байланысқа корреляциялық талдау жүргізілді. No-Till жүйесі көп жағдайда органикалық заттардың жиналуына ықпал ететіні анықталды. Пар алқаптарында Сорг. мен Nжалпы арасында жоғары оң корреляция (r = 0.78), ал Сгк мен Сфк арасында күшті байланыс (r = 0.978) тіркелді. Зерттеу нәтижелері топырақтағы органикалық заттардың жинақталу механизмдерін тереңірек түсінуге және агротехнологиялардың көміртек-азот теңгеріміне әсерін бағалауға мүмкіндік береді.

Кілт сөздер: топырақтың органикалық көміртегі, топырақтағы жалпы азот, Сгк/Сфк арақатынасы, дәстүрлі технология, No-Till, агротехнологиялар, гумус, фракциялық құрамы.

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CORRELATION ANALYSIS OF CHANGES IN ORGANIC CARBON AND HUMUS COMPONENTS UNDER VARIOUS AGRICULTURAL TECHNOLOGIES IN ORDINARY CHERNOZEMS OF KOSTANAY REGION

Abstract

A field experiment conducted on typical chernozem soils of Kostanay Region investigated the effects of different soil tillage systems and crop types on the content of organic carbon, total nitrogen, and the fractional composition of humus. The study was carried out on spring wheat, pea crops, and fallow land under traditional and zero tillage (No-Till) systems. A correlation analysis was performed between the contents of organic carbon and total nitrogen, as well as between humic acid carbon (HAC) and fulvic acid carbon (FAC). It was found that the No-Till system generally promotes the accumulation of soil organic matter. A strong positive correlation between organic carbon and total nitrogen was observed in fallow soils (r = 0.78), and a close relationship was found between HAC and FAC (r = 0.978). The findings contribute to a deeper understanding of the mechanisms of organic matter accumulation and the influence of agrotechnologies on the carbon-nitrogen balance in soils.

Keywords*:* soil organic carbon, total soil nitrogen, HAC/FAC ratio, conventional tillage, No-Till, agrotechnologies, humus, fractional composition.

IRSTI 68.35.53 **DOI** https://doi.org/10.37884/2-2025/38

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OPTIMIZATION OF THE INTRODUCTION OF EXPLANTS OF APPLE TREES OF VARIOUS GENETIC ORIGIN INTO THE INITIAL *IN VITRO* NUTRIENT MEDIUM

Abstract

The research was conducted with the aim of optimizing the introduction of apple clonal rootstocks (M.9, MM.106) and varieties (*Golden Delicious, Zarya Alatau*) into the initial *in vitro*

nutrient medium. As a result, the most favorable time for introducing apple explants into the *in vitro* medium was found to be March - April. A sterilization scheme was developed for one-year-old shoot tips and dormant axillary buds used as initial explants. The most active development of microshoots occurred in the MS-1 nutrient medium. The addition of polyvinylpyrrolidone (PVP) as an antioxidant to the nutrient medium contributed to the inhibition of phenolic compound release and demonstrated that frequent medium renewal was not necessary. The analysis of the obtained data revealed that the development of microshoots depends on the genotypic characteristics of the apple varieties and clonal rootstocks. The highest growth rates of explants were observed in the following apple varieties and clonal rootstocks: *Zarya Alatau* (98% regeneration ability), *Golden Delicious* (77%), and clonal rootstocks M.9 (80%). The lowest growth rate was observed in the clonal rootstock MM.106 (43%). The effect of the duration of the vegetative period of apple varieties and clonal rootstocks on the induction of microshoot growth and development has not been investigated.

Keywords: *apple, cultivar, clonal rootstock, micropropagation, in vitro, microshoot, morphogenesis, regeneration.*

Introduction

In vitro plant cultivation is currently one of the most widely used methods and is extensively applied worldwide to address various practical issues. Its effectiveness lies in the fact that when plants are propagated by seeds, it is impossible to obtain a uniform population with desirable traits. Therefore, *in vitro* propagation is the method of choice to obtain new, fast-growing, quickly ripening, and disease-resistant lines.

