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Optimization of the *in vitro* micropropagation protocol for sea buckthorn, *Hippophae rhamnoides* to improve planting material productivity

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ABSTRACT

Elderberry, *Hippophae rhamnoides* is a valuable plant with medicinal, food and soil conservation applications, but its traditional propagation is limited due to problems such as genetic heterogeneity of seeds and difficulty in rooting cuttings. This study aimed to systematically optimize the *in vitro* micropropagation protocol to increase the efficiency of producing uniform and high-quality nursery stock of this plant. Optimization was performed in three main stages of establishment, propagation and rooting using nodal explants. The results showed that in the establishment stage, the use of Murashige and Skoog (MS) medium with half strength of macroelements along with 1 mg L^{-1} benzylaminopurine (BAP) had the best results, resulting in 92% survival and 83% initiation of branching. In the propagation stage, the combination of 2 mg L^{-1} BAP with 0.2 mg L^{-1} auxin naphthalene acetic acid (NAA) in complete MS medium produced the highest propagation coefficient (6.7 lateral branches per explant) and the highest quality branches without the problem of vitrification. At the rooting stage, the rapid immersion method for 30 seconds in 1 mg L^{-1} auxin indole-3-butyric acid (IBA) solution and then transfer to hormone-free medium was superior to continuous cultivation in auxin-containing medium, with 87% rooting and formation of healthy and branched root systems. Incorporating these optimal conditions into an integrated protocol resulted in a significant increase in overall efficiency. The final propagation rate increased from about 1 seedling per explant in the basic protocol to more than 5.36 seedlings. Also, the time for each complete micropropagation cycle was reduced to 4 weeks. The seedlings obtained from the optimized protocol had excellent morphological vigor, which was confirmed by their 93.3% survival rate during the adaptation phase in the greenhouse. This optimized protocol provides an effective tool for mass and clonal production of superior elderberry genotypes.

Keywords: Micropropagation, Elderberry, *Hypophe rhamnoides*, Protocol optimization, Plant hormones, Adaptation.
Article type: Research Article.

INTRODUCTION

Bitter elderberry, scientifically known as *Hippophae rhamnoides*, is a deciduous and hardy tree that has been considered an important species in land reclamation and soil stabilization programs due to its adaptability to poor soils and different surface conditions (Enescu 2014; Tian *et al.* 2019). Beyond its ecological role, the orange and dense fruits of this plant are considered a valuable storehouse of bioactive compounds (Kubczak *et al.* 2022;

