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# SELECTION OF INITIAL MATERIAL AND IN VITRO CULTIVATION FOR OBTAINING PLANTING MATERIAL FREE FROM POTATO LEAFROLL VIRUS

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***Summary***. *This study investigated the effectiveness of surface sterilization methods for explants of several potato cultivars ("Arizona," "Gala," "Picasso," "Zarzara," and "Santa") under in vitro conditions. Gibberellic acid ($GA_3$), ethanol ($C_2H_5OH$, 70%), mercuric chloride ($HgCl_2$, 0.1%) with Tween-80, sterile distilled water, kinetin (KIN), and benzylaminopurine (BAP) were applied at varying concentrations and exposure durations to assess their effects on explant survival. Two surface sterilization protocols (S1 and S2) were evaluated, with protocol S2 demonstrating superior efficiency, achieving an 80% survival rate. Cultivation of meristem explants in MS1 nutrient medium supplemented with KIN and BAP enhanced the regeneration process. The findings highlight the critical role of apical meristem tissues and optimized surface sterilization protocols in producing virus- and pathogen-free seed potatoes through in vitro techniques.*

***Key word:.*** Potato L virus, PCR, micropropagation, sterilization, gibberellic acid (GA3), ethanol (C2H5OH), mercuric chloride (HgCl2), Murashige and Skoog (MS), KIN and BAP, Arizona, Gala, Picasso, Zarzara, Santa.

# ОТБОР ИСХОДНОГО МАТЕРИАЛА И КУЛЬТИВИРОВАНИЕ IN VITRO ДЛЯ ПОЛУЧЕНИЯ ПОСАДОЧНОГО МАТЕРИАЛА, СВОБОДНОГО ОТ ВИРУСА СКРУЧИВАНИЯ ЛИСТЬЕВ КАРТОФЕЛЯ. (PLRV)

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***Аннотация.*** *В данной статье изучалась эффективность методов поверхностной стерилизации эксплантов различных сортов картофеля («Аризона», «Гала», «Пикассо», «Зарзара», «Санта») при выращивании их в условиях in vitro. В исследовании применялись ГА3 (гиббереллиновая кислота), C2H5OH (70%), HgCl2 (0,1%) + Tween-80, стерильная вода, регуляторы роста KIN и BAP в различных концентрациях и продолжительности воздействия, и анализировалось их влияние на выживаемость эксплантов. Для поверхностной стерилизации эксплантов были протестированы два протокола (S1 и S2), при этом протокол S2 показал более высокую эффективность, достигнув уровня выживаемости 80%. Культивирование меристемных эксплантов в*

*питательной среде MS1 с добавлением KIN и BAP стимулировало процесс регенерации. Результаты исследования подтвердили важность использования апикальной меристемной ткани и эффективных методов поверхностной стерилизации для получения вирусо- и патогенсвободного картофельного семенного материала методом in vitro.*

***Ключевые слова:*** Вирус скручивания листьев картофель, **ПЦР,** микропропагация, стерилизация, гиббереллиновая кислота (ГА3), этанол (C2H5OH), хлорид ртути (II) (HgCl2), среда Мурашиге и Скоога (MS), KIN и BAP, Аризона, Гала, Пикассо, Зарзара, Санта.

## Introduction

The global demand for potato (Solanum tuberosum L.) and potato-based food products has been steadily increasing [1, 2]. Currently, potato is cultivated in nearly 150 countries [3]. As a staple crop, potato has been consumed for centuries and ranks fourth in global production after maize, wheat, and rice [4]. The quality of seed tubers plays a critical role in potato cultivation [5]. However, over time, crop quality has significantly declined due to various infectious diseases that affect plants during the growing season, resulting in considerable yield losses [6].

Potato is highly susceptible to viral infections, which may reduce yields by up to 75%. Individual viruses can cause productivity losses of approximately 40%, while mixed infections may lead to yield reductions of up to 90% [7]. Common viral pathogens include Potato virus X (PVX), Potato virus Y (PVY), Potato virus A (PVA), Potato virus S (PVS), Potato virus M (PVM), and Potato leafroll virus (PLRV), among others [8].

Meristem culture under in vitro conditions is an effective method for producing virus-free plants [9]. Studies have shown that yields from virus-free, meristem-derived plants are approximately 10% higher than those from virus-infected material. Large-scale production of virus-free plants using meristem tissue culture techniques has been successfully implemented in many countries worldwide [9].