Recently, this method has been widely adopted in fruit farming. The production of virus-free fruit, vegetable, field, and ornamental crop seedlings, as well as their rapid propagation, has gained momentum. It is also of great importance for obtaining genetically uniform offspring and propagating highly valuable, elite plants [1].

In general, in fruit farming, the establishment of virus-free mother orchards through *in vitro* processes is crucial.

Plants grown *in vitro* retain all the biosynthetic properties inherent to natural plants, and as such, they are also used for the production of economically valuable substances [2-4]. Thanks to the totipotency of plant cells, new technologies are being developed for plant farming, which simplify and accelerate the selection and propagation of valuable plants and allow for the production of virus-free seedlings [5].

When plants are grown *in vitro*, they undergo various changes at the cellular level, including genetic, morphological, and physiological changes. Even offspring derived from a single seed, that is, a clone of the cell, quickly becomes heterogeneous due to chromosomal variability. Therefore, increasing biological potential involves expanding the gene pool of apple cultivars and clonal rootstocks with new qualitative traits [6-8].

The aim of the study is to optimize the process of introducing and adapting genetically diverse apple cultivars and clonal rootstocks into the initial *in vitro* nutrient medium. To achieve this, the following tasks will be addressed: developing the proper sterilization protocol for microcuttings, selecting the appropriate nutrient medium composition, and monitoring their regenerative ability [9].

The article examines apple cultivars with a focus on their biological potential. In particular, the study involves the cultivars *Golden Delicious* and *Zarya Alatau*. *Golden Delicious* is a widely cultivated variety in the southern regions of Kazakhstan, especially in Almaty oblast. It originated in the United States and is known for its sweet taste, good storability, and high yield. It has several clonal types and is officially included in the State Register of the Republic of Kazakhstan [10].

Zarya Alatau is a domestic Kazakh cultivar developed at the Kazakh Research Institute of Fruit and Vegetable Growing (KazRIFVG) through hybridization of *Suislepskoe* and *Wealthy*. It is well adapted to the local climate and valued for its early ripening, stable yield, frost resistance, and aromatic fruits. This variety is also registered in the State Register of the Republic of Kazakhstan. The article highlights the importance of indicating the origin and official naming of the studied

cultivars in accordance with the national register or breeder's denomination to ensure scientific accuracy and reproducibility.

In this study, two clonal rootstocks were used: M.9 (Malling 9) and MM.106 (Malling-Merton 106), both widely applied in modern apple production systems.

M.9 is a dwarfing rootstock originally developed at the East Malling Research Station in the United Kingdom. It is characterized by its ability to produce small-sized trees, approximately 30–40% of the standard size, and promotes early fruiting (typically within 2–3 years). M.9 requires staking due to weak root anchorage and performs best in well-drained, fertile soils. However, it is susceptible to fire blight (*Erwinia amylovora*), collar rot (*Phytophthora spp.*), and woolly apple aphid (*Eriosoma lanigerum*).

MM.106, a semi-dwarf to semi-vigorous rootstock, was developed through the Malling-Merton breeding program by crossing M.2 with the *Northern Spy* cultivar. It produces trees approximately 60–70% of the standard size, suitable for semi-intensive orchards. MM.106 promotes early bearing (within 3–4 years), provides good root anchorage (staking often unnecessary), and adapts well to a range of soil types. It is resistant to woolly apple aphid but moderately susceptible to collar rot [11-15].

Both rootstocks are included in orchard practices in Kazakhstan, particularly in the southern regions, where they support high-density planting systems and contribute to improved productivity and orchard manageability.

Experimental

The introduction of apple explants into the *in vitro* nutrient medium was carried out at the Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, Biotechnology and Molecular Genetics Laboratory, using the necessary equipment and reagents.

As research objects, two apple cultivars and two clonal rootstocks were selected. For clonal micropropagation, one-year-old shoots with a length of 5–6 cm were used as the initial material. The apical tips of young shoots, ranging in size from 0.5 to 2.0 cm, were taken from branches in early spring (March–April, 2024) from dormant buds, and segments of axillary buds during the active growth phase (May–June, 2024) were also used. In total, 200 explants were introduced into the nutrient medium.

