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IN VITRO MERISTEM CULTURE OF LOCAL POTATO VARIETIES FOR VIRUS-FREE SEED PRODUCTION IN AZERBAIJAN

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SUMMARY

The purpose of the research - In this study, four local potato (Solanum tuberosum L.) cultivars that were adapted to Azerbaijan-Ugur, Vagif, Cenlibel, and Telman were used to produce virus-free planting material using apical meristem culture technique.

The methodology of the research-Apical meristems were excised from sprouted tubers and cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of 6-benzylaminopurine (BAP), kinetin (KIN), and gibberellic acid (GA_3). The effects of hormonal combinations on meristem response, shoot and root regeneration, and virus elimination were evaluated. These findings demonstrate that apical meristem culture, combined with optimized cytokinin and GA_3 treatments, is an effective strategy for rapid in vitro multiplication of healthy, genetically uniform potato plantlets.

The practical importance of the research-This approach offers a practical solution for sustainable seed potato production in Azerbaijan, facilitating the propagation of elite early-maturing cultivars with reduced disease risk.

The results of the research- The results indicated that the type and concentration of cytokinin significantly influenced morphogenic response. The highest regeneration rate (83%) and shortest response time (4–9 days) were observed on MS medium containing 1 mg/L BAP + 0.5 mg/L GA_3, particularly in the Ugur variety. MS medium supplemented with 1 mg/L KIN + 0.5 mg/L GA_3 also showed satisfactory shoot and root induction (68–80%), while lowering kinetin to 0.5 mg/L delayed morphogenesis and slightly reduced regeneration efficiency (70–75%). Virus screening using DAS-ELISA confirmed the production of virus-free plantlets, with clearance rates ranging from 80% to 100% depending on the variety.

The scientific novelty of research: This research lies in the optimization of apical meristem culture and hormone combinations for efficient regeneration and virus elimination in locally adapted Azerbaijani cultivars of Solanum tuberosum. The study establishes a genotype-specific in vitro protocol that ensures high regeneration rates and effective production of virus-free planting material.

Keywords: Apical meristem, virus-free, Solanum tuberosum L., plant growth regulators, meristem culture, in vitro.

Introduction

The increasing global demand for food, combined with climate change and limited agricultural land resources, necessitates the development of innovative and efficient crop improvement strategies. Plant biotechnology provides a wide array of tools that complement and, in some cases, surpass conventional breeding methods. Technologies such as molecular genetics, genome characterization, gene transfer, genome editing, and in vitro regeneration have transformed modern plant breeding into a more precise and accelerated process.

Potato (*Solanum tuberosum* L.) is one of the world's most important food crops and is predominantly propagated vegetatively. While this ensures genetic uniformity, it also facilitates the accumulation and transmission of viral pathogens across generations, resulting in yield losses and reduced tuber quality. Consequently, the production of healthy, pathogen-free seed potatoes represents a critical challenge in potato cultivation systems.

Potato-infecting viruses pose a serious danger to the seed potato industry and inflict substantial harm globally. Once a plant is infected, the virus typically persists until it dies off, and it is transmitted to the progeny by vegetative propagation. The appearance of plants is one way that the viral infection shows up. The biggest risk comes from viral infections in nearby

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potato crops, in groundkeepers from past potato harvests that have persisted in growing in the soil, and from environmental virus reservoirs such weeds (Anikina, 2023).

To eradicate viruses and ensure healthy starting material in potatoes, we can employ the method of sampling and "in vitro" culture of meristems. Meristems are tissues consisting of juvenile cells that maintain their capacity for proliferation throughout the plant's lifespan (Cioloca, 2024). Meristem culture constitutes the basis for acquiring healthy material and is the initial phase in establishing "in vitro" cultures. The dimensions of the explant substantially affect the efficacy of viral elimination in meristem culture techniques. This technique increases the yield of G0 potato seeds by enabling the bulk multiplication of disease-free plantlets (Lahane, 2025). For a very long period, numerous valuable potato types have been revived and utilized in production. This approach has resulted in a more than 42% increase in potato yields (Galeev, 2018). The apical meristem technology has the potential to transform potato agriculture in Azerbaijan by decentralizing seed production and lowering reliance on conventional techniques, therefore resolving yield limitations and farmers' financial difficulties.

