

Research Article

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The influence of explant type and plant growth regulators on callus induction of *Malus niedzwetzkyana*

Malus niedzwetzkyana is a rare and valuable wild apple species characterised by a high anthocyanin content. This species is of considerable interest as a promising source of bioactive compounds. In this study, we investigated the effect of explant type on callus induction and its morphological characteristics. Stems and leaves from 40-day-old *in vitro*-grown plants maintained on Murashige-Skoog (MS) medium were used as explants. The results showed that stem explants had a higher callus induction rate than leaf explants, at 100% and 86.7%, respectively. MS medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) (2.5, 3.0, and 3.5 mg/L) and 6-benzylaminopurine (BAP) (0.5 and 1.0 mg/L) significantly affected callus diameter, fresh and dry weight, and dry matter content ($p < 0.001$). The combination of 3.5 mg/L 2,4-D and 0.5 mg/L BAP in MS medium was optimal, resulting in the formation of a uniform, loose callus with a pinkish-burgundy colour. Thus, our results provide a basis for the establishment of suspension cultures and the biotechnological production of biologically active compounds from *Malus niedzwetzkyana*.

Keywords: callus, *Malus niedzwetzkyana*, plant growth regulators, Murashige-Skoog medium, *in vitro*, auxin, cytokinin.

Introduction

Against the backdrop of the rapid disappearance of natural ecosystems, *in vitro* biotechnological methods are becoming an important tool for the conservation of rare and endangered plant species [1].

One such rare and valuable wild species is *Malus niedzwetzkyana* Dieck, which grows in Central Asia, including Kazakhstan (Karatau and Zailiyskiy Alatau), Kyrgyzstan (Jalal-Abad region) and western China (Xinjiang Uyghur Autonomous Region). This species is of particular interest due to its unique chemical composition and ornamental qualities [2].

A distinctive feature of the *Malus niedzwetzkyana* is the intense red colouration not only of the fruit but also of other parts of the plant—the flowers, leaves, and even the wood. This colouration is due to the high content of anthocyanins—natural pigments from the flavonoid group. These compounds not only impart a bright red colour but also possess powerful antioxidant, anti-inflammatory and anti-cancer properties [3].

These characteristics make the *Malus niedzwetzkyana* of great interest not only as an ornamental and genetic resource of Central Asian flora, but also as a promising source of bioactive compounds for use in the pharmaceutical, food and biotechnology industries. In Kazakhstan, this species is listed in the Red Book, which restricts its widespread use and the collection of wild material [2].

In this context, the development of sustainable biotechnological methods for the cultivation and utilisation of the *Malus niedzwetzkyana* has become particularly important. Such approaches will help to preserve the genetic diversity and natural populations of the species, whilst enabling the production of valuable bioactive compounds without harming ecosystems [4].

One of the modern and promising methods for obtaining valuable compounds from rare or endangered plant species is the use of cell technologies, in particular, the production of callus cultures [5]. In plant tissue culture, the term “callus” refers to a dense mass of undifferentiated cells that form when plant tissue is cultured on a growth medium under controlled conditions *in vitro* [6].

Virtually any part of a plant can be used to produce callus cultures. Explant samples taken from plant tissues grow slowly *in vitro*, forming a cell mass whose colour varies from amorphous and colourless to pale brown [1]. Various callus-inducing factors may be employed to achieve optimal callus formation. Changes in external factors, such as culture medium types, plant growth regulators (PGRs), sucrose concentrations, culture medium pH and incubation temperature, are among the factors that can influence the induction potential and morphology of the induced callus [7].

Despite extensive research into the tissue culture of various species of the genus *Malus niedzwetzkyana*, data on the optimisation of callus formation conditions in *Malus niedzwetzkyana* remain limited. This lack of understanding necessitates the experimental selection of explant type, culture media and PGRs to induce and establish a stable, actively growing callus culture.

This study aimed to investigate the effect of explant type and PGRs concentration (2,4-D and BAP) on the induction and morphological characteristics of *Malus niedzwetzkyana* callus tissue for the subsequent production of suspension cultures and bioactive compounds.

Experimental

Study materials

The research was conducted at the Laboratory of Biotechnology and Plant Breeding at the National Centre for Biotechnology (Astana). *Malus niedzwetzkyana* plants *in vitro* culture, previously obtained by microclonal propagation according to the protocol [8], were used as explants. The *in vitro* shoots were maintained in culture by transferring them to fresh medium of the same composition every 40 days.