The first stage is the preliminary sterilization. In this stage, the explants were soaked in a soap solution for 30 minutes and then rinsed under running water for 40 minutes. The second stage is the actual sterilization. The explants were immersed in a 15% hydrogen peroxide (H_2O_2) solution for 5 minutes and then washed three times with autoclaved distilled water. The subsequent sterilization process was carried out in an aseptic environment (in a laminar flow hood): they were disinfected in 70% alcohol for 2 minutes and treated with a 10% CuSO4 solution for 5 minutes. Afterward, the explants were washed three times with autoclaved distilled water. Finally, the explants were immersed in 10 mg/l ceftriaxone antibiotic solution for 5 minutes, three times.

The third stage involves washing the object from the sterilizing solutions (post-sterilization). In this case, the plant material was washed 3–4 times with special sterile autoclaved water.

The introduction of apple explants into the initial *in vitro* medium was carried out in two different nutrient media (MS-1; MS-2). Polyvinylpyrrolidone (PVP) with an adsorption capacity was added to the Murashige and Skoog (MS-1) nutrient medium at a concentration of 10 g/l. In both nutrient media, the concentrations of macro- and microelements were halved, and 6-benzylaminopurine (BAP) was added at 0.5 mg/l. The media were prepared without agar, meaning they were liquid nutrient media.

The exposure of the explants lasted for 72 hours. The microcuttings were grown at a temperature of 25±2°C in a chamber with a 16-hour photoperiod. The humidity of the air was maintained at 55-60%.

The transfer to a new agar-based nutrient medium was performed after 4 weeks. According to long-term studies, the nutrient media used and the concentrations of growth regulators contained within them are optimal for growing many fruit crops, including apple, under *in vitro* conditions.

The experiments were repeated three times, with 50 explants introduced into the nutrient medium in each trial.

Results and Discussion

According to the studies by I.R. Rakhimbaev and D.K. Dzhumashev, in clonal micropropagation, the apical tips of young shoots and axillary buds are most commonly used. The rate of regeneration *in vitro* depends on the plant species, meaning the genotype has a strong influence [16].

To improve the efficiency of introducing initial explants into the *in vitro* medium, we investigated different options that varied in terms of sterilizing agents, their concentrations, sterilization time, and the modes and stages of sterilization. The best results were obtained through step-by-step sterilization of one-year-old shoots with dormant buds. The process of obtaining sterilized plant material (free from epiphytic and rhizospheric microorganisms) consists of several stages.

The plant part from which the explants are taken is also important for micropropagation. The size of the explant influences its morphogenic ability. The smaller the explant, the lower its ability to undergo organogenesis. Larger explants show greater genetic stability, but in these cases, viruses might remain in their cells [17]. Therefore, antibiotics are used to sterilize plant material that has been damaged by bacterial infections in the internal tissues.

Apple synthesizes significant amounts of phenolic compounds. As a result of their oxidation, tissues darken, and growth is inhibited. Methods to remove these compounds include frequently transferring the explants to a new medium or adding substances that adsorb them to the nutrient medium.

In general, the regeneration capacity of microshoots was considered to depend on the composition and combination of phytohormones in the nutrient medium. The composition of phytohormones in the studied nutrient media remained unchanged.

The initial microcuttings were placed on bridges made of filter paper in test tubes containing the Murashige and Skoog (MS) liquid nutrient medium (Figure 1). It was found that the shoots released phenolic compounds intensively. In the MS-2 liquid medium without PVP, all the microcuttings were transferred to fresh liquid nutrient medium daily until they stopped releasing phenolic compounds, which took about 1–2 weeks. Often, the composition of the liquid medium resembled that of the agar-solidified medium. After 1.5–3 weeks, the surviving explants were transferred to the agar-based MS nutrient medium, which retained its composition.

The survival rate of microcuttings in the MS-2 liquid nutrient medium without PVP reached only 34%. This means that the higher the concentration of phenolic compounds in the nutrient medium, the greater the risk of explant death.