Shoot apical meristem (SAM) culture has proven to be one of the most effective biotechnological approaches for virus elimination in potato. An apical meristem is a group of meristematic (formative)cells organized into a growth center, which occupy the terminal position in a shoot or root and form all organs and primary tissues. Additionally, regeneration results can be greatly enhanced by mixing several plant growth regulators (PGRs) (Jacome 2025). Auxins (IAA, NAA) promote root formation, whereas cytokinins (e.g., BAP, KIN) promote shoot growth. The strength of meristem-based regeneration and general growth dynamics are directly impacted by changes in the content of the nutrient solution (Babajanova, 2025).

Materials and Methods.

The study was conducted at the Agrobiotechnology Laboratory of the Vegetable Research Institute of the Republic of Azerbaijan. Four local potato (*Solanum tuberosum* L.) cultivars: Telman, Ugur, Vagif, and Cenlibel were used as plant material. Selected tubers (weighing 60-80 g) were obtained from the greenhouse facilities of the Vegetable Research Institute. In order to break dormancy, the tubers were treated with 2 ppm gibberellic acid (GA_3) solution for 15 minutes and subsequently incubated in a dark growth chamber at 22 ± 2 °C for 21 days for sprouting.

The Telman cultivar was obtained by individual selection from free pollination of the Alisma cultivar from former Germany. It was officially released in Azerbaijan in 2008. It is a medium-maturing table cultivar with high eating quality. The plants are medium-sized and compact, with moderate branching and multiple stems, and possess a strong root system. Tubers are round to oval in shape, white in color, with purple eyes of medium depth. The vegetation period is 110-120 days. Under irrigated conditions, yield can reach up to 300 centners per hectare. The average weight of marketable tubers is 90-95 g. The flesh is light yellow to whitish, and the starch content ranges from 15 to 16.5% (Vegetable Research Institute Bulletin).

The Vagif variety is an early-maturing genotype with a vegetation period of 95-110 days. The plant forms an upright bush with an average height of about 10 cm, characterized by heart-shaped leaf apices and white conical stamens. Tubers are round to oval in shape, with pale yellow skin and light yellow flesh, shallow eyes, and an average weight of 50-100 g. The productivity of this variety ranges between 400 and 550 sen/ha. In terms of

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biochemical composition, tubers contain 27.0% dry matter, 6.3% total sugar, 14.5% starch, and 111.6 mg/kg nitrate. Vagif shows moderate resistance to fusarium wilt and powdery mildew. From a culinary perspective, the variety is considered high quality for both hot and cold consumption, and the tubers tend to crumble after cooking.

The Ugur variety belongs to the very early maturity group, with a growth period of 80-95 days. Plants form a dense bush approximately 10 cm in height, with glossy light green leaves and white stamens. Tubers are circular, smooth, with light red skin and cream-colored flesh, shallow eyes, and an average weight of 50-90 g. The yield potential of this variety is high, reaching 400-550 sen/ha. Biochemical analysis indicates that tubers contain 31.3% dry matter, 6.5% sugar, 23.7% starch, and 121.6 mg/kg nitrate. Ugur demonstrates good resistance to viral diseases and phytophthora, which increases its adaptability under diverse growing conditions. The variety is distinguished by excellent taste qualities in both hot and cold dishes, and the tubers readily crumble when cooked.

