EVALUATION OF THE REGENERATIVE POTENTIAL OF *PUNICA GRANATUM L CV. DEVEDISHE* APICAL SHOOTS, AFTER FOLLOWING VITRIFICATION AND ENCAPSULATION-VITRIFICATION PROCESSES

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ABSTRACT

Pomegranate (*Punica granatum* L) is a highly significant species, giving rise to numerous local populations and varieties. In recent years, Albanian farmers have shown increasing interest in cultivating foreign pomegranate cultivars with desirable commercial traits, leading to a gradual erosion of autochthonous genetic resources. This irreversible loss is a serious threat to national agriculture, necessitating the development of the development of biotechnological methods for *in vitro* preservation indigenous pomegranate varieties. *In vitro* preservation techniques such as cryopreservation offer long-term preservation. Cryopreservation requires a cryoprotective step to prevent explants mortality under extreme conditions. However, such procedures can cause side effects that must be optimized and evaluated before cryogenic exposure. This study aimed to assess the regenerative potential of pomegranate shoots after vitrification and encapsulation-vitrification processes, in order to identify the most effective cryoprotective procedure ensuring a high regeneration rate. Shoot tips, including

encapsulated ones, were immersed in PVS2 solution [30% (w/v) glycerol, 15% (w/v) ethylene glycol, and 15% (w/v) DMSO] at 0°C for three exposure durations (30, 60, and 90 minutes). After treatment, the explants were inoculated into test tubes containing WPM basal medium supplemented with 1 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L 1-naphthaleneacetic acid (NAA). Explants were periodically monitored for survival rate, contamination, and regeneration into whole plants. The encapsulation-vitrification method was found to be the most effective for preserving the regenerative ability of pomegranate apical shoots, achieving a regeneration percentage of 96.6% compared to 71.1% for the vitrification method. Additionally, the encapsulation-vitrification method demonstrated lower rates of contamination and necrosis in culture, making it more suitable for cryopreservation. Optimizing this pre-treatment step paves the way for further investigations into cryostorage of pomegranate explants.

Keywords: vitrification, encapsulated explants, pomegranate, cryopreservation

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the most economically significant cultivars globally. It belongs to the family Lythraceae, which comprises a single genus (*Punica*) and two species: *P. granatum* and *P. protopunica*. Native to Iran, pomegranate is now widely distributed across the Mediterranean, Asia, Africa, and Europe (Kumar *et al*., 2017). Due to its high adaptability to diverse ecological conditions and its profitability as a crop, pomegranate is commercially cultivated across various climatic zones (Mustafa *et al*., 2018). The fruits are rich in proteins, fiber, carbohydrates, minerals, and antioxidant components, such as phenols, pigments, and tannins. Beyond the high demand for fresh fruit and juices, processed products like pomegranate wine, tea, and candies also enjoy a wide consumer base. Additionally, the fruit's skin and the tree's bark are used in traditional medicine for treating diarrhea, dysentery, and intestinal parasites. They are also valuable in tannin production (Kaur *et al*., 2014). The pharmaceutical industry shows considerable interest in pomegranate due to its bioactive compounds, which have demonstrated anti-cancer and anti-inflammatory properties in laboratory studies (Lansky *et al*., 2007). These compounds have been reported to aid in the treatment of various conditions, including certain types of cancer, cardiovascular diseases, osteoarthritis, rheumatoid arthritis, and other ailments (Zarfeshany *et al*., 2014).

Pomegranate is one of the most widely cultivated fruit trees in Albania, valued both for its nutritional properties and its wood. Pomegranates are primarily grown in the hilly regions of central and southern Albania, with the *Punica granatum* L. Devedishe variety being among the most favoured due to its high productivity, large fruit size, and early maturity (Xhuveli 2012). In recent years, Albanian farmers have expanded pomegranate cultivation by introducing cultivars from other countries. However, this practice has gradually led to the loss of autochthonous genetic resources, which possess excellent agronomic properties. To address this issue, it is essential to develop both short-term and long-term strategies for conserving plant genetic resources. This includes utilizing *in vitro* techniques and establishing genetic banks to preserve cultivars of economic importance. Autochthonous varieties demonstrate exceptional adaptability to local environmental conditions, making them ideal for the development of ecological orchards. This is particularly significant in Albania's mountainous and hilly regions, where enhancing ecotourism is a priority.