It was determined that polyvinylpyrrolidone (PVP) in the MS-1 liquid nutrient medium, as an antioxidant, has a high adsorption capacity for phenolic compounds released from microshoots and serves as a factor inhibiting their release.

In this medium, the survival percentage of the studied microcuttings was higher (Table 1).

Table 1. Regeneration efficiency of apple explants collected in spring and summer

№	Apple varieties	Types of nutrient media			
		MS-1 (Spring)	MS-1 (Summer)	MS-2 (Spring)	MS-2 (Summer)
Apple varieties					
1	*Zarya Alatau*	98	50	82	45
2	*Golden Delicious*	77	40	71	35
Clonal apple rootstocks					
1	M.9	80	45	65	38
2	MM.106	44	15	43	12

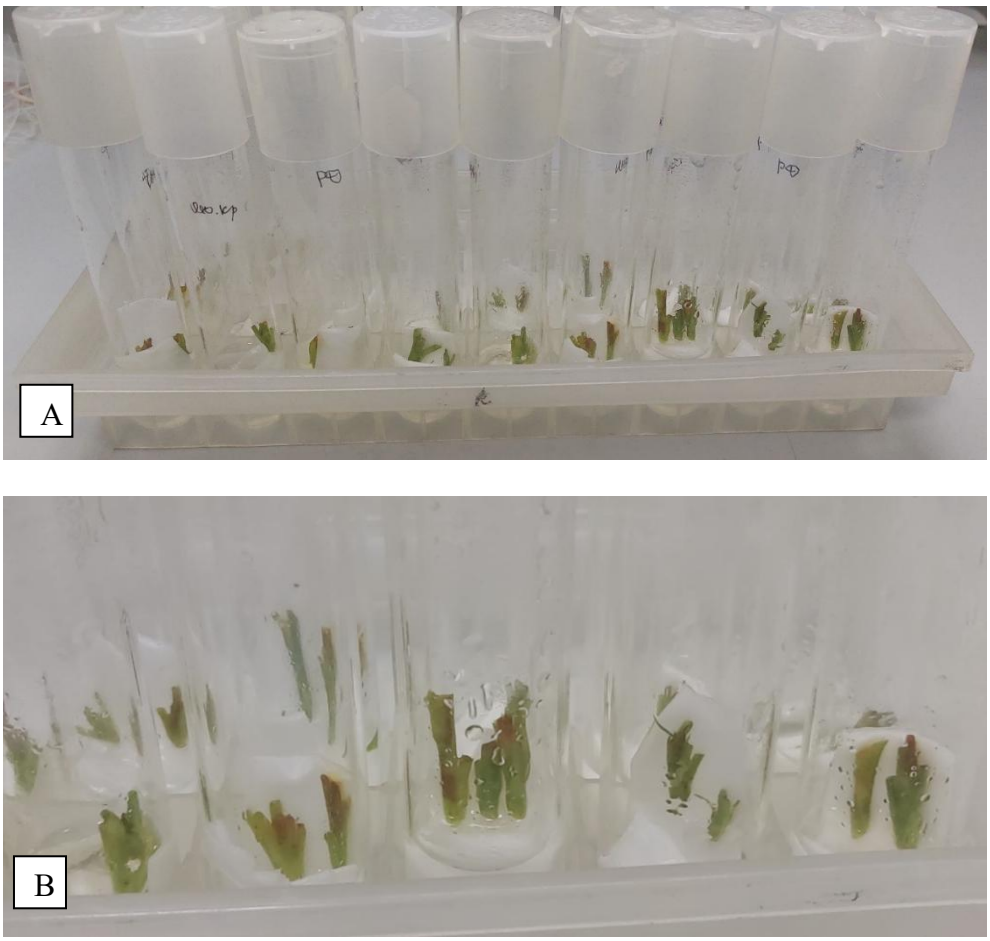

Figure 1. Micro-cuttings in MS-1 liquid medium: A – general view, B – micro-cuttings placed on bridges made of filter paper.