The Cenlibel variety is classified as early-maturing, with a vegetation period of 95-110 days. The plant develops a relatively tall bush, reaching about 25 cm, with dull dark green leaves and a characteristic sickle-shaped leaf apex. Tubers are flat and smooth, with light yellow skin and flesh, shallow eyes, and an average mass of 50-80 g. The productivity of Cenlibel varies between 390 and 550 sen/ha. The chemical composition of tubers includes 26.2% dry matter, 5.0% sugar, 21.8% starch, and 116.8 mg/kg nitrate. This variety shows moderate resistance to fusarium wilt and powdery mildew. In terms of culinary quality, Cenlibel tubers crumble during cooking and are characterized by excellent flavor in both hot and cold consumption.

Micropropagation comprises four sequential stages: (I) the initiation (initial culture) stage, (II) the shoot multiplication stage, (III) the rooting stage, and (IV) the acclimatization stage, in which plantlets are gradually adapted to ex vitro environmental conditions.

Prior to culture initiation, explants are subjected to a disinfection procedure to eliminate external sources of contamination, particularly microorganisms, from the explant surfaces. The washed sprouts were transferred to a laminar airflow cabinet previously sterilized before culture initiation. Surface sterilization was performed by rinsing the sprouts with sterile double-distilled water, followed by immersion in 70% (v/v) ethanol for 1 min and rinsing three times with sterile double-distilled water. The sprouts were then treated with 0.1% (w/v) $HgCl_2$ solution containing three drops of Tween-20 for 4 min. After sterilization, the explants were rinsed four times with sterile double-distilled water to remove all chemical residues. Excess moisture was carefully removed using sterile blotting paper, and the sprouts were placed in sterile Petri dishes.

Apical meristems were excised from the shoot tips of potato sprouts. The primary criteria that frequently affect the efficacy of plant virus elimination are the size of excised meristems, crop cultivar, plant species, and virus species Loebenstein (2001). The isolated meristems were cultured on full-strength and half-strength Murashige and Skoog (MS) (MS, 1962) basal media supplemented with various concentrations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). Growth regulator-free MS medium served as the control.

Cultures were maintained at 24 ± 2 °C under a 16-hour photoperiod with controlled relative humidity. Morphological and physiological parameters—including shoot regeneration frequency, stem length, number of nodes, rooting percentage, number of roots per plant, microtuber formation frequency, and tuber weight—were recorded and analyzed comparatively among treatments and cultivars.

Following three subcultures (approximately 12 weeks), the regenerated plants were screened for Potato virus X (PVX), Potato virus Y (PVY), and Potato leafroll virus (PLRV).

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Plants obtained through meristem culture and propagated by nodal culture were tested using the ELISA method on 2 May 2025 once they reached a certain number and their virus infections were determined. The Double Antibody Sandwich ELISA (DAS-ELISA) method was performed using Multiskan FC (Thermo Scientific) kits. Absorbance values were measured at a wavelength of 405 nm using a microplate reader. Samples showing absorbance values three times higher than the negative control were considered virus-positive and were excluded from the experiment. The results showed that the samples were infected with PVY and PLRV viruses, but did not contain PVX, PVM, PVA, or PVS viruses.

Table 1. Number and virus-free status of seedlings developed in vitro from meristems isolated from infected potato tubers.

Order №	**Variety name**	**Isolated meristem Number**	**In vitro development from meristem**			**Virus clearance rate (%)**
			Total seedling number	Number of seedlings infected with the virus	Number of healthy seedlings	
1	Ugur	25	27	-	27	100.00
2	Cenlibel	32	29	1 (PLRV)	28	96.55
3	Vagif	30	25	3 (PLRV)	22	88.00
4	Telman	28	20	4 (PVY)	16	80.00
	Total	115	101	8	93	92.10

Thermotherapy was applied to sprouted tubers before meristem excision to lower the viral load (PVX, PVY, and PLRV). Over three days, the temperature was raised progressively to 37 ± 1 °C. The sprouts were kept at this temperature for 28 days with a 16-hour photoperiod and 2000 lux of light. The creation of a virus-free zone in the apical region was thought to depend on this stage.From the majority of meristem tissues obtained from the four lines, virus-free material was successfully produced. Considering all lines collectively, the elimination rate was determined to be 80% for PVY and 92.28% for PLRV.

