АУЫЛ ШАРУАШЫЛЫҚ ҒЫЛЫМДАР / СЕЛЬСКОХОЗЯЙСТВЕННЫЕ НАУКИ / AGRICULTURAL SCIENCES

DOI 10.54596/2958-0048-2026-1-199-207
UDK 635.21
IRSTI 68.35.49

EFFECT OF DIFFERENT NUTRIENT MEDIA COMPOSITIONS ON THE MORPHOGENESIS OF POTATO VARIETIES «ROMANO» AND «RIVIERA» IN VITRO CULTURE

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Abstract

Potato (Solanum tuberosum L.) is one of the leading agricultural crops in the Republic of Kazakhstan. However, its productivity is declining due to viral diseases and the ineffectiveness of traditional seed production, necessitating the use of biotechnological methods to obtain improved seed material. determine the optimal conditions for mineral and hormonal nutrition to increase the efficiency of growth and development of microplants of Romano and Riviera varieties in in vitro culture. Cultivation was carried out on modified Murashige-Skoog and White media with varying sterilization, hormonal supplements, and macro- and microelement concentrations. Morphogenesis, root system development, and the multiplication rate were assessed. Variety-specific responses to the medium composition were identified; optimization of mineral and hormonal nutrition contributed to enhanced morphogenesis and an increase in the multiplication rate, which allows for the improvement of technologies for the production of improved planting material.

Keywords: potato, in vitro, nutrient medium, Romano, Riviera, morphogenesis, microclonal propagation, hormonal additives.

«РОМАНО» ЖӘНЕ «РИВЬЕРА» КАРТОП СОРТТАРЫНЫҢ IN VITRO МӘДЕНИЕТІНДЕ МОРФОГЕНЕЗІНЕ ӘРТҮРЛІ ҚОРЕКТІК ОРТАЛАРДЫҢ ӘСЕРІ

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Аңдатпа

Картоп (Solanum tuberosum L.) Қазақстан Республикасының жетекші ауыл шаруашылығы дақылдарының бірі болып табылады, алайда оның өнімділігі вирустық ауруларға және дәстүрлі тұқым шаруашылығының тиімділігінің жеткіліксіздігіне байланысты төмендейді, бұл сауықтыру материалын алудың биотехнологиялық әдістерін қолдану қажеттілігін негіздейді. Зерттеу мақсаты in vitro мәдениетінде Романо және Ривьера сорттарының микроөсімдіктерінің өсуі мен дамуының тиімділігін арттыру үшін минералды және гормоналды тамақтанудың оңтайлы жағдайларын анықтау. Өсіру зарарсыздандыру, гормоналды қоспалар және макро және микроэлементтердің концентрациясы өзгерген Мурасиге-Скуг және Уайт орталарында жүргізілді. Морфогенез, тамыр жүйесінің дамуы және көбею коэффициенті бағаланды. Ортаның құрамына сорттық-спецификалық реакциялар орнатылды; минералды және гормоналды тамақтануды оңтайландыру морфогенездің жоғарылауына және көбею коэффициентінің жоғарылауына ықпал етті, бұл сауықтыру отырғызу материалын өндіру технологиясын жетілдіруге мүмкіндік береді.

Кілт сөздер: картоп, in vitro, қоректік орта, Романо, Ривьера, морфогенез, микроклондық көбейту, гормондық қоспалар.

ВЛИЯНИЕ РАЗЛИЧНЫХ СОСТАВОВ ПИТАТЕЛЬНЫХ СРЕД НА МОРФОГЕНЕЗ КАРТОФЕЛЯ СОРТОВ «РОМАНО» И «РИВЬЕРА» В КУЛЬТУРЕ IN VITRO

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Аннотация

Картофель (*Solanum tuberosum L.*) является одной из ведущих сельскохозяйственных культур Республики Казахстан, однако его продуктивность снижается из-за вирусных заболеваний и недостаточной эффективности традиционного семеноводства, что обусловливает необходимость применения биотехнологических методов получения оздоровленного материала. Цель исследования – определить оптимальные условия минерального и гормонального питания для повышения эффективности роста и развития микрорастений сортов Романо и Ривьера в культуре *in vitro*. Культивирование проводили на модифицированных средах Мурасиге-Скуг и Уайт с варьированием стерилизации, гормональных добавок и концентраций макро- и микроэлементов. Оценивали морфогенез, развитие корневой системы и коэффициент размножения. Установлены сортоспецифические реакции на состав сред; оптимизация минерального и гормонального питания способствовала усилению морфогенеза и повышению коэффициента размножения, что позволяет совершенствовать технологии производства оздоровленного посадочного материала.

