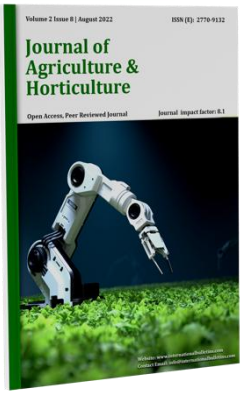

ACCLIMATIZATION OF FATINIA AND KALINA PLANTS MICROCLONALLY PROPAGATED IN VITRO TO EX VITRO CONDITIONS

Khojayev Shomil Tursunovich
Project Leader, Bustonliq Research Experimental Station, Academician M. Mirzaev Research Institute of Horticulture, Viticulture and Winemaking
ORCID ID: 0000-0001-6368-0478
Boyjigitov Fozil Mukhammadiyevich
PhD in Agricultural Sciences, Senior Researcher
ORCID ID: 0009-0007-2586-8497
Allayarov Abdurakhmon Nazaraliyevich
Head of Laboratory
ORCID: 0000-0001-9776-4979
Nurjobov Abbos Utkirovich
Head of Department
ORCID ID: 0009-0002-0479-0727
Yusupova Durdona Olti qizi
Researcher
ORCID: 0009-0001-2724-2830
+998 90 956-01-87
allayarovabduraxmon1986@gmail.com
https://doi.org/10.5281/zenodo.18239890

Abstract: This study investigated the vegetative development and open-field acclimatization of **Fatinia (Physocarpus opulifolius)** and **Kalina (Viburnum spp.)** plants after in vitro propagation and subsequent transplantation into various substrates under greenhouse conditions. Five different substrate mixtures (peat:perlite, peat:biogumus, peat:perlite:sand, soil:biogumus, and soil:peat:biogumus) were tested, and key vegetative parameters such as root number, plant height, and number of leaves were analyzed under each condition. In addition, the growth dynamics of the plants were monitored at 10-day intervals over a 40-day period during the gradual acclimatization to open-field conditions. The results demonstrated that the **peat:perlite:sand (1:1:1)** substrate was the most effective medium for both species, providing significantly higher root formation, vegetative biomass growth, and leaf production compared with the other substrates. In Fatinia, the number of roots reached 8.3 and stem length increased to 34.8 cm within four weeks, whereas in Kalina these values were 7.5 roots and 29.8 cm, respectively. Vegetative development under greenhouse conditions was stable, and the plants exhibited a high level of adaptability to the open environment.

Keywords: Kalina, Fatinia, in vitro, vegetative growth, substrate, perlite, peat, container cultivation, adaptation, greenhouse.

Introduction

Preservation of biological diversity, protection of plant resources, and their rational use are regarded as one of the major global challenges. According to global biodiversity assessments by UNEP, more than 30,000 plant and animal species are currently at risk of extinction due to various factors. In this regard, identifying ornamental, rare, and declining species of natural flora and developing strategies for their conservation are considered among the most pressing scientific issues (FAO statistics, 2023.
http://www.fao.org/faostat/en/#data/QC).

At present, particular attention is being paid to research aimed at developing effective

methods for identifying plant species and ensuring their rapid propagation. In this context, new cultivars and forms of ornamental species have been created. The potential of trees and shrubs in modern landscaping has been assessed, new methods of vegetative propagation have been developed, and optimal techniques for seed propagation have been improved.

Pursuant to Presidential Decree No. PF-46 "On measures to accelerate landscaping activities and to further improve the protection of trees in the Republic," the nationwide program "Yashil Makon" (Green Space) has been implemented across the country.

Woody and shrub plants are not only sources of raw materials and various products but also key factors in improving the natural environment. Plant life activity exerts a direct influence on climate, as plants absorb and neutralize CO_2 and other harmful gases and smoke, and reduce the amount of dust in urban air [8].

Micropropagation has been widely applied for the rapid multiplication of many plant species. However, its broader application has often been limited by high mortality rates when plants are transferred from in vitro conditions to greenhouse or field environments. In many cases, successful micropropagation is achieved by acclimating explants, after which their initial growth under in vitro conditions is followed by transfer to greenhouse or field settings. Under in vitro conditions, plants are grown in relatively airtight specialized containers, typically characterized by higher-than-normal humidity and lower light intensity.

In cell biotechnology, plant propagation in vitro is based on the regenerative capacity of cells, that is, their ability to divide and regenerate tissues [4]. Micropropagation of plants offers significant potential for the conservation of endangered endemic species [1].

