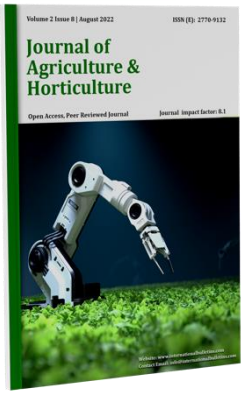

CULTIVATION OF IN VITRO PROPAGATED KIWI (ACTINIDIA DELICIOSA) PLANTS ON DIFFERENT SUBSTRATES

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Abstract

This article presents the results of experiments conducted on the acclimatization of kiwi (Actinidia deliciosa) plants propagated under in vitro conditions using various substrates in a laboratory setting. Among the tested variants, substrates with a high proportion of coconut peat yielded the best results. In particular, in Variant 7, which consisted of 60% coconut peat, 20% vermiculite, 10% perlite, and 10% soil, the plant survival rate reached 86%. In other variants, composed of 60% soil, 10% biohumus, 10% coconut peat, 10% vermiculite, and 10% perlite, plants grew up to an average height of 65 cm.

Keywords: In vitro, kiwi, explant, clonal micropropagation, apical meristem, callus tissue, cytokinin, auxin, phytohormones, acclimatization.

Introduction

In recent years, the cultivation and production volume of fruit crops around the world, including kiwi (Actinidia deliciosa), have been steadily increasing. New varieties such as 'Hayward' and 'Arctic Kiwi' are being planted more widely, with production expanding globally. Kiwi is recognized for its high yield potential and adaptability to various climatic conditions. Countries such as New Zealand, Italy, and Chile are leading the way in advanced technologies and research focused on maximizing kiwi production (Ferguson et al., 2018).

In Uzbekistan, kiwi cultivation is also experiencing growth. However, there is still a significant need for the widespread introduction of new varieties and modern production technologies (Abdullayev, 2021). Although high-yield kiwi varieties are relatively new to Uzbekistan, their adaptability to local climates and the production of high-quality fruits can help meet the growing market demand. Kiwi fruits are valued for their excellent organoleptic properties, rich vitamin content, and antioxidants, making them highly beneficial for consumers (Gao et al., 2019).

Nevertheless, the development of kiwi cultivation in Uzbekistan is hampered by the limited availability of varieties and insufficiently advanced cultivation techniques. This continues to constrain efforts to increase yield potential (Yuldashev et al., 2020).

Enhancing Kiwi Cultivation through Development of New Varieties and Advanced Breeding Methods

The improvement of kiwi (Actinidia deliciosa) cultivation and productivity relies heavily on the development of new varieties and the implementation of advanced breeding techniques. At present, globally popular kiwi varieties such as 'Hayward' and 'Arctic' are widely cultivated due to their climate adaptability, disease resistance, high yields, and superior fruit quality (Ferguson et al., 2018). These varieties are not only in high demand in domestic markets but also enjoy significant export potential (Albuquerque et al., 2015).

In the development of new kiwi varieties, two of the most important traits are resistance to diseases and tolerance to cold climates. Recent breeding efforts have focused on enhancing these traits, resulting in varieties capable of producing high-quality fruits even under low-temperature conditions (Huang et al., 2020).

One of the primary propagation methods for kiwi plants is vegetative propagation, which involves the use of buds or roots to produce new plants. However, the efficiency and speed of this method can sometimes be limited. Therefore, in vitro propagation through tissue culture has gained prominence as a highly effective alternative for kiwi multiplication. This method involves the cultivation of explants (such as leaves, nodes, or roots) in a nutrient medium under sterile laboratory conditions (Murashige & Skoog, 1962).

During in vitro propagation, plant growth regulators such as BAP (6-benzylaminopurine), gibberellic acid (GA_3), and indole-3-butyric acid (IBA) are utilized. These hormones enhance plant growth, accelerate regeneration processes, and ensure the development of high-quality seedlings (Hassan et al., 2015). When used in appropriate concentrations, these regulators facilitate the successful development of shoots and roots in kiwi explants (Camarillo et al., 2016).