In vitro preservation methods provide medium-term preservation through minimal growth and long-term preservation via cryopreservation. Cryopreservation stores plant material at ultra-low temperatures (-196 ºC) in liquid nitrogen tanks, halting biochemical reactions and cellular metabolism by freezing water into ice (Sota *et al*., 2024). Over 15,000 species have been cryopreserved worldwide using *in vitro* techniques, such as droplet vitrification or dormant buds (Zhao *et al*., 2017). Successful preservation and regrowth depend on efficient protocols, including cryoprotection to prevent ice crystal formation, which could damage cells. Cryoprotective substances, combined with MS basal medium (Murashige and Skoog, 1962), dehydrate plant material and facilitate protective processes. Vitrification solidifies liquids into an amorphous, non-crystalline state (Fahy *et al*., 1984). Plant Vitrification Solution (PVS2), a widely used agent, is effective but can cause oxidative stress in explants.

Vitrification is a cryoprotective method that transforms the cytosol into a glassy, amorphous state using various techniques to prevent the formation of intracellular ice, which can be fatal to plant cells and tissues. This is achieved during the rapid reduction of temperatures by directly transferring samples to liquid nitrogen (Osorio-Saenz *et al*., 2011).

The encapsulation-vitrification method, on the other hand, begins with the formation of synthetic seeds, followed by treatment with PVS2. This combined technique, which merges encapsulation-dehydration and

vitrification procedures, has been developed for the cryopreservation of plant germplasm (Matsumoto *et al*., 1995; Liu *et al*., 2009). Synthetic seed production is considered an effective method for propagating and conserving plant species of high economic importance due to their multiple uses and unique properties (Ara *et al*., 2000; Ravi and Anand 2012). Furthermore, encapsulation-vitrification is easier to handle and allows for the treatment of multiple samples simultaneously, compared to single vitrification procedures (Matsumoto *et al*., 1995). However, exposing explants to cryoprotection procedures, particularly in PVS2 solution, can induce severe oxidative stress. The cryoprotective procedure used and the exposure time are critical factors that influence plantlet regeneration, even under normal growth conditions. Plant Vitrification Solution (PVS2), a cryoprotective solution, enables explant survival in cryogenic conditions (-196°C), allowing for successful long-term storage. However, excessive exposure to vitrification solutions can cause high oxidative stress in explants, leading to cell death. The effectiveness of the protocol is closely related to the genotype, and each protocol must be optimized on a case-by-case basis, depending on the plant species being conserved.

This study aims to evaluate the effect of vitrification and encapsulation-vitrification procedures on the regeneration potential of pomegranate shoot tips treated at various exposure times.

2. MATERIAL AND METHODS

Plant material: The primary explants used were *in vitro* plantlets of *Punica granatum L.* Devedishe variety, established from previous experiments (Lala *et al*., 2024).

Vitrification procedure: *In vitro* shoot tips of pomegranate were treated with the filling cryoprotectant solution (2M glycerol + MS solution with 0,4M sucrose) for 25 minutes**.** The filling solution was then removed with a syringe, and the explants were immersed in PVS2 solution [30% (w/v) glycerol, 15% (w/v) ethylene glycol, and 15% (w/v) DMSO] at 0°C. Three exposure times (30, 60 and 90 minutes) in such conditions were tested (Fig. 1 a, b). After each treatment, the PVS2 solution was removed, and the explants were rinsed with a 1.2 M sucrose solution for 20 min.

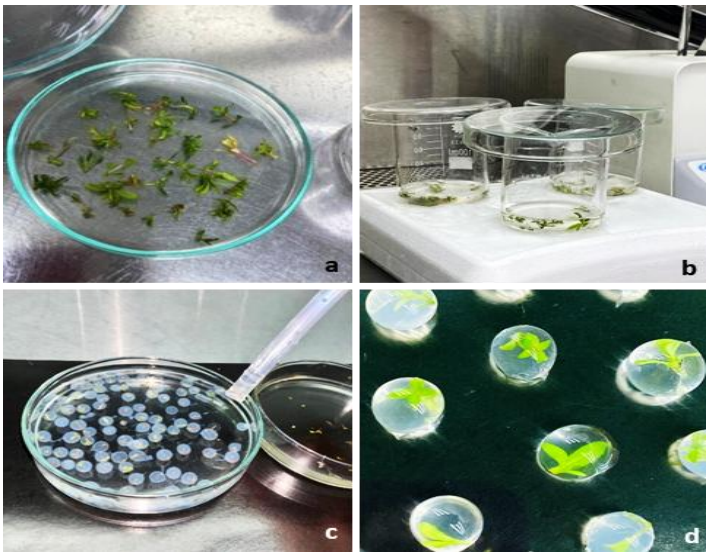


Fig. 1: a) Explants for vitrification. **b)** Vitrification in PVS2 solution at temperature 0°C. **c)** Encapsulation of pomegranate shoots. **d)** Synthetic seeds of pomegranate.