Ключевые слова: картофель, in vitro, питательная среда, Романо, Ривьера, морфогенез, микроклональное размножение, гормональные добавки.

Introduction

Potato (Solanum tuberosum L.) is one of the leading agricultural crops worldwide and occupies a key position in the food security systems of many countries, including the Republic of Kazakhstan [1]. According to the Food and Agriculture Organization of the United Nations (FAO), this crop is cultivated in more than 150 countries worldwide, covering approximately 19 million hectares of agricultural land [2]. Within Kazakhstan's agricultural sector, potato is one of the key crops, serving as a raw material for the processing industry. According to the World Potato Congress report dated 20 February 2020, Kazakhstan ranks 20th globally in potato production (3.807 million tonnes), placing it among the top 25 leading countries [3].

As the world's fourth most important agricultural crop after rice, wheat, and maize, potato plays a key role in global food security. Nevertheless, it is often underestimated, being associated with a high starch content and the risk of obesity. However, modern studies indicate that potato is not only a source of energy but also a rich reservoir of vitamins, minerals, and antioxidants capable of preventing chronic diseases [4].

High nutritional value, versatile use, and a substantial contribution to the population's diet necessitate the stable achievement of high yields of this crop. However, one of the main factors limiting potato productivity remains the widespread occurrence of viral, bacterial and fungal diseases, resulting in a significant reduction in yield and tuber quality [5, 6, 7].

Modern potato seed production is impossible without the application of biotechnological methods, in particular in vitro plant culture, which enables the production of genetically

uniform and phytosanitary planting material [8]. The use of microclonal propagation ensures accelerated multiplication of the initial material and makes it possible to increase the multiplication coefficient by more than twofold compared with conventional methods. It has been established that under complete virus infection, potato yield may decrease by a factor of 2–3 relative to the cultivar's potential yield, underscoring the relevance of developing effective plant sanitation technologies [9].

One of the key factors determining the success of in vitro potato culture is the composition of the nutrient medium. Despite the widespread use of standard media such as Murashige–Skoog medium, plant responses to individual components are distinctly cultivar-specific. Differences in the requirements of potato cultivars for macro- and microelements, vitamins, and growth regulators can significantly affect morphogenesis, shoot growth, root system development, and the multiplication coefficient [10, 11, 12].

Accordingly, a comparative assessment of the effects of different nutrient media and their components on the growth and development of potato cultivars 'Romano' and 'Riviera' in in vitro culture is pertinent. The results obtained will enable the optimization of microclonal propagation conditions, improve the efficiency of producing disease-free planting material, and promote the implementation of biotechnological developments in potato seed production practice [13].

The aim of the study was to evaluate the effect of different nutrient media on the growth and development of the 'Romano' and 'Riviera' cultivars in in vitro culture in order to optimize microclonal propagation.

Research materials

The methodological framework of the study was based on contemporary principles of plant biotechnology, plant physiology, and potato seed production. The study employed in vitro plant tissue culture methods, including explant sterilization, the preparation and modification of nutrient media, and cultivation of plants under controlled conditions.

To analyze the results obtained, laboratory methods were used to assess plant morphometric parameters; methods of comparative analysis of the growth and development of microplants; statistical processing of experimental data to evaluate the statistical significance of the results obtained.

The study material comprised seeds of potato cultivars 'Romano' and 'Riviera'.

The 'Romano' cultivar was bred in the Netherlands in the late 20th century by the breeding company HZPC Holland B.V. It was developed for table use and is adapted to a range of climatic conditions.

'Riviera' is an early-maturing cultivar of Dutch breeding developed by Agrico. It was bred to obtain an ultra-early harvest under a short growing season.

The experiments were conducted in the Biotechnology Laboratory of M. Kozybayev North Kazakhstan University.