Micropropagations of disease free meristem plantlets were done in MS medium. Full-strength MS medium was used at all three stages. According to the requirements of local cultivars, three different concentrations of plant growth regulators (PGRs) were applied at each stage as follows:

- Initiation medium: A) MS (control); B) MS + 0.1 mg/L GA_3; C) MS + 0.5 mg/L 6-benzylaminopurine (BAP); D) MS + 0.5 mg/L BAP + 0.1 mg/L GA_3.
- Multiplication medium: A) Control; B) MS + 0.5 mg/L BAP; C) MS + 1.0 mg/L BAP + 0.5 mg/L kinetin (Kin); D) MS + 2.0 mg/L BAP + 0.5 mg/L Kin.
- Rooting medium: A) Control; B) MS + 0.5 mg/L indole-3-butyric acid (IBA); C) MS + 1.0 mg/L IBA; D) MS + 1.5 mg/L IBA.

The pH of the culture medium was adjusted to 5.8 ± 0.1 using 0.1 N NaOH or HCl, and the medium was sterilized by autoclaving at 121 °C and 15 psi for 20 minutes. All cultures were maintained in a growth room at 25 ± 2 °C under a 16-hour photoperiod with a light intensity of 3000 lux.

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Table 2. Four potato cultivars' genotypic responses to KIN, BPA and GA^3 concentrations and combinations in MS media on the primary response of meristems.

Plant growth regulators (mg/L)	Variety	Days to response	% of meristems responded	Morphogenic response	
				Shoot	Root
MS + 1 mg/L KIN + 0.5 mg/L GA_3	Ugur	4-6	80	+	+
	Vagif	5-8	74	+	+
	Cenlibel	5-7	78	+	+
	Telman	7-10	68	+	+
MS + 0.5 mg/L KIN + 0.5 mg/L GA_3	Ugur	7-9	75	+	+
	Vagif	7-10	72	+	+
	Cenlibel	8-10	74	+	+
	Telman	9-11	70	+	+
MS + 1 mg/L BAP+ 0.5 mg/L GA_3	Ugur	4-5	83	+	+
	Vagif	5-7	78	+	+
	Cenlibel	5-6	76	+	+
	Telman	7-9	70	+	+

+ = Positive responses; − = Negative responses

Results and Discussion.

The presented results demonstrate the effect of different plant growth regulators (KIN, BAP, and GA_3) added to MS medium on the in vitro morphogenic response of apical meristems. The study was conducted on four varieties (Ugur, Vagif, Cenlibel, and Telman), evaluating days to response, percentage of responding meristems, and shoot and root formation.

In the MS medium supplemented with 1 mg/L KIN + 0.5 mg/L GA_3, meristem response was initiated relatively early (4–10 days). The percentage of responding meristems ranged from 68% to 80%, with the highest response observed in the Ugur variety (80%) and the lowest in Telman (68%). These findings indicate that 1 mg/L kinetin is sufficient to effectively stimulate cell division and initiate organogenesis.

In contrast, the medium containing 0.5 mg/L KIN + 0.5 mg/L GA_3 resulted in a delayed response (7–11 days). The percentage of responding meristems varied between 70% and 75%. The reduction in cytokinin concentration slowed the initiation of morphogenesis but did not significantly suppress the overall regeneration capacity. This suggests that cytokinin concentration directly influences meristematic activity and response rate.

The highest morphogenic efficiency was observed in the MS medium supplemented with 1 mg/L BAP + 0.5 mg/L GA_3. In this treatment, the response time was the shortest (4–9 days), and the percentage of responding meristems ranged from 70% to 83%. The highest response (83%) was recorded in the Ugur variety. The superior performance of BAP compared to kinetin may be attributed to its stronger synthetic cytokinin activity, leading to enhanced cell proliferation and shoot induction.