Encapsulation-vitrification procedure: The apical shoots from the in vitro plantlets were isolated under aseptic conditions and immersed in a 3% (w/v) sodium alginate solution and a 0.4 M sucrose solution. Both the explants and the sodium alginate solution were then dropped into a 100 mM $CaCl_2 \cdot 2H_2O$ solution and left for 30 minutes to allow encapsulation (Fig. 1 c, d). The encapsulated explants (synthetic seeds) were then immersed in the PVS2 solution for 30, 60, and 90 minutes at 0°C.

Plantlets recovery medium: After each procedure and treatment, the explants were inoculated into test tubes containing WPM (Lloyd and McCown, 1981) basal medium, supplemented with 1 mg/l of 6-Benzylaminopurine (BAP), 0.1 mg/l of 1-Naphthaleneacetic acid (NAA), 3% sucrose, and 0.7% agar. The pH of the nutrient medium was adjusted to 5.6 before autoclaving.

***In vitro* chamber conditions:** The explants were grown in a controlled environment to mimic their natural habitat. The growth chamber was maintained at a temperature of 25° ± 2°C, with a 16-hour light/24-hour dark photoperiod using cool, white fluorescent light, providing optimal conditions for development. Explants were periodically observed for

survival percentage, possible contamination, and regeneration into whole plants.

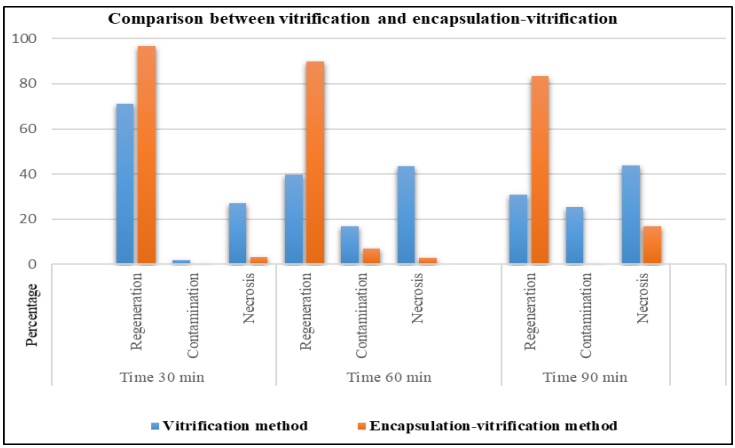
Experimental design: The number of samples was 35 explants for each of the three exposure times (30, 60, and 90 minutes), and the entire procedure for each method tested was performed with three repetitions.

3. RESULTS AND DISCUSSIONS

3.1. Regeneration, contamination and necrosis rates in both procedures after each treatment

Three different exposure times to PVS2 (30, 60, and 90 minutes) were tested to evaluate the regenerative response of non-encapsulated explants and encapsulated explants after treatment with the vitrification solution (Graph 1). The results showed that the combination of encapsulation-vitrification methods proved to be the most suitable for maintaining the regenerative ability of pomegranate apical shoots. The regeneration percentage of explants in the encapsulation-vitrification method was 96.6%, compared to 71.1% for the vitrification method (Fig. 2 a – f).

The process of encapsulation, followed by vitrification, was effective in protecting and stabilizing the explants, ensuring successful regeneration (Fig. 2). The lack of a preliminary encapsulation stage made the explants more susceptible to oxidative stress and damage caused by the vitrification process. The necrosis percentage was 27% for non-encapsulated explants, compared to 3.33% for the encapsulated explants.



Graph. 1: Comparison of regeneration, contamination and necrosis percentages, between the vitrification and encapsulation-vitrification methods.

