mother plants and some of them have observable harmful effects on vines causing necrosis, curling of leaves, streaks in leaves or flowers, shortening the productive life of the vineyard, loss in yield and quality of fruit, reduced growth of vine, incompatibility of graft, decline and death of vine.



Vitis spp–Virus Diseases

There is not a chemical treatment which can eradicate plants of virus infection.

Therefore, *in vitro* propagation as an alternative method for propagating grapevines is very important.

In comparison with the traditional methods of plant propagation the biotechnological method has a number of advantages.

1. large number of healthy (virus free) plants can be produced from a single individual in a short period,
2. Possible to produce and conserve genetically identical plants,
3. Propagation plants during the year not depending of the season of year.

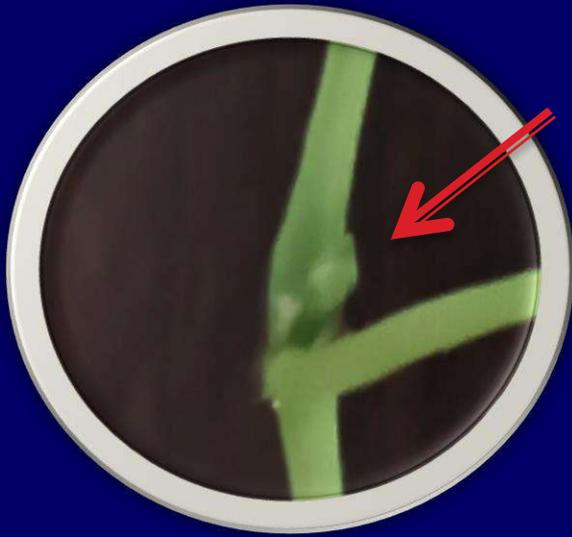
In vitro propagation in grapes is used mostly for the production of pre-basic plants, provided to nurseries.



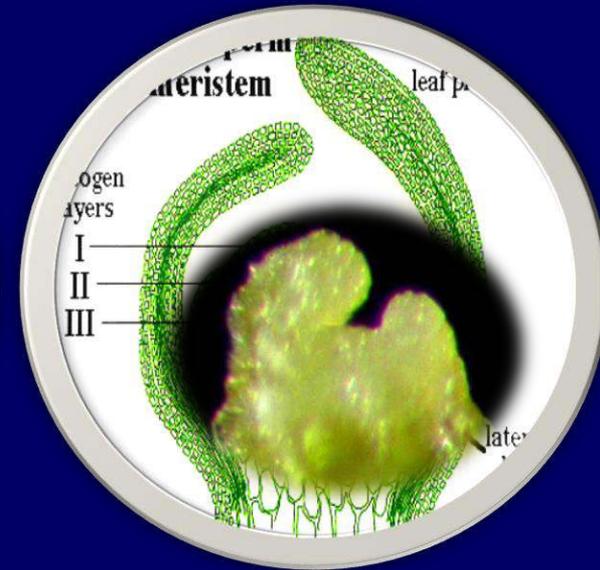
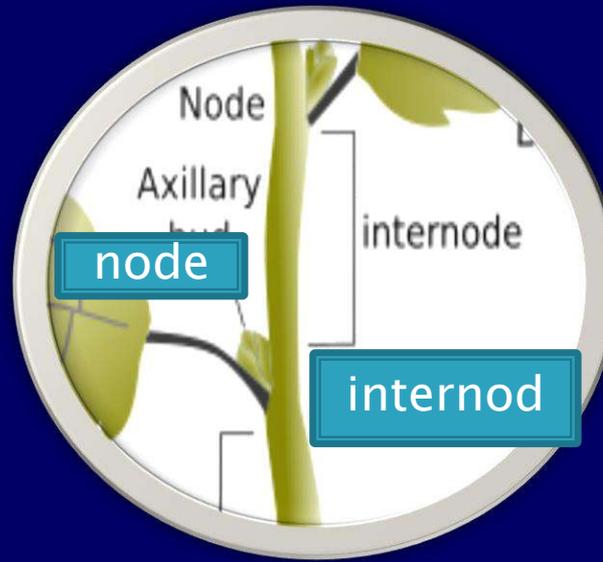
The objective of this study was to establish an efficient protocol for *in vitro* multiplication and rooting, as well as *ex vitro* acclimatization of phylloxera - resistant rootstock cultivar '1103-Paulsen' (*Vitis berlandieri* cv. Rösséguier 2 x *Vitis rupestris* cv. du Lot).