Thus, for the development of clonal apple rootstocks and cultivars in the initial stage, a liquid nutrient medium with half the concentration of macro- and microelements, 0.5 mg/l 6-benzylaminopurine (BAP), 10 g/l polyvinylpyrrolidone (PVP), and without agar, was considered the most suitable medium. The viability of explants was determined based on their growth and development, with the growth rate measured as a percentage (Figure 2).

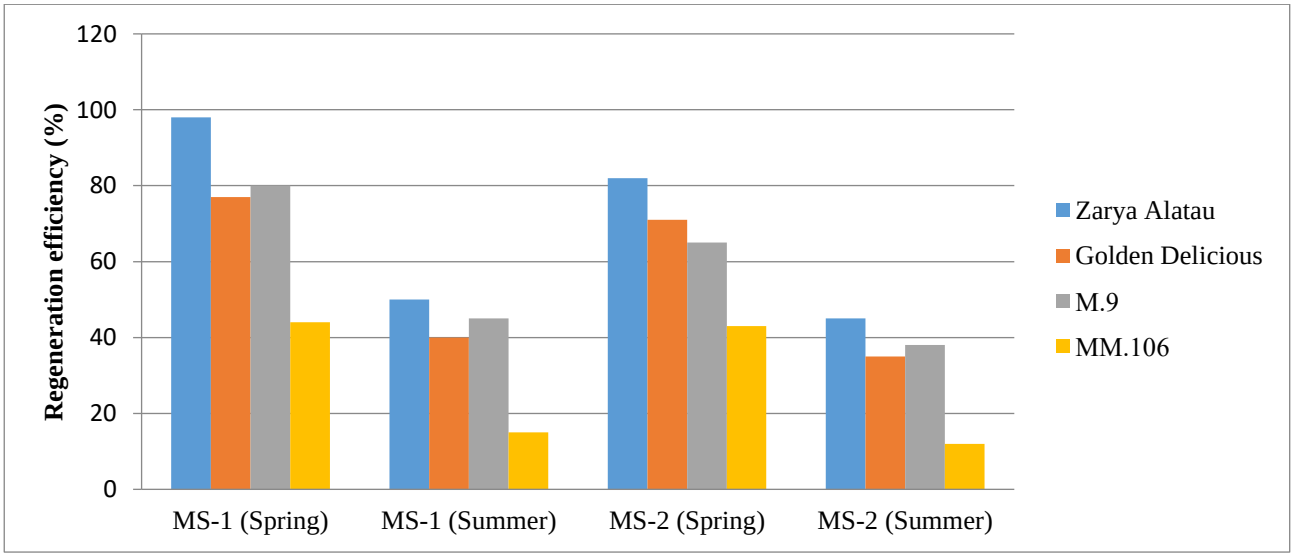

Figure 2. Seasonal comparison of apple explant regeneration

The table presents two nutrient media (MS-1 and MS-2) and regeneration percentages for different apple varieties and clonal rootstocks. We performed a statistical comparison between these two nutrient media (MS-1 and MS-2) using a t-test. *The bar chart below illustrates the significant reduction in regeneration efficiency for explants collected in summer compared to those collected in spring* (Table 2).

Table 2. Comparison of regeneration efficiency in spring and summer (statistical analysis t-test results)

Comparison	**t-value**	**p-value**
MS-1 (Spring vs. Summer)	8.93	0.00296
MS-2 (Spring vs. Summer)	14.10	0.00077

The obtained p-value (0.00296) is significantly below 0.05 ($p = 0.00296 < 0.05$), indicating a statistically significant difference between spring and summer explants. Explants collected in spring demonstrated a markedly higher regeneration efficiency compared to those collected in summer.

The optimal time for introducing apple explants into *in vitro* culture is March–April (spring) rather than May–June (summer). Seasonal factors significantly influence tissue viability, with higher contamination rates and reduced regeneration efficiency observed in explants collected during summer.

The highest survival percentage of microcuttings was observed in the following: *Zarya Alatau* (98% regeneration capacity), *Golden Delicious* (77%), and the clonal rootstock M.9 (80%). The lowest percentage was found in the clonal rootstock MM.106 (43%).

The duration of the vegetative period of the apple cultivars and clonal rootstocks did not significantly affect the induction of microshoot development.