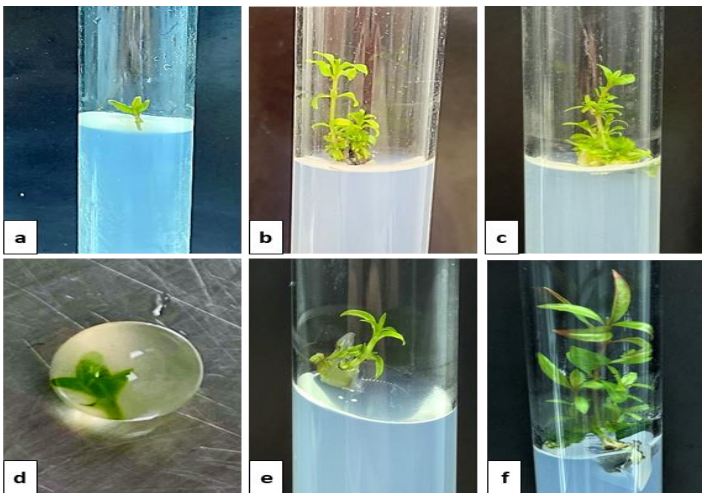


Fig. 2. a) Inoculation of vitrified explants in the recovery medium; b, c,) Conversion of vitrified explants (30, 60 and 90 min) into whole plant after 4 weeks under in vitro conditions after 4 weeks. d) Encapsulation of pomegranate shoot; e, f) Conversion of encapsulated-vitrified explants into whole plant in vitro.

The results also showed that in the vitrification method, the degree of contamination and necrosis increased with longer exposure times to PVS2. As shown in Graphic 2a, as the exposure time to the PVS2 solution increased, the number of regenerating explants decreased. At the same time, with the increase in residence time in the laminar flow, the number of contaminated explants also increased (Graphic 2b). These data demonstrate an increase in the degree of contamination and necrosis with longer exposure times to PVS2. A shorter exposure (30 minutes) resulted in a higher survival rate (71.15%) and a very low contamination rate (1.9%), suggesting that a shorter exposure duration is more suitable for minimizing oxidative stress and increasing explant survival.

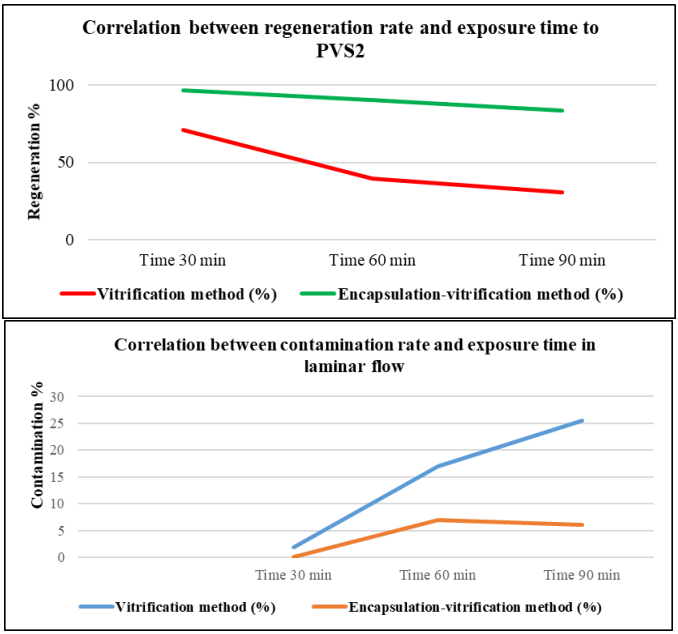
Other results supporting the high regeneration ability of explants after treatment with PVS2 for 30 minutes have been reported in (Vujović *et al*. 2011), who observed a significant increase in the percentage of regeneration (30%) compared to a 10-minute treatment, which resulted in a very low regeneration percentage (5%) in the droplet-vitrification process of apical shoot tips *of Rubus fruticosus L*. and *Prunus cerasifera Ehrh*. Similar to our research, (Nogueira *et al*., 2014) reported the effect of exposure duration to PVS2 on explant regeneration, with a 20-minute

exposure time being the most effective for achieving the highest regeneration percentage of sugarcane shoot tips after cryopreservation.

Also, based on the reports of Panis *et al*., (2011), we can conclude that the time of PVS2 treatment varies considerably between species, but prolonged exposure periods are generally lethal to the cells. The ideal exposure time was 20 minutes for *Rosa hybrida* L. (Halmagyi and Pinker 2006), 30 minutes for *Byrsonima intermedia* A. Juss (Silva *et al*., 2013), 40 minutes for *Musa* spp. (Panis *et al*., 2005), and 60 minutes for *Malus domestica* Borkh. (Condello *et al*., 2011).