Meristems from virus-infected plants typically harbor only minimal concentrations of viruses, making them highly effective for virus elimination in seed potato production. Furthermore, in several regions, the first generation of commercialized transgenic potato varieties has been developed with resistance to herbicides, insects, and pathogens, achieved through the integration of molecular biology approaches with tissue culture techniques. Therefore, ongoing research seeks to identify the most efficient and economically viable strategies to address these challenges [10].

**In vitro experiments** were conducted using apical meristems (0.1–0.2 mm in length) cultured on Murashige and Skoog (MS) nutrient medium. All tested potato cultivars

were found to be free from Potato virus A (PVA), Potato virus M (PVM), and Potato virus Y (PVY), but infected with Potato L virus (PLRV) and Potato virus X (PVX). Thus, the efficiency of virus elimination varied depending on the cultivar [11]. In parallel experiments, meristems of different sizes (0.1–1.0 mm in diameter) excised from roots infected with PVX, PVY, and PVS were cultured in MS nutrient medium supplemented with different combinations of 6-benzylaminopurine (BAP), kinetin (KIN), and $\alpha$-naphthaleneacetic acid (NAA). Meristem survival was influenced by the nutrient medium composition, explant type, and meristem size. Apical meristems demonstrated a higher survival rate (40.5%) compared to lateral meristems (25.9%). Furthermore, smaller meristems (0.11–0.25 mm in diameter) were more effective in producing virus-free potato plantlets than larger ones [12].

Extensive research in potato biotechnology has focused on improving healthy seed tuber production in combination with in vitro microclonal propagation techniques [13]. Plant morphogenesis under artificial conditions is regulated by multiple factors, including culture conditions and explant type, with no universal recommendations available for different plant species. Although efforts have been made to generalize the role of hormones in morphogenesis induction, no single rule describes their effects on bud and root formation [14].

Apical meristem culture enables rapid production of potato plants free from viral, fungal, and bacterial infections, and various nutrient media have been developed for this purpose [15]. Typically, explants measuring 0.1–0.3 mm are excised from actively growing buds. The use of apical meristem tissue significantly reduces viral infection but does not always ensure complete elimination. Virus eradication is most effective when meristem culture is combined with thermotherapy (37–40 °C) or antiviral chemotherapy (e.g., ribavirin, virazole, chitosan, interferon, lamivudine, or cycloferon) targeting major potato viruses such as PLRV, PVY, PVX, PVM, and PVS [16]. To confirm the presence or absence of viruses in regenerated plants, samples are routinely tested using enzyme-linked immunosorbent assay (ELISA) or reverse transcriptase PCR (RT-PCR) for RNA virus detection [15]. At present, regeneration of plants from apical meristem tissue remains the only reliable method for producing virus-free seed potatoes.

## Materials and Methods

This research was carried out in the *"Transgenomics and Tissue Culture"* laboratory at the Genomics and Bioinformatics Center. The objective of the study was to determine the optimal concentrations of cytokinins for plant growth, development, and root

formation, as well as to perform microclonal propagation under in vitro conditions. The experimental material consisted of nodal explants infected with PLRV, identified through real-time PCR, from five potato cultivars: *Arizona, Gala, Picasso, Zarzara,* and *Santa*.

**Preparation of nutrient medium.** Murashige and Skoog (MS) medium [Murashige & Skoog, 1962] was used for microclonal potato seedling production. To prepare 1 L of medium, 5 ml of macronutrient stock solution, 1 ml of micronutrient stock solution, and 10 ml of Fe-EDTA solution were mixed in 800 ml of distilled water maintained at 30–40 °C. Sucrose (30 g/L) and ribavirin (30 mg/L) were added as carbon and antiviral agents, respectively. The pH was adjusted to 5.6–5.8 with 1 M KOH or 1 M HCl. Agar (7 g/100 ml) was used as a solidifying agent, sterilized by boiling, and incorporated into the medium. The prepared medium was aliquoted into sterile containers and autoclaved at 121 °C and 1 atm for 20 minutes.