The studies were carried out on the Scientific Center of Agrobiotechnology of the Armenian National Agrarian University.

Initial explants were taken from new developing shoots of the plants grown under the open field.

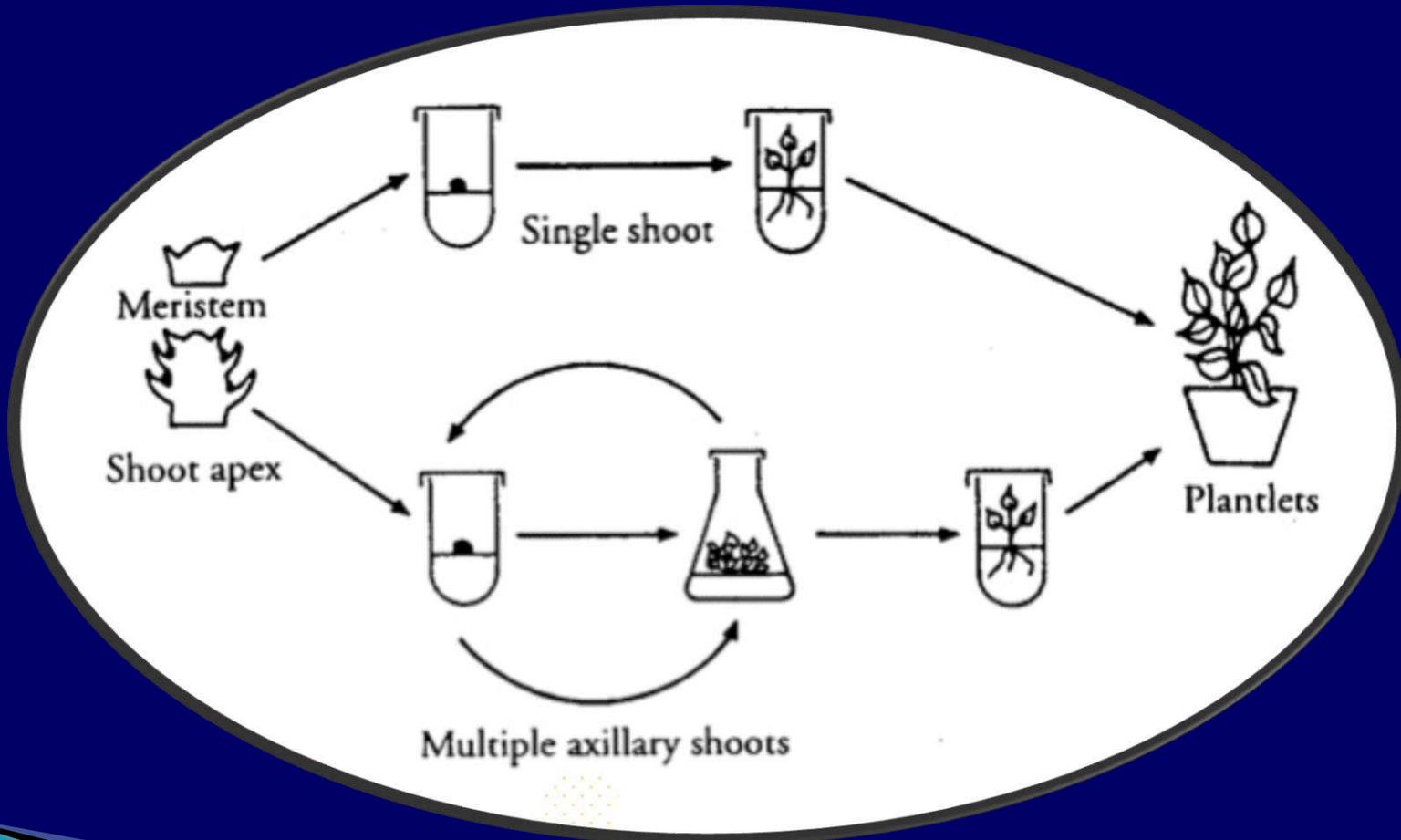


Axillary bud



Apical meristem

In vitro propagation scheme of grapes



The cultures were established from actively growing young shoots.