Preparation and sterilization of seeds. The seeds are washed under running water using a mild detergent. Sterilization is then carried out: the seeds are immersed in 70% ethanol for 2 minutes. After disinfection, the seeds are rinsed repeatedly with sterile water to remove residual reagents. Alternatively, a 10–15% sodium hypochlorite (NaOCl) solution or 0.1% $HgCl_2$ (mercury chloride) may be used; however, hypochlorites are less toxic and are often more effective for potato. All procedures are performed in a laminar flow hood.

After sterilization, the seeds were aseptically introduced into the culture by seeding on nutrient media. Seeding was carried out under sterile conditions in compliance with aseptic

requirements: work surfaces, tools and hands were treated with ethyl alcohol, while the instruments were regularly replaced or additional sterilized at each stage of the manipulations.

Experimental studies were conducted for two years with the periodic introduction of seeds into the culture in the winter and spring period. Cultivation was carried out on Murashige–Skoog and White nutrient media. During the experiment, systematic observations were carried out on the reaction of seeds and the development of seedlings, depending on the composition of the nutrient medium.

Nutrient media. Standard Murashige–Skoog (MS) medium and White medium were used, modified in terms of the concentrations of macro- and microelements, vitamins, and phytohormones (gibberellic acid, cytokinins). The MS medium is characterized by high ionic strength and a high content of nitrogen and vitamins, which stimulates biomass accumulation. The White medium has a low mineral (salt) content and is suitable for root crops.

Cultivation was carried out at 22–25°C, under an illumination of 2000–3000 lx, with a 16/8 h photoperiod. Growth assessment included measurements of plant height, the number of leaves and internodes, root length, and the multiplication coefficient.

Statistical analysis was performed using Statistica 10.0, with calculation of means, standard deviations, and significance of differences according to Student's t-test ($p < 0.05$).

Results of the study

During the experiment, the influence of two nutrient media—the standard Murashige–Skoog (MS) medium and the modified White medium (with altered concentrations of macro- and microelements, vitamins, and phytohormones: gibberellic acid and cytokinins)—on the morphogenesis of potato cultivars 'Romano' and 'Riviera' in in vitro culture was evaluated. The observations showed that the two cultivars exhibited different morphological responses to the medium composition. On the standard Murashige–Skoog (MS) medium, shoot formation and leaf biomass accumulation were moderate, whereas the modified White medium was associated with more active shoot growth, increased leaf size, and stem branching.

The cultivar 'Romano' exhibited more intensive shoot branching on the modified medium, whereas the cultivar 'Riviera' showed more pronounced root system development, particularly on the medium with elevated potassium and phosphorus concentrations.

Table 1. Growth of potato shoots and roots on different culture media

Cultivars	Nutrient medium	Shoot length (cm)	Number of leaves	Number of roots	Leaf color	Shoot branching
'Romano	MS	3,5 ± 0,2	5,0 ± 0,3	5,2 ± 0,6	Green	Average
Romano	White medium	4,3 ± 0,3	6,2 ± 0,4	5,6 ± 0,5	Dark green	High
Riviera	MS	3,2 ± 0,3	4,8 ± 0,3	6,0 ± 0,5	Green	Average
Riviera	White medium	3,9 ± 0,4	5,6 ± 0,4	6,5 ± 0,5	Dark green	Average

An analysis of table 1 shows that the composition of the nutrient medium exerts a significant effect on the morphogenesis of potato cultivars 'Romano' and 'Riviera' in in vitro culture. Both cultivars exhibited greater shoot growth than on the standard MS medium. Thus, the mean shoot length of the 'Romano' cultivar increased by ≈**23%**, from 3.5 ± 0.2 cm on MS to 4.3 ± 0.3 cm on White medium. The 'Riviera' cultivar likewise increased of ≈**22%**, from 3.2 ± 0.3 cm to 3.9 ± 0.4 cm. This indicates a stimulatory effect of the nutrients and phytohormones present in the White medium on shoot development. An increase in leaf number on the White