The Murashige and Skoog (MS, 1962) medium is widely used for in vitro propagation of kiwi, as it contains all the essential nutrients required for plant development. Once explants are introduced into the nutrient medium, they multiply rapidly and produce new plants, contributing to the production of high-quality kiwi seedlings (Duan et al., 2018).

Thus, to further advance kiwi cultivation in Uzbekistan and increase yield levels, it is crucial to introduce new varietys and modern technologies. These developments, in turn, will support the production of competitive, high-demand products for both local and international markets (Santos et al., 2020).

Materials and methods

This study on the clonal micropropagation of kiwi (Actinidia deliciosa) under in vitro conditions was conducted in 2024 at the Biotechnology Laboratory of the Mahmud Mirzayev Research Institute of Horticulture, Viticulture, and Winemaking.

Kiwi (*Actinidia deliciosa*) is one of the fruit crops known for producing large, sweet, and high-quality fruits. This variety is particularly distinguished by its large fruit size, high productivity, and firm structure. Kiwi fruits exhibit a blend of red and green coloration, combining a pleasant balance of sweetness and acidity. They are notable for their large size and delicate skin. The plant grows rapidly and, under optimal conditions, provides high yields. Kiwi typically grows as a round-shaped, perennial vine that flowers in spring and bears fruit in the

summer. The plant adapts well to temperate climates and requires regular irrigation and adequate nutrients for healthy development. Kiwi is widely cultivated in orchards and commercial plantations in many countries.

In vitro propagation of kiwi (*Actinidia deliciosa*), particularly using explants derived from apical meristems or the upper parts of the plant, requires careful sterilization. This process is carried out under controlled conditions, similar to other plant species. The sterilization procedure was implemented as follows:

- **Explant Collection**: Healthy and vigorous leaves or small tissue pieces from the apical parts of the kiwi plant were selected as explants. Only disease-free plant material was used for this purpose.
- **Washing**: The collected explants were first washed for several minutes in soapy water to remove surface fungi and bacteria.
- **Sterilization**: For sterilizing the apical meristems of kiwi, sodium hypochlorite (NaOCl) solutions at concentrations of 0.5%, 1%, 3%, and 5% were used. These solutions effectively eliminated microorganisms, fungi, and bacteria on the plant surface.
- **Duration and Conditions**: The explants were agitated in the NaOCl solution for 10, 15, or 20 minutes using a magnetic stirrer to ensure thorough sterilization.
- **Post-Sterilization Rinsing**: After sterilization, the explants were rinsed 2–3 times with distilled water to remove residual chemicals and prevent damage to the plant tissue, thereby ensuring successful disinfection.

For culture media, both Murashige and Skoog (MS) and Driver and Kuniyuki Walnut (DKW) media with different compositions were used.

Initially, the explants were planted into MS and DKW media supplemented with 30 g/L sucrose and 6.5 g/L agar. The pH was adjusted to 5.6 ± 0.1, and the media were autoclaved at 121°C for 25 minutes.

Young seedlings with well-developed roots were removed from the tubes for **acclimatization** to local soil conditions. Their roots were gently washed to remove any residual DKW media. Subsequently, they were transplanted into mixtures of **coconut peat, vermiculite, and perlite** in various ratios.

The transplanted seedlings were placed into transparent containers that allowed light penetration and stored in a controlled room for **10–14 days**. Temperature and relative humidity were continuously monitored throughout the acclimatization period.

Results And Discussion

The acclimatization phase—transferring in vitro-grown kiwi (*Actinidia deliciosa*) seedlings to external conditions—has a direct impact on their survival rate. At this stage, plant survival largely depends on the composition of the substrate (growing medium) provided, where the type and proportion of components play a critical role. In this study, various substrate combinations were tested to analyze their effects on the survival rate of in vitro-propagated kiwi seedlings.