To prevent microbial contamination, the use of closed containers reduces air circulation, which increases leaf boundary layers and limits the entry of CO_2 as well as the release of gaseous products from the vessels. The culture medium is often supplemented with saccharides as sources of carbon and energy. These additions significantly lower the water potential of the medium and increase the risk of bacterial and fungal contamination. Moreover, plants are usually supplied with high doses of growth regulators. Such conditions may lead to the formation of plants with abnormal morphology, anatomy, and physiology [7,9,10,3].

After transfer from in vitro to ex vitro conditions, plants must acquire the ability to form functional structures adapted to the external environment. In greenhouse conditions, and especially in the open field, light intensity is considerably higher and air humidity is much lower than inside culture vessels. Although the water potential of the substrate is higher than that of sucrose-containing media, plants may rapidly desiccate due to unrestricted water loss from the leaves [5].

In addition, water supply may be limited by the low hydraulic conductivity of roots and by restrictions at the root–shoot junctions [12]. During this stage, a large proportion of plants may perish. Therefore, following ex vitro transplantation, plants generally require several weeks of acclimatization with a gradual reduction in air humidity [11, 13, 2].

Acclimatization units equipped with computer-controlled temperature, humidity, light intensity, CO_2 concentration, and air flow rate have been developed for this purpose [6]. However, studies on the acclimatization of in vitro–grown plants are sometimes characterized by excessive simplification and inappropriate generalization.

Materials and Methods

This study was conducted during 2024–2025 in the Biotechnology Laboratory of the Academic Mahmud Mirzayev Research Institute of Horticulture, Viticulture and Winemaking.

The process of adapting microclonally propagated ornamental plants—*Physocarpus opulifolius* (Fatinya) and *Viburnum* spp. (Kalina)—from in vitro conditions to the external environment represents a critically important stage, since plantlets grown under laboratory conditions are adapted to sterile environments with high

humidity and controlled light and temperature. When transferred to open or semi-natural conditions, such plants may experience physiological stress. The main stages of acclimatization to external conditions are described below.

Initially, the plantlets were maintained in closed containers. Under these conditions, temperature was maintained at 24–26 °C, relative humidity at 80–90%, light intensity at 4000 lux, and illumination was diffused. During the first three days, the containers were kept completely closed. Subsequently, they were opened daily for 15–30 minutes, with the duration of exposure gradually increased. This procedure was carried out for 7–10 days, allowing the plantlets to progressively adapt to ambient air conditions. Proper irrigation management during the acclimatization period was of critical importance.

At the initial stage, the leaves of the plantlets were sprayed with water, while excessive wetting of the substrate was avoided. Maintaining optimal moisture levels prevented root decay. After 10–15 days, the plantlets were supplied with diluted mineral fertilizers for nutritional support. Following 3–4 weeks of acclimatization, the plantlets were transferred to greenhouse conditions. At this stage, they were protected from direct sunlight and the temperature was maintained at a constant level. After 4–5 weeks of further adaptation, the plantlets were transplanted to their permanent planting sites.

Results and Discussion

A large proportion of micropropagated plants fail to survive when transferred from in vitro conditions to greenhouse or open-field environments. Compared with in vitro conditions, greenhouse and field environments are characterized by significantly lower relative humidity, higher light intensity, and the absence of an aseptic environment, which exposes micropropagated plants to considerable stress.

However, the effectiveness of any micropropagation system can only be fully realized when plants propagated from cells and tissues are successfully transferred to environmental conditions in acclimatization facilities. Most species cultivated in vitro require a gradual acclimatization process after transfer to soil in order to ensure sufficient plant survival and vigorous growth.

The influence of substrate composition on the vegetative development of *Physocarpus opulifolius* (Fatinya) and *Viburnum* spp. (Kalina) was therefore investigated. This process was aimed at determining how substrates of different compositions affect key vegetative parameters of these plants, such as root formation, stem growth, and leaf development. This experimental analysis contributes to identifying optimal substrate compositions during the transition from the in vitro stage to the ex vitro phase and to enhancing the plants' adaptive capacity.

Substrates containing soil (for example, soil:biohumus or soil:peat:biohumus mixtures) were relatively heavy, restricted air circulation, retained excessive moisture, and suppressed root development. As a result, the overall vegetative performance of the plants grown in these substrates was reduced.