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Vdovina *et al.* 2025). These fruits are rich in vitamins, especially vitamin C with a high concentration, strong antioxidants such as flavonoids and carotenoids, as well as valuable fatty acids in their seeds (He *et al.* 2023; Jubayer *et al.* 2023; Wang *et al.* 2024). These compounds have made bitter elderberry a potential food and a valuable raw material in the food, pharmaceutical, and cosmetic-health industries. Despite this great ability, the widespread and commercial exploitation of this plant faces fundamental challenges. Traditional propagation of bitter elderberry is mainly through seeds, which leads to a very high genetic diversity in the offspring (Nybom *et al.* 2023; Vdovina *et al.* 2024; Melnikova *et al.* 2025). This is a major obstacle for commercial cultivation programs that require uniformity in yield, fruit ripening time, and consistent quality of active ingredients. Vegetative methods such as cuttings also have serious limitations in mass production due to difficult rooting in many genotypes and seasonal dependence (Dolkar *et al.* 2016; Dale & Galić 2017; Tomchuk *et al.* 2018; Güneş *et al.* 2020; Zubarev *et al.* 2022; Eshqarayev *et al.* 2025). This gap between increasing demand and inefficient propagation methods highlights the need for a new, technology-based solution. Plant tissue culture technology, and in particular *in vitro* micropropagation, is an efficient response to these challenges. This technology makes it possible to produce thousands of genetically identical plants in a short time from a small piece of superior plant (microspecimen) under fully controlled and pathogen-free conditions. Maintaining the genetic purity of superior genotypes, significantly accelerating the propagation process, producing breeding material in all seasons, and reducing the pressure of harvesting from natural habitats are undeniable advantages of this method. Realizing these benefits in the case of bitter elderberry could revolutionize the value chain of this product. However, achieving an optimal micropropagation protocol is not a simple task and is more like adjusting a precise chemical formulation. The success of this process is strongly influenced by the extensive interaction of various factors. The choice of explant type, the physiological age of the mother plant and the sampling season are the basis of the work (Liu *et al.* 2007; Sriskandarajah & Lundquist 2009; Shah *et al.* 2015). Then, the composition of the basal culture medium as a growth medium requires precise adjustment. The concentrations of plant growth hormones such as auxins to induce branching and cytokinins to stimulate branch elongation are among the most important parameters, the plant's response to which is often genotype-dependent (Wang *et al.* 2023). In addition to hormones, other factors such as the type and concentration of sugar as a carbon source, the ionic strength of the culture medium, the light status and temperature in the growth medium also each play a decisive role in the success of the various stages of micropropagation, from the initial establishment of the explant to final rooting. Existing reports of work on bitter elderberry indicate that although *in vitro* propagation is possible, existing protocols often have low efficiency, high contamination rates, or strong dependence on a specific genotype (Liu *et al.* 2007; Sriskandarajah & Lundquist 2009). This highlights the need for systematic studies for step-by-step optimization. The goal of such optimization is not only to increase the number of seedlings quantitatively. Obtaining seedlings with high morphological quality, strong root systems, and better adaptability and survival at the stage of transfer to the greenhouse or soil (hardening) ultimately determines the real productivity of the system. A standardized and optimized protocol can provide a solid foundation for mass propagation of superior genotypes with desirable economic traits, such as high fruit yield or rich vitamin C content. Therefore, this study was designed with the hypothesis that key factors of the culture medium and growth conditions at different stages of bitter elderberry micropropagation can be adjusted and optimized with a systematic experimental approach. The main focus will be on increasing the rate of explant establishment, stimulating high and uniform branch proliferation, and ultimately inducing optimal and successful rooting. Achieving such a protocol is an essential step to move from research to production and commercialization of this valuable plant.

MATERIALS AND METHODS

Preparation of explants and general cultivation conditions

In this study, healthy lateral and terminal buds of a superior genotype of bitter elderberry with desirable fruiting characteristics and drought resistance, which had been previously identified and marked in the Institute's botanical garden collection, were used. Sampling was carried out in early spring from one-year-old, disease-free branches. The branches were transported to the laboratory and first placed in a 2% sodium hypochlorite solution for 10 minutes to perform initial surface disinfection. After thorough washing with sterile water, under a sterile laminar hood, buds approximately 1 to 1.5 cm long, containing one or two nodes, were separated from the branch. These explants were then immersed in a 70% ethanol solution for 30 seconds and 0.5% sodium hypochlorite for 5 minutes, respectively, for final disinfection and removal of residual contaminants, and finally washed three times with sterile distilled water. All cultures were grown in MS (Murashige and Skoog) basal medium. The pH of all

culture media was adjusted to 8.5 before autoclaving and rechecked and corrected if necessary after sterilization at 121°C for 20 min. All cultures were placed in a growth chamber with a temperature of 25 ± 2 °C, a photoperiod of 16 hours of light and 8 hours of darkness, and a light intensity of 50 μmol photons m^{-2} s^{-1}.