**Sterilization of explants.** All instruments, glassware, and culture media were sterilized using wet steam (autoclaving) or dry heat. Petri dishes, glassware, and metal instruments were wrapped in foil and sterilized at 180 °C for 40 minutes. Flasks, cotton, gauze, and filter paper were sterilized by autoclaving at 120 °C and 2 atm for 30 ± 5 minutes. Media dispensed into tubes or flasks were sterilized at 120 °C and 0.7–0.8 atm for 30 ± 5 minutes.

Nodal explants (15 per variety) were washed under running water and treated with 0.10 g/L gibberellic acid ($GA_3$) solution for 2 hours. After rinsing, the explants were incubated in sterile paper bags at 24 °C for two weeks. Buds (1.0–1.5 cm shoots) emerging from nodes were collected into sterile containers with distilled water and rinsed for 5 minutes. Surface sterilization was carried out following the protocol of Butenko (1990), involving two sterilization regimes (S1 and S2).

To minimize the harmful effects of phenolic compound exudation, explants were treated with 100 mg/L ascorbic acid solution for 10 minutes and rinsed three times with sterile distilled water prior to in vitro culture.

**Culture conditions.** MS medium was used at all experimental stages, with pH adjusted to 5.7. Explants and regenerated shoots were cultured in a growth chamber under a 16/8 h light/dark photoperiod at 25 ± 2 °C and 3000 lux light intensity. Arizona, Gala, Picasso, Zarzara, and Santa.

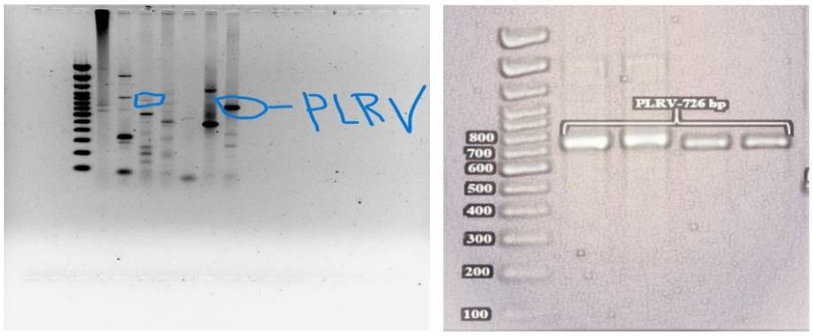
Explants were cultivated in the following nutrient medium composition: Murashige and Skoog (1962), sucrose –

## Result and discussion

According to the conducted research results, real-time PCR tests were performed on the potato varieties

8%, agar-agar – 0.7%, kinetin – 0.5 mg/L; 1.0 mg/L; 2.0 mg/L, BAP – 0.5 mg/L; 1.0 mg/L; 2.0 mg/L, and kinetin – 1.0 mg/L + BAP – 0.5 mg/L. The plants were grown under conditions of +20 to +22°C temperature, a photoperiod of 16:8 hours, and light intensity of 2000–3000 lux.

During the study, in order to obtain virus-free microshoots through in vitro cultivation, fifteen nodal explants of uniform size were selected from each potato variety under investigation. These nodes were treated with gibberellic acid ($GA_3$) at a concentration of 0.10 g/L for two hours to induce bud initiation, after which they were incubated for two weeks.




**1)** **2)**

**Fig. 1. Electropherogram of the PCR product of potato plant nodes infected with PLRV.**
**In Figure 1: M – Marker 2000 bp DNA ladder plus; 1 – Zarzara; 2 – Pikasso.**
**In Figure 2: M – Marker 2000 bp DNA ladder plus; 1 – Gala; 2 – Arizona; 3 – Santa; 4 – Zarzara variety.**

Gibberellic acid ($GA_3$) is a commonly used, broad-spectrum plant growth regulator that accelerates plant growth and development by promoting cell elongation and division. As a result of $GA_3$ treatment, maximum bud initiation and development were observed across all experimental varieties. However, notable variation in the extent of bud development was recorded among cultivars, with some exhibiting higher and others lower levels of growth. These differences may be associated with the inherent dormancy period of the nodal tissues.

Overall, $GA_3$ treatment effectively stimulated bud sprouting in all tested varieties, with a 100% bud emergence rate recorded in nodal explants. Among the tested cultivars, *Gala* and *Santa* displayed relatively higher bud numbers and greater bud length compared to the other varieties (Table 1, Figure 2).