Conclusion

As a result of the study, it was determined that the most suitable time for introducing apple cultivars and clonal rootstocks of different genetic origins into the initial *in vitro* nutrient medium is March-April. The results showed that in March, one-year-old shoots with axillary buds were sterilized as follows: washing in soapy solution for 30 minutes; rinsing with running water for 40 minutes; washing with a 15% hydrogen peroxide solution for 5 minutes; rinsing three times with autoclaved distilled water. Under aseptic conditions, the sterilization procedure included: soaking in 70% alcohol for 2 minutes; treatment with a 10% CuSO4 solution for 5 minutes; washing three times with autoclaved distilled water; and treatment in a 10 mg/l ceftriaxone antibiotic solution for 5 minutes, three times.

In the initial phase, the most suitable medium for the development of clonal apple rootstocks and cultivars was a liquid nutrient medium with half the concentration of macro- and microelements, 0.5 mg/l 6-benzylaminopurine (BAP), 10 g/l polyvinylpyrrolidone (PVP), and without agar.

The inclusion of an antioxidant in the nutrient medium contributed to the inhibition of phenolic compounds. Analysis of the obtained data showed that the development of microcuttings is highly dependent on the genotypic characteristics of the cultivar and clonal rootstocks. The highest survival percentages of microcuttings were observed in the following: *Zarya Alatau* (98% regeneration capacity), *Golden Delicious* (77%), and clonal rootstock M.9 (80%). The lowest percentage was found in clonal rootstock MM.106 (43%).

The duration of the vegetative period of the apple cultivars and clonal rootstocks did not affect the induction of microshoot development, and this requires further investigation.

In conclusion, the liquid culture medium without agar, consisting of 6-benzylaminopurine (BAP) at 0.5 mg/l and polyvinylpyrrolidone (PVP) at 10 g/l, was found to be the most suitable medium for initial *in vitro* propagation. The results of this medium revealed high regeneration capacity in the Zarya Alatau variety.

Acknowledgements: This article was carried out within the framework of the project " Study of biodiversity and development of techniques for *ex situ* conservation of genetic resources of fruit

and nut plants" (Project code BR21882024). The funding was provided by the Science Committee of the Ministry of Science and Higher Education.

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ГЕНЕТИКАЛЫҚ ШЫҒУ ТЕГІ ӘРТҮРЛІ АЛМА ЭКСПЛАНТТАРЫН БАСТАПҚЫ *IN VITRO* ҚОРЕКТІК ОРТАСЫНА ЕНГІЗУДІ ОҢТАЙЛАНДЫРУ

Аңдатпа

Зерттеу жұмысы алманың клондық телітушілері (M.9, ММ.106) мен сорттарын (*Zarya Alatau, Golden Delicious*) бастапқы *in vitro* қоректік ортасына енгізуді оңтайландыру мақсатында жүргізілді. Нәтижесінде алма эксплантарын *in vitro* ортасына енгізудің ең қолайлы уақыты наурыз − сәуір айлары екені анықталды. Бастапқы эксплант ретінде қолданылған бір жылдық жас өркендердің ұштары және ұйқыдағы қолтық бүршіктерді залалсыздандыру схемасы әзірленді. Ең белсенді микроөскіндердің дамуы MS−1 қоректік ортасында жүрді. Ал антиоксидант ретінде қосылған поливинилпирролидон (ПВП) қоректік ортаға фенолдық қосылыстардың шығу деңгейінің тежелуіне ықпал етті және қоректік ортаны жиі жаңартуды қажет етпейдігін көрсетті. Алынған мәліметтерді талдау барысында, микроөскіндердің дамуы алма сорттары мен клондық телітушілердің генотиптік ерекшеліктеріне тәуелді екендігін айқындады. Эксплантардың өсу деңгейінің ең жоғары пайызы келесі алманың сорттары мен клондық телітушілерінде байқалды: *Zarya Alatau* (98% регенерация қабілетіне ие), *Golden Delicious* (77%), клондық телітушілерден M.9 (80%). Өсу деңгейі ең төмен пайыз көрсеткіші клондық телітуші ММ.106 (43%) орын алды. Алма сорттары мен клондық телітушілерінің вегетациялық кезеңінің ұзақтығы, микроөскіндердің өсу және дамуының индукциясына әсері зерттелмеген.