Longer exposures (60 and 90 minutes) showed a significant increase in necrosis and contamination, significantly reducing the survival rate. These results are important for optimizing vitrification protocols, suggesting that a delicate balance must be maintained between exposure duration and maintaining the health of the explants. Furthermore, these data highlight the importance of controlling experimental conditions to minimize the negative effects of oxidative stress and contamination during the vitrification process.

In contrast, in the encapsulation-vitrification method, exposure times of 30 and 60 minutes showed similar results, with a high percentage of regeneration and a low percentage of necrosis. In both of these conditions, the contamination rate was low, indicating that these exposure periods are effective in regenerating the explant sites. Vitrification for 90 minutes showed a slightly lower percentage of regeneration (83.3%) and a higher percentage of necrosis (10.7%), although the exposure time is still effective because the percentage of regeneration remains relatively high, albeit slightly more susceptible to necrosis. After 30 minutes of exposure to PVS2, there were no signs of contamination, making this exposure time more effective than other durations in the encapsulation-vitrification procedure. Analyzing that exposure at different times resulted in low percentages of contamination and necrosis, we conclude that encapsulation increases the resistance of the explants, making them less sensitive to oxidative stress and damage caused by the vitrification process.



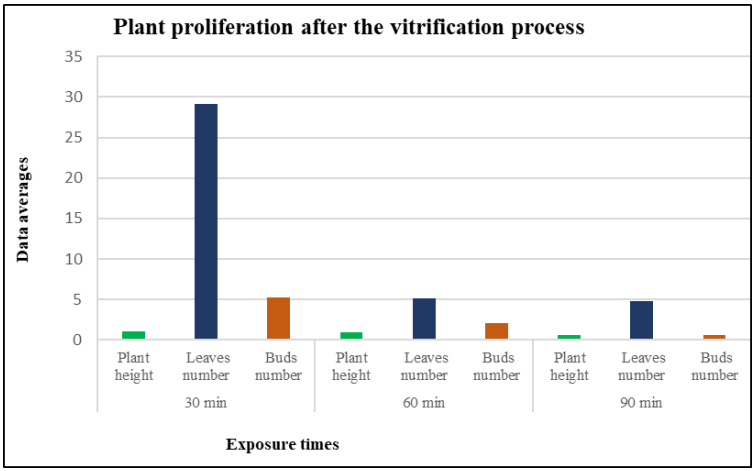
Graphic 2. Comparison between regeneration and contamination rates in both procedures. **a)** Correlation between regeneration rate and exposure time to PVS2 solution **b)** Correlation between contamination rate and exposure time in laminar flow of explants.

Similarly to our research, Wang *et al*. (2005) reported a high regeneration rate (85%) of raspberry shoots using encapsulation-vitrification methods for cryopreservation. The efficiency of the encapsulation-vitrification method compared to the vitrification method has also been studied by Kumaria *et al*. (2012), who reported that the maximum regeneration (66%) was achieved after cryopreservation of Cymbidium eburneum L. using encapsulation-vitrification, while the regeneration using vitrification was the lowest (50%).

In addition, encapsulation-vitrification was much easier to handle and could be used to treat a large number of samples simultaneously, compared to the vitrification procedure, as also noted by Matsumoto *et al*. (1995). These results demonstrate a high potential for the preservation of pomegranate using encapsulation technology, and these findings can serve as a solid foundation for future implementation of long-term preservation protocols via cryopreservation using encapsulation-vitrification methods.

3.2. Proliferation of pomegranate *Punica granatum L* cv. Devedishe shoots after different pre-treatments

The average values of the morphometric data show that the rate of growth and development of the vitrified explants decreases with the increase in the time of exposure to PVS2 (Graphic 3). Exposure to PVS2 solution for 30 minutes has been identified as the most efficient time for regenerating explants with low levels of contamination and necrosis. This result suggests that a shorter period of exposure to the PVS2 solution minimizes oxidative damage and stress, thus increasing regeneration success. According to the graph, we can see that the averages of plant length, the number of leaves, and the number of shoots of pomegranate plants regenerated after 4 weeks under in vitro conditions are higher in the treatment for 30 minutes in the PVS2 solution. These data further confirm that the 30-minute exposure time to the PVS2 vitrifying solution ensures more complete regeneration of the explants compared to the other two exposure times. Additionally, the encapsulated explants treated for 30 minutes in PVS2 regenerated better into whole plants compared to other treatment times (Figure 3). The regenerated plants showed very good development in terms of plant height and number of leaves. The use of the hormonal ratio in favor of cytokinins in the WPM recovery medium promoted the development of many side shoots, which will enable the production of a large number of plants in the micropropagation stage.