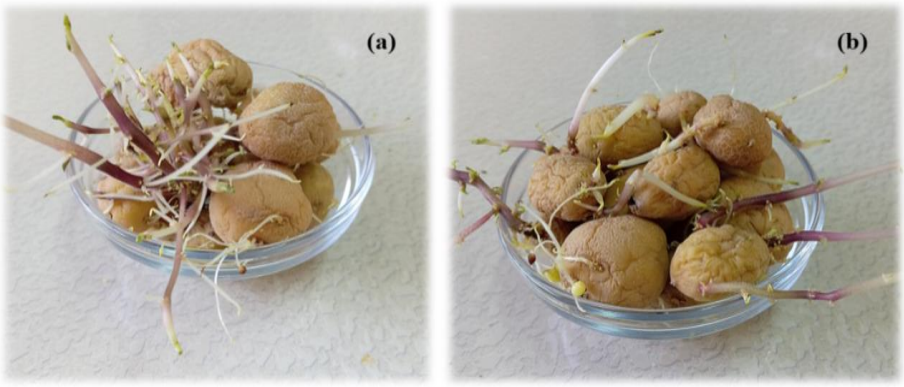



**Fig. 2. Bud formation and development on nodes under the influence of gibberellic acid (GA3). a) Gala variety. b) Santa variety.**

One of the important factors in working with isolated tissues during in vitro plant cultivation is adherence to sterile techniques. The nutrient medium, rich in essential organic compounds and substrates, provides a favorable environment for the growth of microorganisms Table 1.

Table 1.

**The effect of gibberellic acid (GA3) on bud formation from tubers of the experimental varieties.**

| Research Types | Number of Nodes, pcs | Percentage of Bud Formation, % | Number of Formed Buds, pcs | Bud Length, cm |
|---|---|---|---|---|
| Arizona | 15 | 100 | 6,73±0,88 | 3,73±0,59 |
| Gala | 15 | 100 | 7,53±0,99 | 4,06±0,37 |
| Pikasso | 15 | 100 | 6,93±0,79 | 3,26±0,37 |
| Zarzara | 15 | 100 | 7,00±0,84 | 3,40±0,43 |
| Santa | 15 | 100 | 7,13±0,74 | 4,00±0,32 |

One of the critical factors in working with isolated plant tissues under in vitro conditions is strict adherence to sterile techniques. The nutrient medium, enriched with essential organic compounds and substrates, provides a favorable environment not only for plant tissue growth but also for the proliferation of microorganisms. Therefore, surface sterilization of explants is a key prerequisite for successful in vitro culture.

**Surface sterilization of explants.** In this study, various concentrations of sterilizing agents were tested to assess the levels of bacterial and fungal contamination and to ensure the recovery of sterile explants. The effects of sterilizing treatments were evaluated in two experimental variants (S1 and S2). For each variant, 50 explants were used, and the efficiency of sterilization was analyzed.

In the S1 treatment (0.20 min in 70% ethanol, followed by 5.0 min in 0.1% $HgCl_2$ supplemented with Tween-80, and rinsing with sterile distilled water), no significant differences in bacterial or fungal contamination were

observed across explants of the tested potato cultivars (Figure 3a).