Effect of explant type on callus induction

To induce callus, leaves and stems of *Malus niedzwetzkyana in vitro* culture were used as explants to select the most suitable type of explant. Stem and leaf segments measuring 0.3–0.5 cm, taken from plants grown *in vitro*, were inoculated onto Murashige-Skoog (MS) basal medium [9] containing 30 g/l sucrose and 7 g/l agar, with 2,4-dichlorophenoxyacetic acid (2,4-D) (2.5 mg/L) and 6-benzylaminopurine (BAP) (0.5 mg/L). All chemicals used for analysis were supplied by Sigma-Aldrich (St. Louis, MO). Explantlets grown on MS medium without PGRs were used as a control. Before adding agar, the pH of the medium was adjusted to 5.8, and it was autoclaved at 121 °C for 15 minutes. Cultivation was carried out in a controlled-environment room at a temperature of 25±2 °C with a 12-hour photoperiod and relative humidity of 60–65 %. After 30 days of cultivation, morphological observation was carried out to select a suitable type of explant.

The effect of PGRs concentration on callus formation

To optimise the hormonal composition of the culture medium, the effect of various concentrations of PGRs 2,4-D (2.5; 3.0; 3.5 mg/L) and BAP (0.5; 1.0 mg/L) on the induction of callus formation in stem explants of *Malus niedzwetzkyana* was investigated. The stems were cut into segments measuring 0.3–0.5 cm and inoculated onto MS medium containing 30 g/l sucrose and 7 g/l agar. A total of six treatment were studied: I — (2,4-D 2.5 mg/L + BAP 0.5 mg/L); II — (2,4-D 3.0 mg/L + BAP 0.5 mg/L); III — (2,4-D 3.5 mg/L + BAP 0.5 mg/L); IV — (2,4-D 2.5 mg/L + BAP 1.0 mg/L); V — (2,4-D 3.0 mg/L + BAP 1.0 mg/L); VI — (2,4-D 3.5 mg/L + BAP 1.0 mg/L). All treatments were cultured in a climate-controlled chamber at a temperature of 25±2 °C with a 12-hour photoperiod and relative humidity of 60–65 %. After 40 days of culture, callus growth parameters were measured.

Measurement of callus growth parameters and statistical analysis

To assess the morphogenetic potential, the colour and texture of the callus were determined visually. The growth parameters of the induced callus were assessed by fresh weight (FW), dry weight (DW) and dry matter (DM). To determine dry weight, the fresh callus was dried in an oven at 45 °C until a constant weight was achieved (Binder ED115, Tuttlingen, Germany). The dry matter of callus was calculated using the formula:

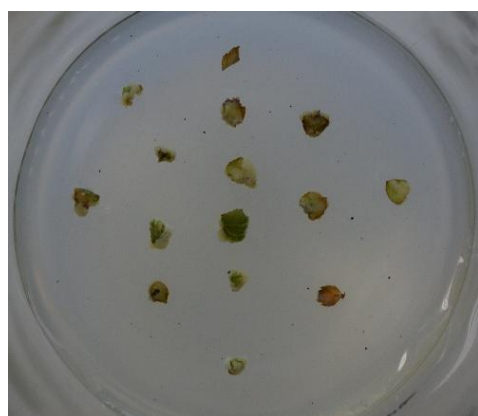
$$DM (\%) = (DW/FW) \times 100.$$

Statistical analysis was performed using Python. The data obtained were analysed using one-way analysis of variance (ANOVA). Tukey's HSD test was used for pairwise comparison of mean values. Results are presented as mean ± standard deviation. Differences between treatments were considered statistically significant at $p < 0.05$.

Results and Discussion

Effect of explant type on callus induction

The first signs of cell dedifferentiation and callus formation were observed 10–15 days after inoculation, manifesting as cell swelling and proliferation at the cut surfaces of the explants. No callus formation was observed on the control MS medium without the addition of PGRs. On the MS medium (2,4-D — 2.5 mg/L and BAP — 0.5 mg/L), the extent of callus formation varied depending on the type of explant. On leaf explants, callus formed in several zones: along the edges of the leaf blade and in its central part (Fig. 1). On stem explants, callus tissue began to develop from the transverse cuts on both sides. A comparative analysis showed that callus formation was significantly more pronounced on stem explants: the callus induction rate was 100 % for stems and 86.7 % for leaves. Consequently, stem explants were used for further studies.



a — callus from a leaf explant



b — callus from a stem explant



c — leaf explant under a microscope
($\times 15$)



d — stem explant under a microscope
($\times 15$)

Figure 1. Callus induction from leaf and stem explants of *Malus niedzwetzkyana*

The effect of PGRs on the growth and development of callus cultures

Various combinations of the PGRs 2,4-D (2.5, 3.0 and 3.5 mg/L) and BAP (0.5 and 1.0 mg/L) had a marked effect on the morphological and growth characteristics of callus tissue. All PGR combinations tested resulted in 100 % callus induction from stem explants of *Malus niedzwetzkyana*.