A total of nine different substrate mixtures were evaluated during the study. These substrates were composed of components such as **soil, biohumus, coconut peat, vermiculite**, and **perlite**, with the proportion of each component varying across the tested variants. The overall survival percentage of the seedlings was calculated for each combination.

The results showed that the **variants containing higher proportions of coconut peat** provided the best outcomes. Notably, **Variant 7**, consisting of **60% coconut peat, 20%**

vermiculite, 10% perlite, and 10% soil, demonstrated the **highest survival rate of 86%**. Similarly, **Variant 8**, which contained **50% coconut peat**, yielded a **75% survival rate**.

These findings suggest that **coconut peat is the most favorable substrate component** for acclimatizing in vitro-grown kiwi plants. The reason lies in its excellent aeration capacity, high water retention ability, and relatively sterile environment, which creates optimal conditions for plant survival during the transition phase.

Table 1. Survival rates of kiwifruit (Actinidia deliciosa) plants exposed to different substrates during the in vitro acclimation phase.

№	SUBSTRATE					
	Soil percentage (in proportion)	**Biohumus percentage (in proportion)**	**Coconut peat percentage (in proportion)**	**Vermiculite percentage (in proportion)**	**Perlite percentage (in proportion)**	**Plant survival (%)**
1	7/10	0/10	0/10	2/10	1/10	56%
2	5/10	0/10	2/10	2/10	1/10	63%
3	6/10	1/10	1/10	1/10	1/10	55%
4	0/10	6/10	1/10	2/10	1/10	28%
5	0/10	5/10	1/10	2/10	2/10	35%
6	1/10	4/10	1/10	2/10	2/10	40%
7	1/10	0/10	6/10	2/10	1/10	86%
8	1/10	1/10	5/10	2/10	1/10	75%
9	0/10	3/10	3/10	2/10	2/10	48%

On the other hand, the survival rate of seedlings was significantly lower in substrate variants with a high proportion of biohumus. For instance, **Variant 4** (60% biohumus) and **Variant 5** (50% biohumus) demonstrated survival rates of only **28% and 35%**, respectively. This suggests that the **high organic content and elevated microbiological activity** in biohumus may have created an unfavorable environment for in vitro-grown plantlets. Since the root systems of such plantlets are not yet fully developed, **excessive biological activity** likely reduced their chances of survival.

Moderate survival rates were observed in **soil-based substrates**. For example, **Variant 2** (50% soil, 20% coconut peat, 20% vermiculite) recorded a **63% survival rate**, while **Variants 1 and 3** had survival rates of approximately **55–56%**. Thus, although a **mixture of soil and coconut peat** produced intermediate results, **substrates dominated by coconut peat** yielded **significantly higher survival rates** and are therefore more favorable for maximum efficiency.

Overall, based on the findings, the following conclusions can be drawn:

- **Higher proportions of coconut peat** in the substrate lead to **maximum survival rates** of in vitro-grown kiwi seedlings;
- **High levels of biohumus** have **negative effects** due to intense biological activity;
- **Soil, vermiculite, and perlite** are **beneficial as supplementary components**, but should **not be the main substrate base**.

The most effective substrate mixture identified in the study was composed of **60% coconut peat, 20% vermiculite, 10% perlite, and 10% soil**, which provided an **86% survival rate**. These results have **practical significance**, especially for selecting optimal substrates when transferring kiwi (*Actinidia deliciosa*) from laboratory to open or commercial conditions.

During the **vegetative growth phase** of *Actinidia deliciosa*, particularly under greenhouse conditions, the **growth rate and development** of the plants are largely dependent on the **substrate composition**. In this study, seedling height (in cm) was measured in each substrate combination to evaluate the **growth dynamics**. The tested substrates consisted of various proportions of **soil, biohumus, coconut peat, vermiculite**, and **perlite**.