Table 1. **Effect of substrates on the vegetative development of Fatinia and Kalina plants**

Substrates	Fatinia plant			Kalina plant		
	Number of roots (pcs)	Plant height (cm)	Number of leaves (pcs)	Number of roots (pcs)	Plant height (cm)	Number of leaves (pcs)

Peat + perlite (1:1)	6.2	21.7	7.3	4.6	19.7	5.7
Peat + biogumus (2:1)	5.6	18.3	6.4	4.2	16.5	4.6
Peat + perlite + sand (1:1:1)	6.8	24.6	9.5	5.4	25.8	8.2
Soil + biogumus (1:1)	2.7	11.2	3.3	3.2	12.7	4.5
Soil + peat + biogumus (1:1:1)	3.1	12.1	4.7	3.6	14.8	4.2

During the acclimatization of *Physocarpus opulifolius* (Fatinya) and *Viburnum* spp. (Kalina) plants to ex vitro conditions, the applied substrates had a significant effect on their rooting, growth, and leaf development. In the experiment, the following substrate mixtures were evaluated: peat–perlite (1:1), peat:biohumus (2:1), peat:perlite:sand (1:1:1), soil:biohumus (1:1), and soil:peat:biohumus (1:1:1).

In *Physocarpus opulifolius*, the best results were obtained in the peat:perlite:sand (1:1:1) substrate, where plants developed an average of 6.8 roots, reached a plant height of 24.6 cm, and produced 9.5 leaves. These values were higher than those recorded in other substrates, indicating that this substrate composition provides favorable aeration and moisture-retention capacity. The second-best performance was observed in the peat–perlite (1:1) substrate, in which plants formed an average of 6.2 roots and attained a height of 21.7 cm.

Similarly, for *Viburnum* spp., the peat:perlite:sand (1:1:1) substrate proved to be the most effective, yielding an average of 5.4 roots, a plant height of 25.8 cm, and 8.2 leaves. This substrate ensured optimal development of both the root system and aboveground biomass of Kalina seedlings. High values were also recorded in the peat–perlite (1:1) substrate, where plants developed an average of 4.6 roots and reached a height of 19.7 cm.

Soil-based substrates (soil:biohumus and soil:peat:biohumus) resulted in comparatively lower performance in both plant species. For example, in *Physocarpus opulifolius* grown in the soil:biohumus (1:1) substrate, the average number of roots was 2.7, plant height was 11.2 cm, and the number of leaves was 3.3. In *Viburnum* spp., the same substrate produced plants with an average of 3.3 roots and a height of 13.6 cm. The limited aeration capacity and potentially higher salt content of these substrates may have restricted vegetative growth.

Overall, peat-based substrates, particularly those combined with perlite and sand, provided the highest vegetative performance for both plant species. These substrates play a crucial role in promoting root formation, enhancing vegetative biomass development, and improving the acclimatization capacity of plants during the transition from in vitro to ex vitro conditions.

Table 2. **Vegetative development of Fatinia and Kalina plants during adaptation to open field conditions under greenhouse conditions**

Observation date	**Fatinia plant**				**Kalina plant**			
	Number of roots (pcs)	Root length (cm)	Plant height (cm)	Number of leaves (pcs)	Number of roots (pcs)	Root length (cm)	Plant height (cm)	Number of leaves (pcs)

10 May	6.9	4.5	24.7	9.3	5.6	5.7	19.7	5.7
20 May	7.8	5.3	25.8	10.3	6.2	6.4	21.3	6.3
30 May	7.9	5.9	29.7	12.8	6.9	7.8	24.7	6.8
10 June	8.3	6.8	34.8	14.9	7.5	8.6	29.8	7.9

The dynamics of vegetative development of *Physocarpus opulifolius* (Fatinya) and *Viburnum* spp. (Kalina) plants acclimatized to open-field conditions under greenhouse environments were monitored at 10-day intervals, and changes in the number of roots, stem length, and number of leaves were recorded. Observations covered a 40-day period from May 10 to June 10.

In *Physocarpus opulifolius*, on May 10 the average number of roots was 6.9, plant height was 24.7 cm, and the number of leaves was 9.3. During the following ten days, these parameters increased markedly, with the number of roots rising to 7.8 and plant height reaching 25.8 cm. By May 30, plants produced an average of 7.9 roots, attained a height of 29.7 cm, and developed 13.2 leaves. By June 10, the number of roots increased to 8.3, plant height reached 34.8 cm, and the number of leaves rose to 14.9.

A similar growth trend was observed in *Viburnum* spp. During the initial ten-day period, an average of 5.7 roots, a plant height of 19.7 cm, and 5.7 leaves were recorded. On May 20, these values increased to 6.3 roots, 25.3 cm in height, and 6.4 leaves. By May 30, the number of roots reached 6.7, plant height increased to 27.9 cm, and the number of leaves rose to 7.6. By June 10, Kalina seedlings exhibited 7.5 roots, a stem length of 29.8 cm, and 7.9 leaves.