As shown in Table 1, all PGR combinations significantly affected callus diameter, fresh weight (FW), dry weight (DW) and dry matter (DM) ($p < 0.001$). The largest callus diameter (13.20 ± 3.76 mm) and the highest dry weight (0.045 ± 0.019 g) were observed in treatment I (2,4-D 2.5 mg/L + BAP 0.5 mg/L). The highest fresh weight was recorded in treatment II (2,4-D 3.0 mg/L + BAP 0.5 mg/L) and amounted to 0.342 ± 0.204 g. With an increase in PGR concentration, a gradual decrease in all growth parameters was observed: the minimum values for diameter (5.93 ± 2.55 mm), fresh weight (0.048 ± 0.033 g) and dry weight (0.014 ± 0.007 g) were recorded in treatment VI (2,4-D 3.5 mg/L + BAP 1.0 mg/L).

The dry matter content showed the opposite trend: in treatments I-III, the dry matter was at its lowest (12.09–16.03 %), indicating a high water content and a loose callus structure. In treatments IV-VI, a significant increase in dry matter (31.97–34.75 %) was observed, indicating the formation of a denser and more compact callus. Thus, increasing the BAP concentration from 0.5 to 1.0 mg/L had a more pronounced inhibitory effect on callus growth parameters and promoted the formation of denser, more compact tissue compared with an increase in 2,4-D concentration.

Table 1

**Effect of various combinations of plant growth regulators (2,4-D and BAP)
on the fresh weight, dry weight and dry matter of callus**

Treatment	2,4-D (mg/L)	BAP (mg/L)	Callus diameter (mm)	Fresh callus weight (g)	Dry callus weight (g)	Dry matter (%)	Callus colour and texture
I	2,5	0,5	13,20±3,76	0,301±0,133	0,045±0,019	16,03±5,92	Yellowish with white flecks, heterogeneous
II	3,0	0,5	12,80±3,57	0,342±0,204	0,039±0,017	12,56±2,83	Creamy yellow with pink and burgundy flecks, heterogeneous
III	3,5	0,5	10,80±4,11	0,194±0,109	0,023±0,015	12,09±4,05	Creamy yellow with pink and burgundy flecks, crumbly
IV	2,5	1,0	6,93±1,87	0,070±0,039	0,022±0,008	33,10±6,44	White and yellow with brown patches, mottled
V	3,0	1,0	6,07±0,96	0,061±0,059	0,017±0,016	34,75±18,10	Greenish-yellow with purple flecks, dense
VI	3,5	1,0	5,93±2,55	0,048±0,033	0,014±0,007	31,97±5,37	Green and greenish-white, compact
Significance			***	***	***	***	
***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$. The data are presented as the mean ± standard deviation, n = 15.							

Visual inspection revealed that the calluses in all treatments were predominantly yellowish or greenish yellow in colour (Fig. 1a, c). With an increase in the concentration of 2,4-D to 3.0 mg/L in combination with both 0.5 mg/L BAP (treatment II) and 1.0 mg/L BAP (treatment V), the appearance of a pronounced reddish-purple hue was observed. With a further increase in the concentration of 2,4-D to 3.5 mg/L (treatments III and VI), the intensity of pigmentation decreased; however, in treatments with 1.0 mg/L BAP (VI), an intensification of the greenish colour and the appearance of dense nodular structures were noted, indicating the onset of differentiation processes. The best texture loose, uniform, creamy-yellow with pinkish-burgundy flecks was demonstrated by treatment III (2,4-D 3.5 mg/L + BAP 0.5 mg/L), making it the most suitable for further cultivation.

In terms of structure, the callus tissues of treatments I-III (BAP 0.5 mg/L) had a heterogeneous and loose structure, with treatment III easily breaking down into individual cells and cell aggregates. Increasing the BAP concentration to 1.0 mg/L (treatments IV-VI) led to the formation of a dense callus structure; furthermore, organogenesis of the callus tissue was observed.