In all treatments, both shoot and root formation were observed (+/+), indicating that the hormonal balance used was suitable for direct organogenesis. The presence of 0.5 mg/L GA_3 likely promoted shoot elongation and contributed positively to the completion of morphogenic development.

Overall, the results indicate that the type and concentration of cytokinin play a decisive role in the in vitro regeneration of apical meristems. Among the tested treatments, 1 mg/L BAP + 0.5 mg/L GA_3 proved to be the most effective combination, providing a higher regeneration percentage and shorter response time. Additionally, genotypic differences among

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varieties were observed, suggesting that morphogenic potential is influenced by genetic factors.

Result. Tissue culture studies have concluded that not every variety shows the same response to the same medium. Varietal differences were evident in all treatments. The variety Ugur consistently exhibited the fastest response and highest regeneration percentage, indicating a higher morphogenic competence. In contrast, Telman showed delayed responses and lower regeneration frequencies, highlighting the strong influence of genotype on in vitro meristem culture performance.

Overall, the results indicate that the type and concentration of cytokinin play a decisive role in the in vitro regeneration of apical meristems. Among the tested treatments, 1 mg/L BAP + 0.5 mg/L GA_3 proved to be the most effective combination, providing a higher regeneration percentage and shorter response time. Additionally, genotypic differences among varieties were observed, suggesting that morphogenic potential is influenced by genetic factors.

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AZƏRBAYCAN ÜÇÜN VİRUSDAN AZAD TOXUM İSTEHSALI MƏQSƏDİLƏ YERLİ KARTOF SORTLARININ İN-VİTRO MERİSTEM KULTURASI

XÜLASƏ

Tədqiqatın məqsədi – Bu tədqiqatda Azərbaycana uyğunlaşdırılmış dörd yerli kartof (*Solanum tuberosum L.*) sortu – Uğur, Vaqif, Çənlibel və Telman – apikal meristem kulturası üsulundan istifadə etməklə virusdan azad əkin materialının əldə olunması üçün istifadə edilmişdir.
Tədqiqatın metodologiyası – Apikal meristemlər cücərmiş yumrulardan ayrılmış və müxtəlif konsentrasiyalarda 6-benzilaminopurin (BAP), kinetin (KIN) və gibberellin turşusu (GA_3) əlavə olunmuş Murashige və Skoog (MS) qida mühitində kultivasiya edilmişdir. Hormon kombinasiyalarının meristemin reaksiyasına, zoğ və kök regenerasiyasına, həmçinin virusların eliminasiyasına təsiri qiymətləndirilmişdir. Nəticələr göstərmişdir ki, apikal meristem kulturasının optimallaşdırılmış sitokinin və GA_3 tətbiqi ilə birlikdə istifadəsi sağlam və genetik cəhətdən homogen kartof bitciklərinin sürətli *in vitro* çoxaldılması üçün effektiv strategiyadır.
Tədqiqatın praktik əhəmiyyəti – Bu yanaşma Azərbaycanda davamlı toxumluq kartof istehsalı üçün praktik həll yolu təklif edir və xəstəlik riskini azaltmaqla elit, tez yetişən sortların çoxaldılmasına imkan yaradır.
Tədqiqatın nəticələri – Nəticələr göstərmişdir ki, sitokininin tipi və konsentrasiyası morfogenetik reaksiyaya əhəmiyyətli dərəcədə təsir göstərmişdir. Ən yüksək regenerasiya faizi (83%) və ən qısa reaksiya müddəti (4–9 gün) 1 mq/L BAP + 0,5 mq/L GA_3 tərkibli MS mühitində, xüsusilə Uğur sortunda müşahidə edilmişdir. 1 mq/L KIN + 0,5 mq/L GA_3 əlavə olunmuş MS mühiti də qənaətbəxş zoğ və kök əmələgəlməsi (68–80%) göstərmişdir. Kinetinin 0,5 mq/L-ə endirilməsi morfogenez prosesini ləngitmiş və regenerasiya effektivliyini bir qədər azaltmışdır (70–75%). DAS-ELISA üsulu ilə aparılan virus testləri 80%-dən 100%-ə qədər dəyişən göstəricilərlə virusdan azad bitciklərin əldə olunduğunu təsdiq etmişdir.
Tədqiqatın elmi yeniliyi – Bu tədqiqatın yeniliyi Azərbaycana uyğunlaşdırılmış *Solanum tuberosum* sortlarında effektiv regenerasiya və virusların eliminasiyası üçün apikal meristem kulturasının və hormon kombinasiyalarının optimallaşdırılmasındadır. Tədqiqat genotipə spesifik *in vitro* protokolunu müəyyən etmiş və yüksək regenerasiya göstəriciləri ilə virusdan azad əkin materialının səmərəli istehsalını təmin etmişdir