According to the experimental data, the **highest growth rate** was recorded in **Variant 3**.

Table 2. Growth dynamics of kiwi (Actinidia deliciosa) plants under the influence of different substrates in greenhouse conditions.

№	SUBSTRATE					
	Soil percentage (in proportion)	Biohumus percentage (in proportion)	Coconut peat percentage (in proportion)	Vermiculite percentage (in proportion)	Perlite percentage (in proportion)	Plant height (cm)
1	7/10	0/10	0/10	2/10	1/10	46 cm
2	5/10	0/10	2/10	2/10	1/10	53 cm
3	6/10	1/10	1/10	1/10	1/10	65 cm
4	0/10	6/10	1/10	2/10	1/10	27 cm
5	0/10	5/10	1/10	2/10	2/10	32 cm
6	1/10	4/10	1/10	2/10	2/10	38 cm
7	1/10	0/10	6/10	2/10	1/10	36 cm
8	1/10	1/10	5/10	2/10	1/10	45 cm
9	0/10	3/10	3/10	2/10	2/10	38 cm

The substrate consisting of 60% soil, 10% biohumus, 10% coconut peat, 10% vermiculite, and 10% perlite yielded the tallest plants, with an average height of 65 cm. This finding indicates that when soil is the dominant component and other materials such as biohumus, coconut peat, vermiculite, and perlite are added in smaller proportions, plant growth is significantly enhanced.

The next best results were observed in Variant 2 (53 cm) and Variant 1 (46 cm). In both cases, soil was the major component, supplemented by coconut peat, vermiculite, and perlite, resulting in moderate to good growth. Notably, Variant 2, comprising 50% soil, 20% coconut peat, 20% vermiculite, and 10% perlite, ensured relatively stable plant development.

Interestingly, despite achieving the highest survival rate in in vitro acclimatization, Variant 7, which contained 60% coconut peat, 10% soil, 20% vermiculite, and 10% perlite, led to only 36 cm of plant height under greenhouse conditions. This suggests that coconut peat, while favorable for survival, may lack sufficient nutrients or structural properties to support optimal growth in greenhouse environments.

Similarly, substrates rich in biohumus negatively affected plant growth. For example:

- Variant 4 (60% biohumus): 27 cm
- Variant 5 (50% biohumus): 32 cm
- Variant 6 (40% biohumus): 38 cm

These low growth outcomes may be attributed to excess organic compounds or salts present in biohumus, which can inhibit early root development in delicate in vitro-derived seedlings.

A moderate outcome was recorded in Variant 8, which contained 50% coconut peat, and 10% each of soil, biohumus, vermiculite, and perlite. This combination supported plant growth up to 45 cm, but still underperformed compared to soil-dominant mixtures, likely due to the high share of coconut peat.

In Variant 9, where coconut peat and biohumus were each at 30%, and the remaining components at 20%, the resulting average plant height was 38 cm, indicating medium performance.

- The best vegetative growth of *Actinidia deliciosa* under greenhouse conditions was achieved with soil-based substrates, particularly Variant 3, which featured 60% soil and equal lower shares of other components, producing plants up to 65 cm tall.
- High proportions of coconut peat or biohumus had negative effects on growth. Notably, biohumus exceeding 40% resulted in marked reductions in plant height.
- Perlite and vermiculite improved substrate aeration and physical structure, but were most effective in moderate amounts (10–20%).
- Overall, for stable growth of kiwi under greenhouse conditions, a nutrient-rich, aerated, and moisture-retentive substrate with soil as the primary component provides the best results.

Conclusion

In conclusion, it was found that soil, vermiculite, and perlite are useful as additional components, but they should not be the main components. The most effective substrate composition was identified as 6/10 coconut peat, 2/10 vermiculite, 1/10 perlite, and 1/10 soil, which provided 86% survival rate and created favorable conditions for growth and development. Such findings are of practical importance for selecting the optimal substrate, especially when transferring kiwi plants from laboratory conditions to open environments for commercial and scientific propagation.