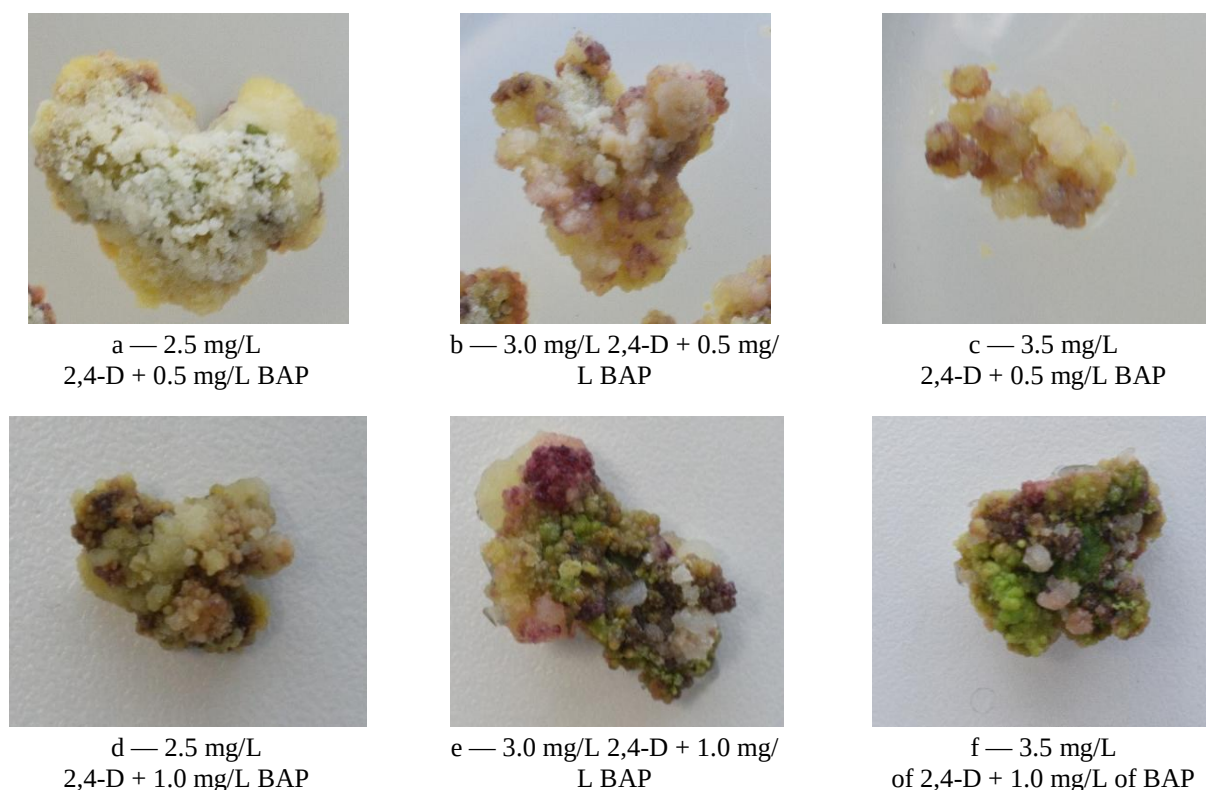


Figure 2. Callus formation on MS medium supplemented with different combinations of 2,4-D and BAP

A detailed morphological examination of the calli revealed differences in texture and colouration between the treatments. The calli of treatment III (2,4-D 3.5 mg/L + 6-BAP 0.5 mg/L) had a uniform, loose, granular texture that easily disintegrated into individual cell aggregates, and were cream-yellow in colour with pinkish-burgundy inclusions (Fig. 1c). In contrast, the calli of treatment II (2,4-D 3.0 mg/L + 6-BAP 0.5 mg/L) had a denser, heterogeneous texture in the form of large aggregates with pink-burgundy inclusions (Fig. 1b). It should be noted that the pink-burgundy colour of the calli in both treatments is due to the presence of anthocyanins, which accumulate in *Malus niedzwetzkyana*. Although treatment II demonstrated the highest fresh weight (0.342 ± 0.204 g), treatment III was preferable for suspension culture.

The calli from the treatments with a higher concentration of BAP (1.0 mg/L) differed significantly in morphology. Treatment IV (2,4-D 2.5 mg/L + BAP 1.0 mg/L) was characterised by a denser, heterogeneous texture with brownish areas, which may indicate oxidative processes in the callus tissue (Fig. 1d). Treatment V (2,4-D 3.0 mg/L + BAP 1.0 mg/L) was distinguished by the most pronounced anthocyanin pigmentation with intense purple spots, as well as the presence of green nodular structures, indicating the onset of organogenesis processes. In treatment VI (2,4-D 3.5 mg/L + BAP 1.0 mg/L), a greenish colouration predominated with a dense, compact callus structure, indicating enhanced cell differentiation at high concentrations of both growth regulators. Thus, increasing the BAP concentration to 1.0 mg/L generally contributed to the compaction of callus tissue and the intensification of differentiation processes, making these treatments less suitable for obtaining suspension cultures compared to treatment III.

The choice of explant type is one of the key factors influencing the efficiency of callus induction [10]. In this study, stem explants of *Malus niedzwetzkyana* demonstrated a higher morphogenetic capacity compared to leaf explants. Callus induction was 100 % for stem explants and 86.7 % for leaf explants. This is consistent with the study by Zhang et al. (2020), which showed that callus induction from stem explants of the wild apple (*Malus sieversii*) occurred more rapidly and with higher efficiency compared to leaf explants, whereas leaf explants were highly sensitive to medium composition and died in the absence of auxin [11]. The greater ability of stem explants to form callus can be explained by the large number of meristem-like cells in the vascular tissues of the stem, which are known to possess a greater capacity for dedifferentiation [12].