Graph. 3: Plant proliferation after the vitrification process with the PVS2 solution.

The use of WPM as a recovery medium, with a hormonal ratio of 10:1 for BAP and NAA, was effective in the development and regeneration of explants after both treatment procedures. Similar results for the efficiency of WPM basal medium in the proliferation of *Punica granatum* L. explants were also reported in (Samir *et al*., 2009).

In addition to viability after treatments, in germplasm cryopreservation, it is also important that the treated explants are able to produce plants identical to the donor plant (Chen *et al*., 2011). In our study, regeneration occurred directly, without the intermediate callus stage, which is often undesirable due to its potential for causing genetic instability in the regenerants.

4. CONCLUSIONS

The findings are particularly important for the development of efficient cryopreservation protocols through vitrification and encapsulation-vitrification methods. This experimental platform demonstrated that the combination of encapsulation-vitrification methods was the most suitable for maintaining the regenerative ability of pomegranate apical shoots. The regeneration percentage of explants in the encapsulation-vitrification method was 96.6%, compared to 71.1% in the vitrification method. Additionally, the encapsulation and vitrification method was considered more suitable for cryopreservation of explants, as it resulted in lower percentages of contamination and necrosis compared to the vitrification method. With an exposure time of 30 minutes to PVS2, there were no signs of contamination; therefore, this time is considered the most efficient treatment duration for the encapsulation-vitrification procedure, also yielding the highest percentage of regeneration and the lowest percentage of necrosis in culture. Furthermore, encapsulation-vitrification was much easier to handle and could be used to treat a large number of samples simultaneously, compared to the vitrification procedure. Therefore, we can conclude that the use of this method can assist in the preservation of genetic diversity and improve plant crops, providing sustainable solutions to plant conservation and regeneration challenges.

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REFERENCES

Ara H, Jaiswal U, Jaiswal VS. 2000. Synthetic seed: prospects and limitations. *Current Science,* **78(12):** 1438-44. http://www.jstor.org/stable/24104316.

Chen X-L, Li J-H, Xin X, Zhang ZE, Xin PP, Lu XX. 2010. Cryopreservation of in vitro-grown apical meristems of *Lilium* by droplet-vitrification. *South African Journal of Botany*, **77(2):** 397-403. https://doi.org/10.1016/j.sajb.2010.10.005.

Condello E, Caboni E, Andre E, Piette B, Druart R, Swennen R, Panis B. 2011. Cryopreservation of apple in vitro axillary buds using droplet-vitrification. *Cryo Letters*, **32(2)**: 175-85. PMID: 21766147.

Fahy G, MacFarlane, DR, Angell CA, Meryman HT. 1984. Vitrification as an approach to cryopreservation. *Cryobiology,* **21(4):** 407-426.

Halmagyi A, Pinker I. 2006. Plant regeneration from Rosa shoot tips cryopreserved by a combined droplet-vitrification method. *Plant Cell, Tissue and Organ Culture*, **84:** 145-153, 2006. DOI: 10.1007/s11240-005-9012-z.

Kanwar K, Joseph J, Deepika, R. 2010. Comparison of *In vitro* regeneration pathways in *Punica granatum* L. *Plant Cell, Tissue and Organ Culture*, **100**: 199–207; https://doi.org/10.1007/s11240-009-9637-4.

Kaur C, Pal RK, Kar A, Gadi C, Sen S, Kumar P, Chandra R, Jaiswal S, Khan I. 2014. Antioxidant Activity of Indian Pomegranate Varieties. *Journal of Food Biochemistry*, **38:** 397-406. https://doi.org/10.1111/jfbc.12066.

Kumar R, Jakhar ML, Verma R, Jat, HM. 2017. Pomegranate Micropropagation - A Review. *International Journal of Pure & Applied Bioscience*, **5(5):** 1138-1149.

Kumaria S, Gogoi K, Tandon P. 2012. A comparative study of vitrification and encapsulation-vitrification for cryopreservation of protocorms of Cymbidium eburneum L., a threatened and vulnerable orchid of India. *Cryo Letters*, **33(6):** 443-452.