Fungal and bacterial contamination of grapevine explants taken from the field is a serious problem. Therefore, explants surface disinfection is the first main stage in establishing their culture.



Effect of different sterilizing agents with various concentrations and duration times were studied in order to find out the best variant with least toxic effect on *in vitro* culture establishment.

The results showed that the maximum aseptic cultures with the highest percentage of explants survival (67.5 %) were obtained when nodal segments treated with 1.0 % sodium hypochlorite (NaClO) for 10 min + 70 % ethanol for 1.0 min.

For the culture initiation Gamborg (B5) medium was better than Murashige and Skoog (MS), Nitsch and Woody plant (WPM) media which showed rapid growth of axillary bud within 6 days.

Numerous combinations of 6 -Benzylaminopurin (BAP), 6-furfuryl aminopurine (kinetin) and Giberrellic acid (GA3) were carried out to optimize the proliferation phase.

The highest shoot proliferation (4.30 ± 0.35) was obtained in medium fortified with 0.5 mg/L BAP + 0.2 mg/L Kin and the highest shoot length (4.50 cm) was obtained at 0.5 mg/L BAP + 0.8 mg/L GA₃.



0.5 mg/L BAP + 0.8 mg/L GA₃

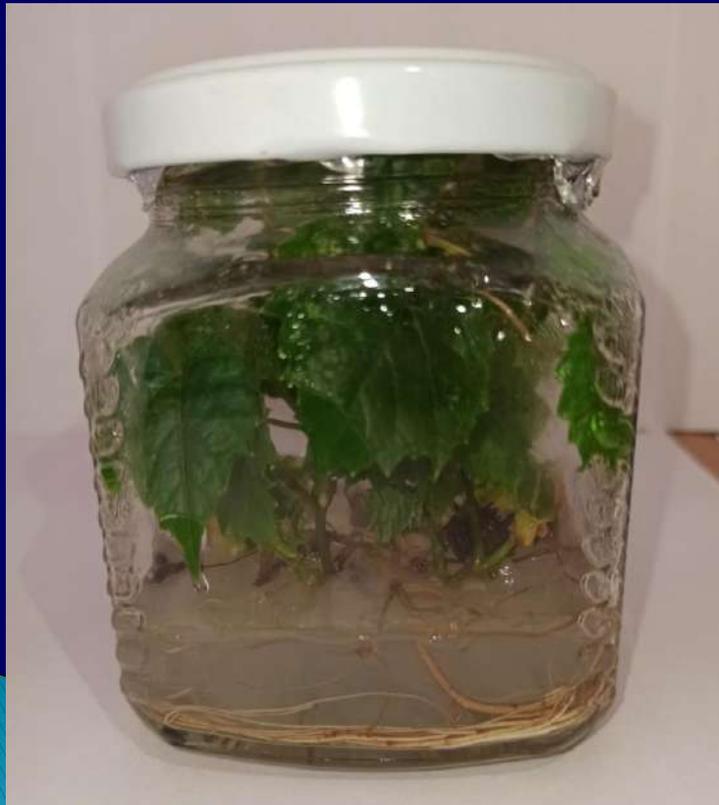
Addition of GA₃ along with BAP enhanced shoot elongation in all the explants.

Auxin is the most common phytohormone used for *in vitro* rooting.

For *in vitro* rooting shoots were cultured on ½-strength B5 medium containing different concentrations of Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA).

The plant growth condition in the culture room were 16 h photoperiod, 3000 lux light intensity and a relative humidity of 60 ± 5.0 %.

'1103-Paulsen' rooted well in all rooting combinations, but the highest rooting (100%) was observed in medium containing 0.2 mg/l IBA + 0.1 mg/l IAA.



Acclimatization of *in vitro* plants to *ex vitro* is very hard and stressful phase during micro-propagation. *In vitro* well-developed rooted plantlets with 6-8 cm height were acclimatized *ex vitro*.

The maximum percentage (83.0%) of plant survival was on the potting medium consisted of equal quantities of perlite, soil.





In vitro plants in Aeroponic system

It was found out that the productivity of plant regeneration and root formation depends on the used phytohormones and their concentrations.

This protocol would serve for mass multiplication, germplasm conservation and in vitro crop improvement programmes.

Thank YOU