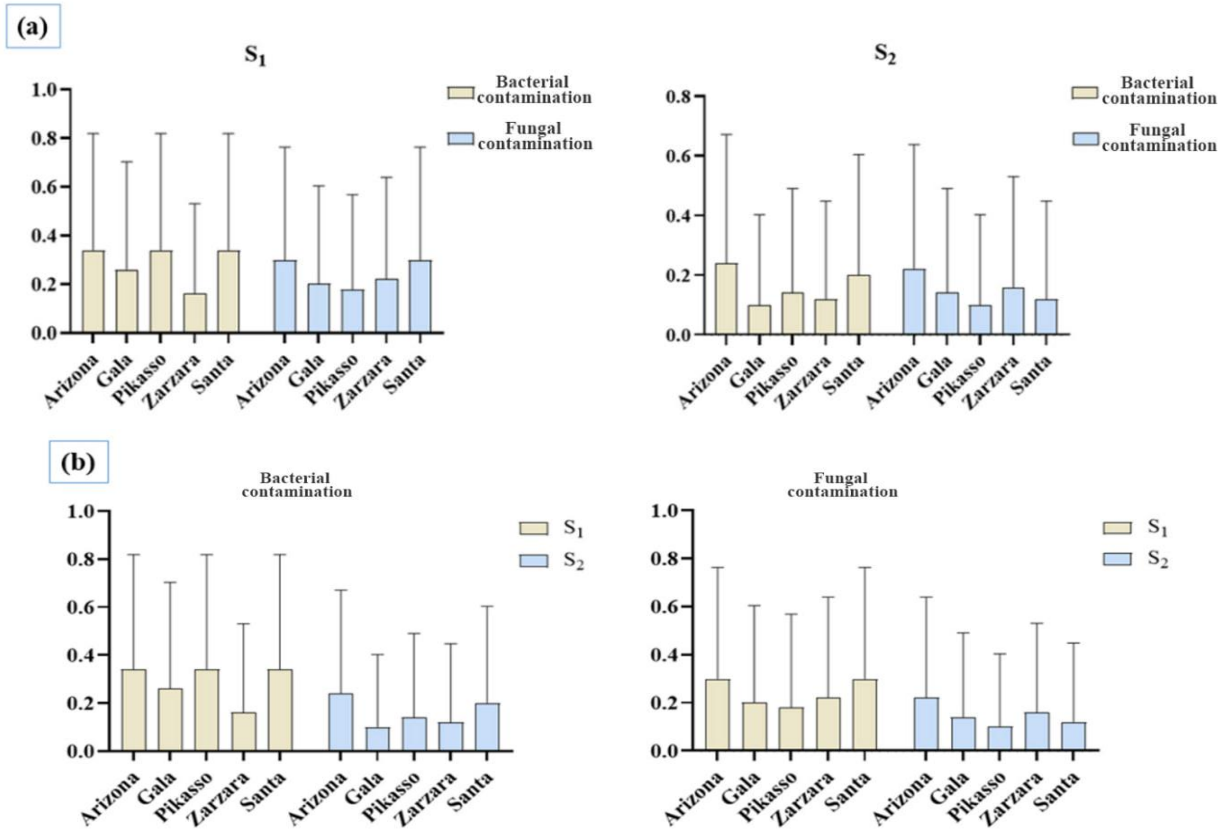



**Fig. 3. Surface sterilization of explants from experimental varieties.**
**S1:** (0.20 min. 70% $C_2H_5OH$, 5.0 min. 0.1% $HgCl_2$ + Tween-80, Sterile $H_2O$).
**S2:** (0.20 min. 70% $C_2H_5OH$, 10 min. 5% NaOCl + Tween-80, Sterile $H_2O$).

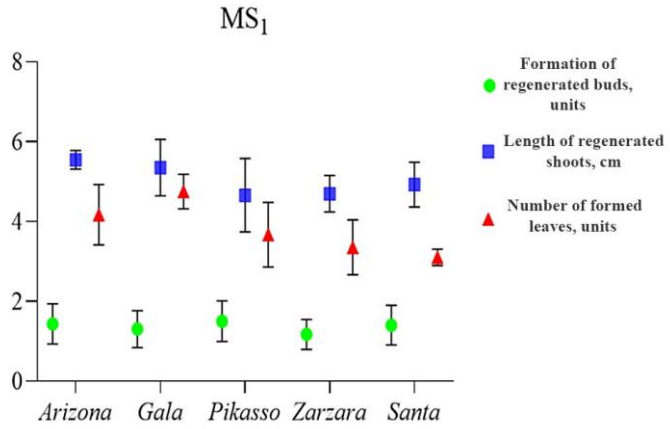



**Fig. 4. The effect of growth regulators KIN (4.0 mg/L) and BAP (2.0 mg/L) on the regeneration of meristem explants of experimental varieties.**

In the explants of the *Arizona*, *Picasso*, and *Santa* cultivars, bacterial contamination levels were found to be similar. Likewise, fungal contamination levels in *Arizona* and *Santa* explants showed comparable results. The lowest bacterial contamination was recorded in *Zarzara*, whereas the lowest fungal contamination occurred in *Picasso*. Among all cultivars, the highest survival rate of explants was observed in *Gala*, reaching 56.0% (Figure 3).

In the S2 treatment (0.20 min in 70% ethanol, followed by 10 min in 5% NaOCl with Tween-80, and rinsing with sterile distilled water), no significant differences in contamination levels were observed among the experimental cultivars. However, the overall results of this treatment were 1.0–1.5 times higher compared with S1. In particular, *Gala* explants exhibited the lowest bacterial contamination (0.10 ± 0.30) and relatively low fungal contamination (0.14 ± 0.35), which corresponded with the highest explant survival rate (80.0%) among all tested cultivars.