Кілт сөздер: алма, сорт, клондық телітуші, микроклондық көбейту, *in vitro*, микроөскін, морфогенез, регенерация.

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ОПТИМИЗАЦИЯ ВВЕДЕНИЯ ЭКСПЛАНТАТОВ ЯБЛОНЬ РАЗЛИЧНОГО ГЕНЕТИЧЕСКОГО ПРОИСХОЖДЕНИЯ В НАЧАЛЬНУЮ *IN VITRO* ПИТАТЕЛЬНУЮ СРЕДУ

Аннотация

Исследовательская работа была проведена с целью оптимизации введения клоновых подвоев(M.9, ММ.106) и сортов яблонь (*Zarya Alatau, Golden Delicious*) в начальную *in vitro* питательную среду. В результате было установлено, что наиболее подходящее время для введения эксплантатов яблонь в *in vitro* среду — март-апрель. Была разработана схема

стерилизации для верхушек однолетних побегов и спящих боковых почек, использованных в качестве исходных эксплантатов. Наиболее активное развитие микроотростков происходило в питательной среде MS-1. Добавление поливинилпирролидона (ПВП) в качестве антиоксиданта в питательную среду способствовало задержке выделения фенольных соединений и показало, что частая замена среды не требуется. Анализ полученных данных показал, что развитие микроотростков зависит от генотипических особенностей сортов яблонь и клоновых подвоев. Наибольший процент роста эксплантатов был зафиксирован в следующих сортах яблонь и клонированных подвоев: *Zarya Alatau* (98% способность к регенерации), *Golden Delicious* (77%), клонированные подвои М.9 (80%). Наименьший процент роста был зарегистрирован у клонированного подвоя ММ.106 (43%). Длительность вегетационного периода сортов яблонь и клоновых подвоев не оказывала влияния на индукцию роста и развития микроотростков и требует дальнейших исследований.

Ключевые слова: яблоня, сорт, клонированный двойник, микроклональное размножение, *in vitro*, микросаженец, морфогенез, регенерация.

МРНТИ 68.05.29:68.05.35:68.05.37 **DOI** https://doi.org/10.37884/2-2025/39

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РАЗРАБОТКА КАРТ ПРИГОДНОСТИ СЕЛЬСКОХОЗЯЙСТВЕННЫХ КУЛЬТУР ПО КАТЕГОРИЯМ ЗЕМЕЛЬ ПРИ ПРОЕКТИРОВАНИИ АЛСЗ В СТЕПНОЙ ЗОНЕ КОСТАНАЙСКОЙ ОБЛАСТИ

Аннотация

В статье представлена комплексная агроэкологическая оценка земель Костанайской области с использованием геоинформационных технологий (ГИС) и статистического анализа для проектирования адаптивно-ландшафтных систем земледелия. Агроэкологическая оценка земель была проведена 2024 году на черноземах южных Костанайской области, Костанайского района на площади 1500 га, для проведения анализа, были отобраны почвенные образцы с глубины 0-20 см по элементарным участкам в 25 га. На основе агроэкологических групп и видов земель спроектированы карты пригодности сельскохозяйственных земель по категориям.

Созданы электронные картограммы содержания гумуса, нитратного азота, подвижного фосфора, калия, pH почвенной среды и серы. Также проведен корреляционный анализ, который выявил сильную положительную связь между содержанием гумуса (r = 0.978), нитратным азотом (r = 0.965), подвижным фосфором (r = 0.930) и подвижным калием (r = 0.942) с урожайностью. Регрессионный анализ подтвердил, что содержание гумуса влияет на 95.7% вариации урожайности, а содержание фосфора и калия — на 86.4% и 88.7%, соответственно. Внедрение АЛСЗ способствует устойчивому управлению земельными ресурсами, повышению урожайности и сохранению плодородия почв в условиях континентального климата Костанайской области.