Plant growth regulators play a key role at all stages of plant development, influencing cell growth, differentiation, physiological responses and the production of secondary metabolites. Under *in vitro* conditions,

they are one of the key factors controlling callus formation and cell proliferation, and their optimal type and concentration must be determined experimentally for each species, genotype and explant [13]. Auxins and cytokinins are essential for cell division and morphogenesis. Auxins are considered the primary inducers of callus formation, promoting cell wall softening through acidification and activating the expression of genes associated with growth and development. Cytokinins complement this effect by directly regulating the cell cycle, thereby maintaining sustained cell division and morphogenetic activity [14].

According to the results of our study, a low concentration of BAP (0.5 mg/L) is more effective than a high concentration (1.0 mg/L). The 2,4-D and BAP had a marked and statistically significant effect on all the callus formation parameters studied ($p < 0.001$). The auxin 2,4-D, in combination with a low concentration of cytokinin (BAP 0.5 mg/L), stimulated active cell proliferation and the formation of loose, well-growing callus tissue. Increasing the BAP concentration to 1.0 mg/L had a marked inhibitory effect on growth parameters, which is consistent with the general pattern that in cultures containing low concentrations of cytokinin and high concentrations of auxin, callus cultures grow excessively, and the callus structure becomes soft and loose, but as the concentration of cytokinin increases, the callus tissue becomes stiffer [15].

Studies conducted on *Abies koreana* showed that a combination of the NAA, 2,4-D and BAP had a positive effect on callus formation. The callus proliferation coefficient at a high concentration of 2,4-D (3.0 mg/L) was high, amounting to 1147.6 % compared to the initial mass of fresh callus [16]. A comparison with data on closely related species of the *Rosaceae* family shows that optimal concentrations and PGR types vary significantly depending on the genotype. Thus, in studies by Menbari et al. (2021), it was found that the maximum callus induction rate (100 %) was achieved on MS medium containing 2.0 mg/L 2,4-D and 1.0 mg/L BAP. The same combination of PGRs also resulted in the highest fresh and dry callus biomass (2.754 g and 0.37 g, respectively). Furthermore, callus formation occurred more rapidly compared with other combinations. The authors also noted that this combination yielded the best callus quality, characterised by a loose texture and a yellowish-white colour [17].

The results obtained are consistent with the literature on the key role of the auxin-to-cytokinin ratio in the regulation of callus formation. The best indicators of fresh callus mass and texture were achieved using 2,4-D at a concentration of 2.5-3.5 mg/L in combination with BAP at 0.5 mg/L, which is slightly higher than the concentrations described for other species of the genus *Malus*. This appears to reflect the genetic characteristics of *Malus niedzwetzkyana*.

Treatment III (2,4-D 3.5 mg/L + BAP 0.5 mg/L) was identified as optimal not only in terms of the morphological characteristics of the callus loose texture and low dry matter content, but also in terms of its suitability for the subsequent establishment of suspension cultures. Thus, treatment III ensures the production of callus most suitable for establishing suspension cultures and studying the bioactive compounds of this species.

Conclusion

This study investigated callus formation in leaf and stem explants of *Malus niedzwetzkyana*. It was found that stem explants possess higher morphogenetic activity compared to leaf explants and demonstrate 100 % callus induction. The optimal PGR composition of the medium for callus formation was determined. Among the PGR combinations tested, the MS medium supplemented with 2,4-D at a concentration of 3.5 mg/L and BAP at a concentration of 0.5 mg/L proved to be the most promising for the cultivation of uniform, loose callus tissue. The pinkish-burgundy colour of the calli formed, due to the accumulation of anthocyanins, demonstrates the high biosynthetic potential of this species and opens up prospects for the use of *Malus niedzwetzkyana* callus and suspension cultures for the biotechnological production of biologically active substances.

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Conflict of interest

The authors declare no conflict of interest.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Kakimzhanova A.A.** — conceptualization, investigation, writing draft; **Yessimseitova A.K., Dyussembekova D.A., Islamova S.S., Nurtaza A.S.** — data curation, analysis, methodology, data collection; **Maller A.V.** — investigation, data analysis.