medium was observed in both cultivars: 'Romano'— by ≈**24%**, from 5.0 ± 0.3 to 6.2 ± 0.4 leaves; 'Riviera'— by ≈**17%**, from 4.8 ± 0.3 to 5.6 ± 0.4 leaves. An increase in leaf number is indicative of enhanced photosynthetic activity and overall biological productivity of plants on a nutrient-rich medium. **Root system development:** Cultivar 'Romano' showed a moderate increase in root number by ≈**8%**, from 5.2 ± 0.6 to 5.6 ± 0.5, whereas cultivar 'Riviera' exhibited a more pronounced rhizogenic capacity, increasing by **8%**, from 6.0 ± 0.5 to 6.5 ± 0.5. This indicates cultivar-specific differences: 'Riviera' responds more actively to improved culture conditions with respect to root formation. **Leaf color:** Foliage coloration was more intense on White medium: leaves of both cultivars were dark green compared with the green coloration observed on MS medium. The intense leaf coloration indicates good plant health and elevated chlorophyll content, reflecting the positive effects of micronutrients and phytohormones in the medium. **Active shoot branching was observed on** White medium, which stimulated shoot branching in the 'Romano' cultivar, increasing it from "medium" on MS to "high" on White. At the same time, the 'Riviera' cultivar maintained moderate shoot branching, with a concomitant emphasis on root system development.

The modified White medium positively affects morphogenesis in potatoes of both cultivars, stimulating shoot growth, increasing leaf number, intensifying leaf biomass coloration, and promoting stem branching (table 2). Cultivar-specific differences were observed: 'Romano' is more responsive to stimulation of shoot branching, whereas 'Riviera' is more responsive to root system development. These data indicate the need to select an optimal nutrient medium composition, taking cultivar-specific characteristics into account, to ensure effective microclonal propagation and morphogenetic development of potato in in vitro culture.

Table 2. Morphological characteristics of microplants

Cultivar	Nutrient medium	Stem thickness (mm)	Leaf length (cm)	Leaf color	Shoot branching
Romano	MS	2,1 ± 0,1	1,8 ± 0,1	Green	Average
Romano	White medium	2,5 ± 0,2	2,3 ± 0,2	Dark green	High
Riviera	MS	1,9 ± 0,1	1,7 ± 0,1	Green	Average
Riviera	White medium	2,2 ± 0,1	2,0 ± 0,1	Dark green	Average

Based on table 2, it may be noted that analysis of the morphological characteristics of microplants showed that the composition of the nutrient medium exerts a marked effect on stem thickness, leaf length, leaf color, and shoot branching in the potato cultivars 'Romano' and 'Riviera' under in vitro culture. On the modified White medium, an increase in stem thickness was observed in both cultivars. The 'Romano' cultivar increased stem thickness by ≈**19%** from 2.1 ± 0.1 mm on Murashige–Skoog (MS) medium to 2.5 ± 0.2 mm on White medium. The 'Riviera' cultivar exhibited growth of ≈**16%** from 1.9 ± 0.1 mm to 2.2 ± 0.1 mm. Thicker stems indicate improved mechanical strength of the plants and a favorable effect of nutrients and phytohormones on shoot structural development. An increase in leaf blade length on White medium was recorded in both cultivars. In 'Romano', leaf length increased by ≈**28%** from 1.8 ± 0.1 cm to 2.3 ± 0.2 cm, and in 'Riviera' — by ≈**18%** from 1.7 ± 0.1 cm to 2.0 ± 0.1 cm. This indicates more active photosynthetic development and higher overall biological productivity of

the microplants when cultured on an improved nutrient medium. Leaves on MS medium were green, whereas those on White medium were dark green. Such coloration indicates a high chlorophyll content and good plant health. The more intense leaf coloration indicates the positive effect of the micronutrients and phytohormones present in the modified medium on plant metabolism. **Shoot branching:** The 'Romano' cultivar exhibited a significant increase in branching, from "medium" on MS to "high" on White medium, whereas the 'Riviera' cultivar maintained moderate branching on both media.

Thus, the 'Romano' cultivar is more responsive to the stimulation of shoot branching, whereas 'Riviera' is characterized by a greater orientation toward root system formation, as also shown in table 1.