Lala Y, Meta K, Çuko B, Sota V, Benelli C, Lambardi L, Kongjika E. 2024. Synthetic seeds conversion to plantlets in *Punica granatum* L. cv. Devedishe after short-term storage at 4°C. The 2nd Conference of Cost Action CA21157 – COPYTREE: "In vitro culture of woody crops: Problem solving by new approaches", Jūrmala, Latvia. 22 – 24, April, 2024, Book of Proceedings ISBN: 978-9934-38-084-6, pg. 82 – 89.

Larrosa M, González-Sarrías A, Yáñez-Gascón MJ, Selma MV, Azorín-Ortuño M, Toti S, Tomás-Barberán F, Dolara P, Espín JC. 2009. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. *The Journal of nutritional biochemistry*, **21(8):**717-25. doi: 10.1016/j.jnutbio.2009.04.012. Epub 2009 Jul 18. PMID: 19616930.

Lloyd G, McCown B. 1980. Commercially feasible micropropagation of mountain laurel (*Kamlia latifolia*). *International Plant Propagation Society, Combined Proceedings*, **30:** 421–427.

Matsumoto T, Sakai A, Takahashi C, Yamada K. 1995. Cryopreservation of *in vitro*-grown meristems of wasabi (*Wasabia japonica*) by encapsulation-vitrification method. *Cryo Letters.* **16:** 189-196.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant.*, 15:473–497.

Nogueira GF, Pasqual M, Everson J. 2014. Survival of sugarcane shoot tips after cryopreservation by droplet-vitrification. Pg.1-4. DOI: 10.1590/S0100-204X2013001100014. https://doi.org/10.1590/S0100-204X2013001100014.

Osorio -Saenz A, Mascorro -Gallardo JO, Rodríguez De LA OJL, López CM, González-Arnao MT. 2011. Cryopreservation of chrysanthemum shoot-tips (Dendranthema grandiflorum Kitam) by encapsulation-dehydration and vitrification. *Revista Chapingo Serie Horticultura, XVII, Edición Especial* **2, 17**: 33-43. v17nspe2a4 PDF (www.scielo.org.mx).

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

Ravi D, Anand P. 2012. Production and applications of artificial seeds: A review. *International Research Journal of Biological Sciences*, **1:** 74-78.

Samir Z, El-Agamy Rafat AA, Mostafa Mokhtar M, Shaaban Marwa TE. 2009. *In vitro* Propagation of Manfalouty and Nab El-gamal pomegranate cultivars. *Research Journal of Agriculture and Biological Sciences*, **5 (6):**1169-1175.

Silva LC, Paiva R, Swennen R, Andrè E, Panis B. 2013. Shoot-tip cryopreservation by droplet-vitrification of *Byrsonima intermedia* A. Juss.: A woody tropical and medicinal plant species from Brazilian cerrado. *Cryo Letters*, **34**: 338-348.

Sota V, Kongjika E, Gashi B. 2023. Plant Biotechnology: *In vitro* Methodology and Protocols. Tirana: Albanian Academy of Sciences 204-205.

Vujovic T, Sylvestre I, Ružić D, Engelmann F. 2011. Droplet-vitrification of apical shoot tips of *Rubus fruticosus* L. and *Prunus cerasifera* Ehrh. *Scientia Horticulturae*, **130:** 222-228. DOI: 10.1016/j.scienta.2011.06.049.

Wang Q, Laamanen J, Uosukainen M. 2005. Cryopreservation of in vitro-grown shoot tips of raspberry (*Rubus idaeus* L.) by encapsulation–vitrification and encapsulation–dehydration. *Plant Cell Reports*, **24:** 280–288.

Xhuveli L. 2012. Albania, the domestication country for pomegranate (Punica granatum L.). *Genetic Resources and Crop Evolution*, **59**:1605–1610. DOI 10.1007/s10722-012-9915-6.

Zarfeshany A, Asgary S, Javanmard SH. 2014. Potent health effects of pomegranate. *Advanced biomedical research*, **25 (3): 100:** 1-7. https://doi.org/10.4103/2277-9175.129371. PMID: 24800189; PMCID: PMC4007340.

Zhao G, Fu J. 2016. Microfluidics for cryopreservation. *Biotechnology advances*, **35(2):** 323-336. doi: 10.1016/j.biotechadv.2017.01.006. Epub 2017 Jan 30. PMID: 28153517; PMCID: PMC6236673.