When comparing S1 and S2, no statistically significant differences were detected between treatments (Figure 3b). Nevertheless, both sterilization protocols proved effective for surface sterilization of potato explants.

**Initial stage – explant regeneration (shoot formation).** Sterile explants that survived surface sterilization were transferred to MS1 nutrient medium, where they were cultured for three weeks to induce regeneration and shoot formation (Figure 4).

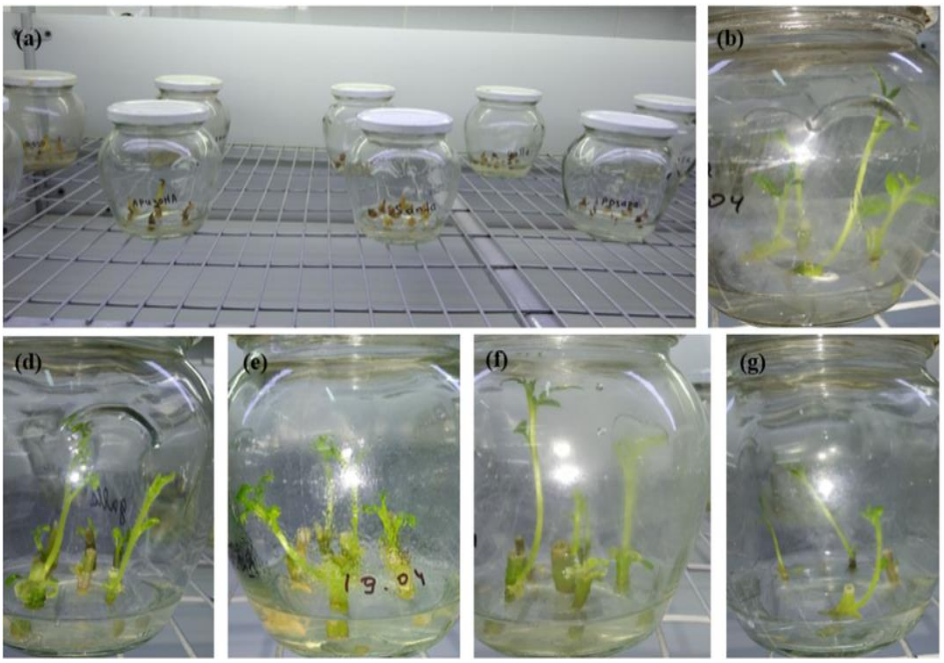



**Fig. 5. Regeneration (shoot formation) of explants from experimental varieties.**
(a) Initial culture, (b) Arizona variety, (d) Gala variety, (e) Picasso variety, (f) Zarzara variety, (g) Santa variety

According to the experimental results, the type and concentration of phytohormones in the nutrient medium exerted a positive influence on the explants of all tested potato cultivars. Complete (100%) regeneration was achieved in explants of all varieties, with no statistically significant differences observed among them (Figure 4). Nevertheless, certain cultivar-specific variations were recorded. The highest regeneration index was obtained in *Picasso* explants (1.50 ± 0.50). The longest shoots were observed in *Arizona* explants (5.54 ± 0.23 cm), while the greatest number of leaves

was produced in *Gala* explants (4.75 ± 0.43) (Figure 5).

Cytokinins play a crucial role in the development of vegetative organs in plants and are widely used in tissue culture to stimulate leaf and root formation. In vitro propagation of potatoes using various plant growth regulators has demonstrated positive outcomes, particularly with the application of kinetin in the *Diamant* variety. Literature analyses confirm that different plant growth regulators exert significant effects on the initiation and development of the potato root system.

**Conclusion.** The present study demonstrated that the use of apical meristem tissue in the in vitro cultivation of potato plants is a highly effective approach for protecting plants from fungal diseases, viral infections, and other bacterial and phytopathogenic agents. This method enables the production of virus-free planting material with high regeneration potential.

The findings emphasize the critical role of tissue culture in modern plant biology and biotechnology. This approach not only provides a reliable platform for obtaining pathogen-free seed potatoes but also contributes to improved economic efficiency in potato production. Furthermore, it establishes a solid foundation for future research and innovations in potato breeding and biotechnology.

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