Açar sözlər: Apikal meristem, virusdan azad, *Solanum tuberosum L.*, bitki böyümə tənzimləyiciləri, meristem kulturası, *in vitro*.

КУЛЬТУРА МЕРИСТЕМ IN VITRO МЕСТНЫХ СОРТОВ КАРТОФЕЛЯ ДЛЯ ПРОИЗВОДСТВА ВИРУСОСВОБОДНОГО СЕМЕННОГО МАТЕРИАЛА В АЗЕРБАЙДЖАНЕ

РЕЗЮМЕ

Цель исследования – В данном исследовании для получения вирусо-свободного посадочного материала с использованием техники культуры апикального меристема были использованы четыре местные сорта картофеля (*Solanum tuberosum L.*), адаптированные к Азербайджану: Угур, Вагиф, Дженлибель и Телман.

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Методология исследования – Апикальные меристемы были выделены из проросших клубней и культивированы на среде Мурасиге и Скуга (MS), обогащённой различными концентрациями 6-бензиламинопурина (BAP), кинетина (KIN) и гиббереллиновой кислоты (GA_3). Оценивались эффекты комбинаций гормонов на реакцию меристемы, регенерацию побегов и корней, а также на удаление вирусов. Результаты показывают, что культура апикального меристема в сочетании с оптимизированным применением цитокинина и GA_3 является эффективной стратегией для быстрого *in vitro* размножения здоровых и генетически однородных картофельных растений.

Практическая значимость исследования – Этот подход представляет собой практическое решение для устойчивого производства семенного картофеля в Азербайджане, облегчая размножение элитных раннеспелых сортов с сниженным риском заболеваний.

Результаты исследования – Результаты показали, что тип и концентрация цитокинина существенно влияют на морфогенетический ответ. Наибольшая скорость регенерации (83%) и самое короткое время отклика (4–9 дней) наблюдались на среде MS, содержащей 1 мг/л BAP + 0,5 мг/л GA_3, особенно у сорта Угур. Среда MS с 1 мг/л KIN + 0,5 мг/л GA_3 также обеспечивала удовлетворительную индукцию побегов и корней (68–80%), в то время как снижение концентрации кинетина до 0,5 мг/л замедляло морфогенез и слегка снижало эффективность регенерации (70–75%). Скрининг вирусов методом DAS-ELISA подтвердил получение вирусо-свободных растений с показателями очистки от 80% до 100% в зависимости от сорта.

Научная новизна исследования – Новизна данного исследования заключается в оптимизации культуры апикального меристема и комбинаций гормонов для эффективной регенерации и удаления вирусов у местных азербайджанских сортов картофеля. Исследование разработало генотип-специфический *in vitro* протокол, обеспечивающий высокую скорость регенерации и эффективное производство вирусо-свободного посадочного материала.

Ключевые слова: Апикальный меристем, вирусо-свободный, *Solanum tuberosum L.*, регуляторы роста растений, культура меристема, *in vitro*.

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