References

- 1 Efferth T. Biotechnology applications of plant callus cultures / T. Efferth // Engineering. — 2019. — Vol. 5, No. 1. — P. 50–59. <https://doi.org/10.1016/j.eng.2018.11.006>
- 2 Kolchenko M. Wild *Malus niedzwetzkyana* Dieck ex Koehne as a genetic resource for fire blight resistance / M. Kolchenko, A. Nurtaza, A. Pozharskiy, D. Dyussembekova, A. Kapytina, G. Nizamdinova, M. Khusniddinova, A. Taskuzhina, A. Kakimzhanova, D. Gritsenko // Horticulturae. — 2023. — Vol. 9, No. 10. — P. 1066. <https://doi.org/10.3390/horticulturae9101066>
- 3 Khoo H.E. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits / H.E. Khoo, A. Azlan, S.T. Tang, S.M. Lim // Food & Nutrition research. — 2017. — Vol. 61, No. 1. — P. 1361779. <https://doi.org/10.1080/16546628.2017.1361779>
- 4 Мамонов Л.К. Степень изученности видов, родов и семейств флоры Казахстана и перспективы дальнейших исследований / Л.К. Мамонов, Р.А. Музычкина, Н.Г. Гемеджиева, Ю.И. Васильев, Г.Т. Ситпаева, Н.А. Рябушкина, Г.С. Муканова // Введение в фитохимические исследования и выявление биологической активности веществ растений. — Алматы, 2008. — С. 24.
- 5 Babich O. Evaluation of the conditions for the cultivation of callus cultures of *Hyssopus officinalis* regarding the yield of polyphenolic compounds / O. Babich, S. Sukhikh, A. Pungin, L. Astahova, E. Chupakhin, D. Belova, A. Prosekov, S. Ivanova // Plants. — 2021. — Vol. 10, No. 5. — P. 915. <https://doi.org/10.3390/plants10050915>
- 6 Sidik N.J. A Mini review of plant tissue culture: the role of media optimization, growth regulators in modern agriculture, callus induction and the applications / N.J. Sidik, H.M. Agha, A.A. Alkamil, M. M.S. Alsayadi, A.A. Mohammed // AUIQ Complementary Biological System. — 2024. — Vol. 1, No. 2. — P. 96–109. <https://doi.org/10.70176/3007-973X.1019>
- 7 Osman N.I. Effects of variations in culture media and hormonal treatments upon callus induction potential in endosperm explant of *Barringtonia racemosa* L. / N.I. Osman, N.J. Sidik, A. Awal // Asian Pacific Journal of Tropical Biomedicine. — 2016. — Vol. 6, No. 2. — P. 143–147. <https://doi.org/10.1016/j.apjtb.2015.10.007>
- 8 Nurtaza A. Micropropagation of the endangered species *Malus niedzwetzkyana* for conservation biodiversity in Kazakhstan / A. Nurtaza, G. Magzumova, A. Yessimseitova, V. Karimova, A. Shevtsov, D. Silayev, V. Lutsay, Y. Ramankulov, A. Kakimzhanova // In Vitro Cellular & Developmental Biology. — 2021. — Vol. 57, No. 6. — P. 965–976. <https://doi.org/10.1007/s11627-021-10174-4>
- 9 Murashige T. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures / T. Murashige, F. Skoog // Physiologia plantarum. — 1962. — Vol. 15, No. 3. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- 10 Rahayu S. The effect of types and concentrations of auxins on callus induction of *Centella asiatica* / S. Rahayu, I. Roostika, N. Bermawie // Nusantara Bioscience. — 2016. — Vol. 8, No. 2. — P. 283–7. <https://doi.org/10.13057/nusbiosci/n080224>
- 11 Zhang Y. An efficient in vitro regeneration system from different wild apple (*Malus sieversii*) explants / Y. Zhang, T.A. Bozorov, D.X. Li, P. Zhou, X.J. Wen, Y. Ding, D.Y. Zhang // Plant Methods. — 2020. — Vol. 16, No. 1. — P. 56. <https://doi.org/10.1186/s13007-020-00599-0>
- 12 Kaňuková Š. Establishment of stem cell-like cells of *Sida hermaphrodita* (L.) rusby from explants containing cambial meristems / Š. Kaňuková, M. Gubišová, L. Klčová, D. Mihálik, J. Kraic // International Journal of Molecular Sciences. — 2022. — Vol. 23, No. 14. — P. 7644. <https://doi.org/10.3390/ijms23147644>
- 13 Song W. Improvement of culture conditions and plant growth regulators for *in vitro* callus induction and plant regeneration in *Paeonia lactiflora* Pall / W. Song, Y. Song, X. Liu, X. Zhang, R. Xin, S. Duan, S. Guan, X. Sun // Plants. — 2023. — Vol. 12, No. 23. — P. 3968. <https://doi.org/10.3390/plants12233968>
- 14 Hazrati R. Factors affecting the growth, antioxidant potential, and secondary metabolites production in hazel callus cultures / R. Hazrati, N. Zare, R. Asghari-Zakaria, P. Sheikhzadeh, M. Johari-Ahar // AMB Express. — 2022. — Vol. 12, No. 1. — P. 109. <https://doi.org/10.1186/s13568-022-01449-z>
- 15 Rahmati Z. et al. Optimization of culture medium for *in vitro* callogenesis in *Taxus baccata* L. and *T. brevifolia* Nutt / Z. Rahmati, V. Payam Nour, K. Ghasemi Bezdi, P. Ebrahimi // Forest and Wood Products. — 2017. — Vol. 70, No. 3. — P. 381–391.
- 16 Guo G. Explant, medium, and plant growth regulator (PGR) affect induction and proliferation of callus in *Abies koreana* / G. Guo, B.R. Jeong // Forests. — 2021. — Vol. 12, No. 10. — P. 1388. <https://doi.org/10.3390/f12101388>
- 17 Menbari A. Establishment of callus and cell suspension cultures of Granny Smith apple fruit and antityrosinase activity of their extracts / A. Menbari, B. Bahramnejad, M. Abuzaripoor, E. Shahmansouri, M. A. Zarei // Scientia Horticulturae. — 2021. — Vol. 286. — P. 110222. <https://doi.org/10.1016/j.scienta.2021.110222>