References:

1.Abdullayev, A. (2021). Introduction of modern technologies and varieties in kiwi cultivation. Academy of Agriculture of Uzbekistan.
2.Albuquerque, P., Ribeiro, R., & Mendes, M. (2015). Kiwi plant breeding and technology improvement. Fruit Science Journal, 45(2), 120–130.
3.Basu, S., Ghosh, P., & Dutta, D. (2016). Effect of different concentrations of BAP and IBA on in vitro propagation of kiwifruit. International Journal of Plant Biology, 7(3), 1–6.
4.Camarillo, I., Valdes, J., & Garcia, F. (2016). In vitro propagation methods: Microclonal propagation in kiwi plant. Plant Cell Reports, 35(7), 1605–1612.
5.Chandra, R., Meena, S., & Agarwal, A. (2015). Role of plant growth regulators in in vitro propagation of horticultural crops. Science Progress, 98(2), 214–225.
6.Dawood, M., Al-Dosary, M., & Khalaf, N. (2017). In vitro sterilization techniques for plant tissue culture: A review. International Journal of Advanced Research, 5(3), 1737–1744.

7.Duan, Q., Zhang, Z., & Liu, J. (2018). Kiwi cultivation and adaptation of new varieties to climate. Agricultural Research Review, 22(1), 49–58.
8.Ferguson, A., Mcneil, D., & Hayward, G. (2018). Kiwi breeding: New varieties and innovative technologies. New Zealand Journal of Horticultural Science, 46(4), 350–366.
9.Gao, W., Zhou, X., & Wang, J. (2019). Organoleptic properties and benefits of kiwi fruits. Food Chemistry, 245, 563–572.
10.Hassan, M., Al-Aswad, A., & Ahmed, M. (2015). In vitro propagation and role of plant growth regulators. Biotechnology Advances, 33(6), 977–983.
11.Hassan, R., Iqbal, A., & Uddin, M. (2021). In vitro propagation of kiwi (Actinidia deliciosa) and its rooting response to various plant growth regulators. Plant Cell Tissue and Organ Culture, 146(1), 1–8.
12.Huang, Y., Zhan, D., & Chen, X. (2020). Improving disease immunity in kiwi plants. Horticultural Science, 55(3), 200–210.
13.Mithila, K., Shikha, K., & Rani, S. (2019). Effect of sterilization agents on in vitro cultures of medicinal plants. Plant Cell Reports, 38(4), 421–431.
14.Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3), 473–497.
15.Narayana, A., Ramesh, G., & Kiran, P. (2017). Effect of plant growth regulators on root induction and shoot multiplication of Actinidia deliciosa in vitro. Journal of Applied Horticulture, 19(2), 115–120.
16.Nizam, M., Arshad, M., & Saeed, T. (2014). In vitro culture of kiwifruit: Effect of media composition on regeneration and rooting. Indian Journal of Biotechnology, 13(4), 434–439.
17.Pshikhacheva, T. (2012). Kiwi yield and breeding processes. Journal of Fruit Production, 39(2), 121–128.
18.Razi, M., Shamsi, K., & Shah, R. (2020). Effect of BAP concentration on shoot proliferation and rooting of kiwi (Actinidia deliciosa) in vitro. Horticultural Science, 45(1), 22–30.
19.Santos, J., Kumar, R., & Patel, D. (2020). New technologies and production efficiency in kiwi cultivation. International Journal of Agricultural Research, 18(4), 235–248.
20.Singh, R., Sharma, M., & Patil, M. (2018). Effect of auxins and cytokinins on in vitro regeneration of Actinidia deliciosa. Acta Horticulturae, 1215, 89–95.
21.Yuldashev, F., Bazarov, S., & Kamolov, R. (2020). Development trends in kiwi cultivation in Uzbekistan. Journal of Horticultural Science, 45(1), 23–30.