These results indicate that when acclimatization under greenhouse conditions is carried out gradually, both plant species exhibit consistent development of the root system and vegetative biomass. In particular, during the 30–40 day period, root formation and leaf emergence increased significantly. This confirms the successful adaptation of the plants to the new environment and their high survival rate following transplantation.

Conclusion

The conducted studies demonstrated that the vegetative development of *Physocarpus opulifolius* and *Viburnum* spp. during the transition from in vitro conditions to greenhouse and open-field environments is directly dependent on substrate composition and the duration of the acclimatization period. Among the tested substrates, the peat:perlite:sand (1:1:1) mixture was identified as the most effective. This substrate promoted enhanced root formation, greater plant height, and increased leaf production, thereby facilitating the successful transition of plants to the ex vitro stage.

Furthermore, phenological observations carried out over a 40-day period confirmed the stable development of both plant species under greenhouse conditions. Root number and vegetative growth parameters increased at each 10-day interval. By June 10, *Physocarpus opulifolius* exhibited 8.3 roots, a stem length of 34.8 cm, and 14.9 leaves, while *Viburnum* spp. developed 7.5 roots, a stem length of 29.8 cm, and 7.9 leaves.

The results indicate that the use of lightweight, well-aerated substrates combined with a stepwise acclimatization strategy enhances plant survival and ensures stable development of both the root system and aboveground biomass. This approach demonstrates high efficiency for the mass production of micropropagated plantlets and their successful transfer to open-field conditions.

References:

1.Bramwell, D. (1990). The role of in vitro cultivation in the conservation of endangered species. In Conservation Techniques in Botanic Gardens (pp. 3–16). Koeltz Scientific Books, Königstein, Germany.

2.Bolar, J. P., Norelli, J. L., Aldwinckle, H. S., & Hanke, V. (1998). An efficient method for rooting and acclimation of micropropagated apple cultivars. HortScience, 37, 1251–1252.
3.Budendorf-Joosten, J. M. C., & Woltering, E. J. (1994). Components of the gaseous environment and their effects on plant growth and development in vitro. Plant Growth Regulation, 15, 1–16.
4.Davronov, Q. D. (2008). Biotexnologiya: Ilmiy, amaliy va uslubiy asoslari [Biotechnology: Scientific, practical, and methodological foundations]. Tashkent, Uzbekistan. 418 p.
5.Desjardins, Y. (1995). Photosynthesis in vitro: On the factors regulating CO_2 assimilation in micropropagation systems. Acta Horticulturae, 393, 45–61.
6.Hayashi, M., Nakayama, M., & Kozai, T. (1988). An application of the acclimatization unit for growth of carnation explants, and for rooting and acclimatization of the plantlets. Acta Horticulturae, 230, 189–194.
7.Kozai, T. (1991). Micropropagation under photoautotrophic conditions. In P. C. Debergh & R. H. Zimmerman (Eds.), Micropropagation: Technology and Application (pp. 447–469). Kluwer Academic Publishers, Dordrecht–Boston–London.
8.Xakimova, M., Turdiev, S., & Babadjanova, L. (2021). Bioecology and traditional medicinal importance of sea buckthorn (Hippophae) with ornamental and useful properties. Academic Research in Educational Sciences Scientific Journal, Impact Factor (SJIF) 5.723.
9.Pospíšilová, J., Solárová, J., & Čatský, J. (1992). Photosynthetic responses to stresses during in vitro cultivation. Photosynthetica, 26, 3–18.
10.Pospíšilová, J., Čatský, J., & Šesták, Z. (1997). Photosynthesis in plants cultivated in vitro. In M. Pessarakli (Ed.), Handbook of Photosynthesis (pp. 525–540). Marcel Dekker, New York–Basel–Hong Kong.
11.Preece, J. E., & Sutter, E. G. (1991). Acclimatization of micropropagated plants to the greenhouse and field. In P. C. Debergh & R. H. Zimmerman (Eds.), Micropropagation: Technology and Application (pp. 71–93). Kluwer Academic Publishers, Dordrecht–Boston–London.
12.Fila, G., Ghashghaie, J., Hoarau, J., & Cornic, G. (1998). Photosynthesis, leaf conductance and water relations of in vitro cultured grapevine rootstock in relation to acclimatization. Physiologia Plantarum, 102, 411–418.
13.Kadleček, P. (1997). Effect of pretreatment by irradiance and exogenous saccharose under in vitro conditions on photosynthesis and growth of tobacco (Nicotiana tabacum L.) plants during acclimatization after transfer to soil (Diploma thesis). Charles University, Department of Plant Physiology, Prague.
14.FAO. (2023). FAOSTAT database. Retrieved from: https://www.fao.org/faostat/en/#data/QC