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Недзвецкий алмасының (*Malus niedzwetzkyana*) каллус индукциясына эксплант түрі және өсімдік гормондарының әсері

Недзвецкий алмасы (*Malus niedzwetzkyana*) — антоцианиннің жоғары мөлшерімен сипатталатын сирек кездесетін және бағалы жабайы түр. Бұл түр биоактивті қосылыстардың перспективалы көзі ретінде елеулі қызығушылық тудырады. Осы зерттеуде эксплант түрінің каллус индукциясына және оның морфологиялық сипаттамаларына әсері зерттелген. 40 күн *in vitro* жағдайында Мурасиге-Скуг (МС) коректік ортасында өсірілген өсімдіктердің сабағы мен жапырақтары эксплант ретінде алынды. Нәтижелер көрсеткендей, сабақ экспланттары жапырақ экспланттарына қарағанда каллус түзу қабілетін жоғарырақ көрсетті, тиісінше 100 % және 86,7 %. 2,4-дихлорофеноксиацетикалық қышқылдың (2,4-D) әртүрлі концентрациялары бар МС ортасы (2,5; 3,0; 3,5 мг/л) және 6-бензиламинопурин (БАП) (0,5; 1,0 мг/л) концентрациялары диаметрге, жана және құрғақ салмаққа, сондай-ақ құрғақ зат мөлшеріне ($p < 0,001$) елеулі әсер етті. МС ортасы мен 3,5 мг/л концентрациядағы 2,4-D және 0,5 мг/л БАП үйлесімі оңтайлы, бұл қызғылт-кою қызыл түсті біртекті әрі борпылдақ каллустың түзілуін қамтамасыз етті. Осылайша, алынған нәтижелер Недзвецкий алмасының суспензиялық өсірінділерін алу және биологиялық белсенді заттарды биотехнологиялық жолмен өндіру үшін негіз бола алады.

Кілт сөздер: каллус, *Malus niedzwetzkyana*, өсу реттегіштері, Мурасиге-Скуг ортасы, *in vitro*, ауксин, цитокинин.

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Влияние типа экспланта и гормонов растений на индукцию каллуса яблони Недзвецкого (*Malus niedzwetzkyana*)

Яблоня Недзвецкого (*Malus niedzwetzkyana*) — редкий и ценный дикорастущий вид, который характеризуется высоким содержанием антоцианов. Этот вид представляет значительный интерес как перспективный источник биологически активных веществ. В настоящем исследовании мы изучали влияние типа экспланта на индукцию каллуса и его морфологические характеристики. В качестве эксплантов использовали стебли и листья 40-дневных растений, выращенных в условиях *in vitro*, которые культивировали на среде Мурасиге-Скуга (МС). Результаты показали, что экспланты стебля демонстрировали более высокую способность к образованию каллуса по сравнению с эксплантами листьев — 100 % и 86,7 % соответственно. Среда МС с различными концентрациями 2,4-дихлорофеноксиуксусной кислоты (2,4-D) (2,5; 3,0; 3,5 мг/л) и 6-бензиламинопурина (БАП) (0,5; 1,0 мг/л) оказала значительное влияние на диаметр, свежую и сухую массу, а также содержание сухого вещества ($p < 0,001$). Сочетание среды МС с 2,4-D в концентрации 3,5 мг/л и БАП 0,5 мг/л являлось оптимальным, что обеспечивало образование однородного и рыхлого каллуса розово-бордового цвета. Таким образом, полученные нами результаты служат основой для получения суспензионных культур и биотехнологического получения биологически активных веществ яблони Недзвецкого.

Ключевые слова: каллус, *Malus niedzwetzkyana*, регуляторы роста, среда Мурасиге-Скуга, *in vitro*, ауксин, цитокинин.

References

- 1 Efferth, T. (2019). Biotechnology applications of plant callus cultures. *Engineering*, 5(1), 50–59. <https://doi.org/10.1016/j.eng.2018.11.006>
- 2 Kolchenko, M., Nurtaza, A., Pozharskiy, A., Dyussembekova, D., Kapytina, A., Nizamdinova, G., ... & Gritsenko, D. (2023). Wild *Malus niedzwetzkyana* Dieck ex Koehne as a genetic resource for fire blight resistance. *Horticulturae*, 9(10), 1066. <https://doi.org/10.3390/horticulturae9101066>
- 3 Khoo, H.E., Azlan, A., Tang, S.T., & Lim, S.M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition research*, 61(1), 1361779. <https://doi.org/10.1080/16546628.2017.1361779>
- 4 Mamonov, L.K., Muzychkina, R.A., Gemedzhieva, N.G., Vasiliev, Yu.I., Sitpaeva, G.T., Ryabushkina, N.A., & Mukanova, G.S. (2008). Stepen izuchennosti vidov, rodov i semeistv flory Kazakhstana i perspektivy dalneishikh issledovaniy [The degree of study of species, genera and families of the flora of Kazakhstan and prospects for further research]. *Vvedenie v*

fitokhimicheskie issledovaniia i vyivlenie biologicheskoi aktivnosti veshchestv rastenii — Introduction to phytochemical research and identification of biological activity of plant substances, 24. Almaty [in Russian].