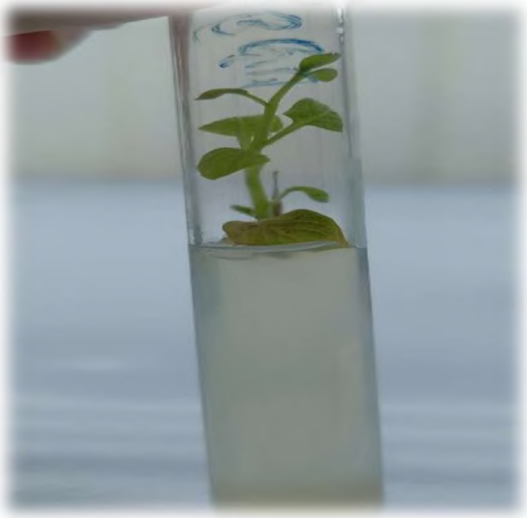

Figure 1 – Romano — MS nutrient medium

Figure 2 – Romano — White medium

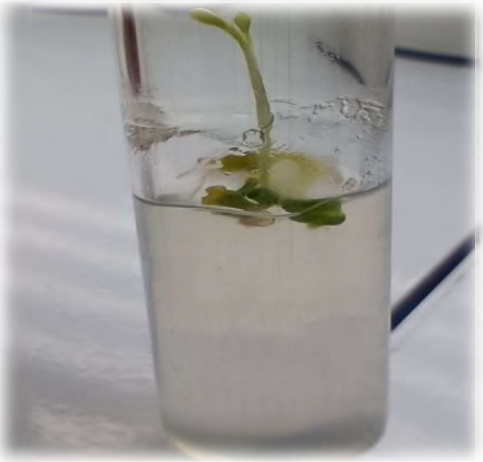

Figure 3 – Riviera — MS nutrient medium

Figure 4 – Riviera — White medium

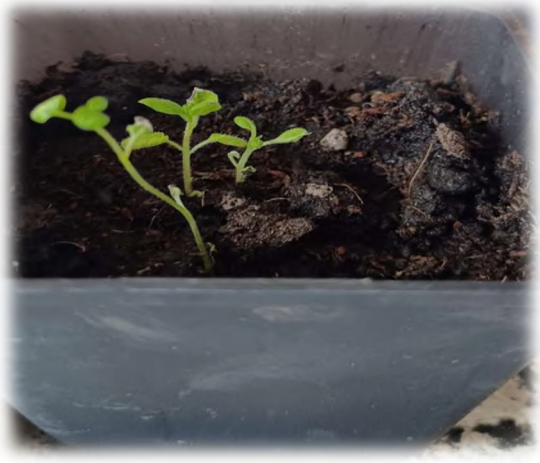

Figure 5 – Regenerant plant Romano

Figure 5 – Regenerant plant Riviera

The modified White medium exerts a positive effect on the morphological traits of microplants of both cultivars. The following are observed in this medium:

- increased stem thickness and leaf length,
- more intense green leaf coloration,
- enhanced shoot branching in the cultivar ‘Romano’.

The results underscore cultivar-specific characteristics of morphogenesis: the cultivar ‘Romano’ exhibits an active response to shoot stimulation, whereas the cultivar ‘Riviera’ predominantly focuses on root system development and moderate branching. These data confirm the importance of optimizing nutrient medium composition in a cultivar-specific manner to ensure efficient in vitro cultivation of potato microplants and to improve their morphological characteristics.

Discussion

The results show that potato morphogenesis in vitro is strongly dependent on the composition of the nutrient medium. Modified White medium, enriched with phytohormones and with altered element concentrations, stimulated shoot growth, stem branching, and the development of leaf biomass in both cultivars.

Cultivar-specific differences were as follows: ‘Romano’ exhibited more active shoot branching, whereas ‘Riviera’ showed a greater capacity for root formation. This is consistent with findings reported in the literature on the effects of macronutrients and phytohormones on morphogenesis in tuber crops.

Leaf color and overall cultivar morphology also served as indicators of plant health: an intensely green coloration and sturdy shoots indicate a favorable medium composition.

Conclusion

1. The nutrient medium composition has a significant effect on the morphogenesis of the potato cultivars ‘Romano’ and ‘Riviera’ under in vitro culture conditions.

2. The modified White medium enhances shoot growth, leaf biomass, and stem branching relative to the standard MS medium.

3. The cultivar ‘Romano’ is more responsive to branching stimulation, whereas ‘Riviera’ is more responsive to root system development.

4. Optimization of the nutrient medium composition increases the efficiency of microclonal propagation of potato and its morphogenetic activity.

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