5 Babich, O., Sukhikh, S., Pungin, A., Astahova, L., Chupakhin, E., Belova, D., ... & Ivanova, S. (2021). Evaluation of the conditions for the cultivation of callus cultures of *Hyssopus officinalis* regarding the yield of polyphenolic compounds. *Plants*, 10(5), 915. <https://doi.org/10.3390/plants10050915>

6 Sidik, N.J., Agha, H.M., Alkamil, A.A., Alsayadi, M.M. S., & Mohammed, A.A. (2024). A Mini review of plant tissue culture: the role of media optimization, growth regulators in modern agriculture, callus induction and the applications. *AUIQ Complementary Biological System*, 1(2), 96–109. <https://doi.org/10.70176/3007-973X.1019>

7 Osman, N.I., Sidik, N.J., & Awal, A. (2016). Effects of variations in culture media and hormonal treatments upon callus induction potential in endosperm explant of *Barringtonia racemosa* L. *Asian Pacific Journal of Tropical Biomedicine*, 6(2), 143–147. <https://doi.org/10.1016/j.apjtb.2015.10.007>

8 Nurtaza, A., Magzumova, G., Yessimseitova, A., Karimova, V., Shevtsov, A., Silayev, D., ... & Kakimzhanova, A. (2021). Micropropagation of the endangered species *Malus niedzwetzkyana* for conservation biodiversity in Kazakhstan. *In Vitro Cellular & Developmental Biology*, 57(6), 965–976. <https://doi.org/10.1007/s11627-021-10174-4>

9 Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, 15(3). <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>

10 Rahayu, S., Roostika, I., & Bermawie, N. (2016). The effect of types and concentrations of auxins on callus induction of *Centella asiatica*. *Nusantara Bioscience*, 8(2), 283–7. <https://doi.org/10.13057/nusbiosci/n080224>

11 Zhang, Y., Bozorov, T. A., Li, D. X., Zhou, P., Wen, X. J., Ding, Y., & Zhang, D. Y. (2020). An efficient in vitro regeneration system from different wild apple (*Malus sieversii*) explants. *Plant Methods*, 16(1), 56. <https://doi.org/10.1186/s13007-020-00599-0>

12 Kaňuková, Š., Gubišová, M., Klčová, L., Mihálik, D., & Kraic, J. (2022). Establishment of Stem Cell-like Cells of *Sida hermaphrodita* (L.) Rusby from Explants Containing Cambial Meristems. *International Journal of Molecular Sciences*, 23(14), 7644. <https://doi.org/10.3390/ijms23147644>

13 Song, W., Song, Y., Liu, X., Zhang, X., Xin, R., Duan, S., ... & Sun, X. (2023). Improvement of Culture Conditions and Plant Growth Regulators for In Vitro Callus Induction and Plant Regeneration in *Paeonia lactiflora* Pall. *Plants*, 12(23), 3968. <https://doi.org/10.3390/plants12233968>

14 Hazrati, R., Zare, N., Asghari-Zakaria, R., Sheikhzadeh, P., & Johari-Ahar, M. (2022). Factors affecting the growth, antioxidant potential, and secondary metabolites production in hazel callus cultures. *AMB Express*, 12(1), 109. <https://doi.org/10.1186/s13568-022-01449-z>

15 Rahmati, Z., Payam Nour, V., Ghasemi Bezdi, K., & Ebrahimi, P. (2017). Optimization of culture medium for *in vitro* callogenesis in *Taxus baccata* L. and *T. brevifolia* Nutt. *Forest and Wood Products*, 70(3), 381–391.

16 Guo, G., & Jeong, B.R. (2021). Explant, medium, and plant growth regulator (PGR) affect induction and proliferation of callus in *Abies koreana*. *Forests*, 12(10), 1388. <https://doi.org/10.3390/f12101388>

17 Menbari, A., Bahranejad, B., Abuzaripoor, M., Shahmansouri, E., & Zarei, M. A. (2021). Establishment of callus and cell suspension cultures of Granny Smith apple fruit and antityrosinase activity of their extracts. *Scientia Horticulturae*, 286, 110222. <https://doi.org/10.1016/j.scienta.2021.110222>

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