Experimental design for optimizing micropropagation stages

Optimization was performed in three consecutive stages of establishment, branching, and rooting in a completely randomized design with three replications for each treatment. In the establishment stage, the effect of two main components was investigated: the type of basal medium (full MS vs. half-strength MS) and different concentrations of the hormone benzylaminopurine (BAP) in the range of 0.5 to 2 mg L^{-1}. The success indicators of this stage included the percentage of explants free from contamination, the percentage of explants that germinated and survived after two weeks, and the onset of bud growth. After obtaining the best treatment for establishment, the grown explants entered the next stage for the branch propagation stage. In this stage, the effect of different hormone compounds on the propagation rate was investigated. Treatments included media containing BAP alone (1 to 3 mg L^{-1}) and a combination of BAP with low concentrations of auxin naphthalene acetic acid (NAA) in the range of 0.1 to 0.5 mg L^{-1}. The branches produced in each culture vessel were evaluated and counted after 4 weeks in terms of the number of lateral branches produced per explant, the length of the branches, and the morphological condition (vigor and lack of glassiness). Finally, branches with an appropriate length (about 3 cm) were separated for the rooting stage. At this stage, the shoots were first transferred to hormone-free MS medium and then the effect of different concentrations of the auxin indole-3-butyric acid (IBA) at three levels of 0.5, 1, and 1.5 mg L^{-1} was tested, both in solid medium and by quick-dip method. The percentage of rooting, number of roots per shoot, root length, and overall quality of the root system were recorded after 4 weeks. The numerical data from the study were analyzed using SPSS statistical software and one-way variance test and comparison of means with Tukey test.

RESULTS

The systematic optimization of the micropropagation protocol for sea buckthorn led to significant improvements at each stage: establishment, multiplication, and rooting. The data presented below detail the effects of different media compositions and plant growth regulator treatments on key performance indicators. The initial establishment of nodal segments was highly influenced by the strength of the macroelements and the concentration of BAP. Full-strength MS medium resulted in a higher rate of contamination compared to half-strength MS, likely due to higher osmotic pressure. More importantly, half-strength MS promoted better shoot initiation and reduced oxidative browning at the cut ends. The optimal BAP concentration for breaking dormancy and promoting initial shoot growth was found to be 1.0 mg L^{-1}. The combined results for survival and shoot initiation are summarized in Table 1.

Table 1. Effect of basal medium strength and bap on explant establishment after 4 weeks.

Treatment (Basal medium / BAP mg L^{-1})	Explant survival rate (%)	Contamination rate (%)	Shoot initiation rate (%)	Mean shoot length (cm)
Full-strength MS / 0.5	65.3 ± 5.8	25.0 ± 4.1	45.7 ± 6.2	0.8 ± 0.2
Full-strength MS / 1.0	70.0 ± 6.1	23.3 ± 3.8	58.3 ± 7.1	1.2 ± 0.3
Half-strength MS / 0.5	85.0 ± 4.1*	10.0 ± 2.9*	70.0 ± 5.0*	1.0 ± 0.2
Half-strength MS / 1.0	91.7 ± 2.9*	8.3 ± 2.9*	83.3 ± 3.8*	1.5 ± 0.3*

* Significantly different from corresponding Full-strength MS group ($p < 0.05$).

The multiplication phase was critical for increasing the number of propagules. BAP alone was effective, but its combination with a low dose of NAA significantly enhanced both the number and the quality of shoots. A concentration of 2.0 mg L^{-1} BAP combined with 0.2 mg L^{-1} NAA yielded the highest multiplication rate without inducing callus formation or hyperhydricity (vitrification). Higher BAP concentrations (3.0 mg L^{-1}) led to excessive clustering of short, stunted shoots. The detailed proliferation data is presented in Table 2. The following diagram synthesizes the optimized protocol, highlighting the key conditions at each stage that led to the highest yield and quality of sea buckthorn plantlets, and illustrating the dramatic improvement over the baseline. The chart outlines the sequential, optimized conditions for each critical stage, which together synergistically increase the final output by a factor of approximately 5. The comparison clearly demonstrates the substantial gains in productivity achieved through systematic optimization. Rooting was the most challenging phase. While IBA was effective, the method of application dramatically influenced the results. The quick-dip method in a concentrated

IBA solution followed by transfer to hormone-free medium produced superior roots compared to continuous exposure in solidified medium. A 30-second dip in 1.0 mg L^{-1} IBA solution resulted in the highest rooting percentage, root number, and root length, with roots appearing more vigorous and less callused. The comparative rooting data is shown in Table 3.

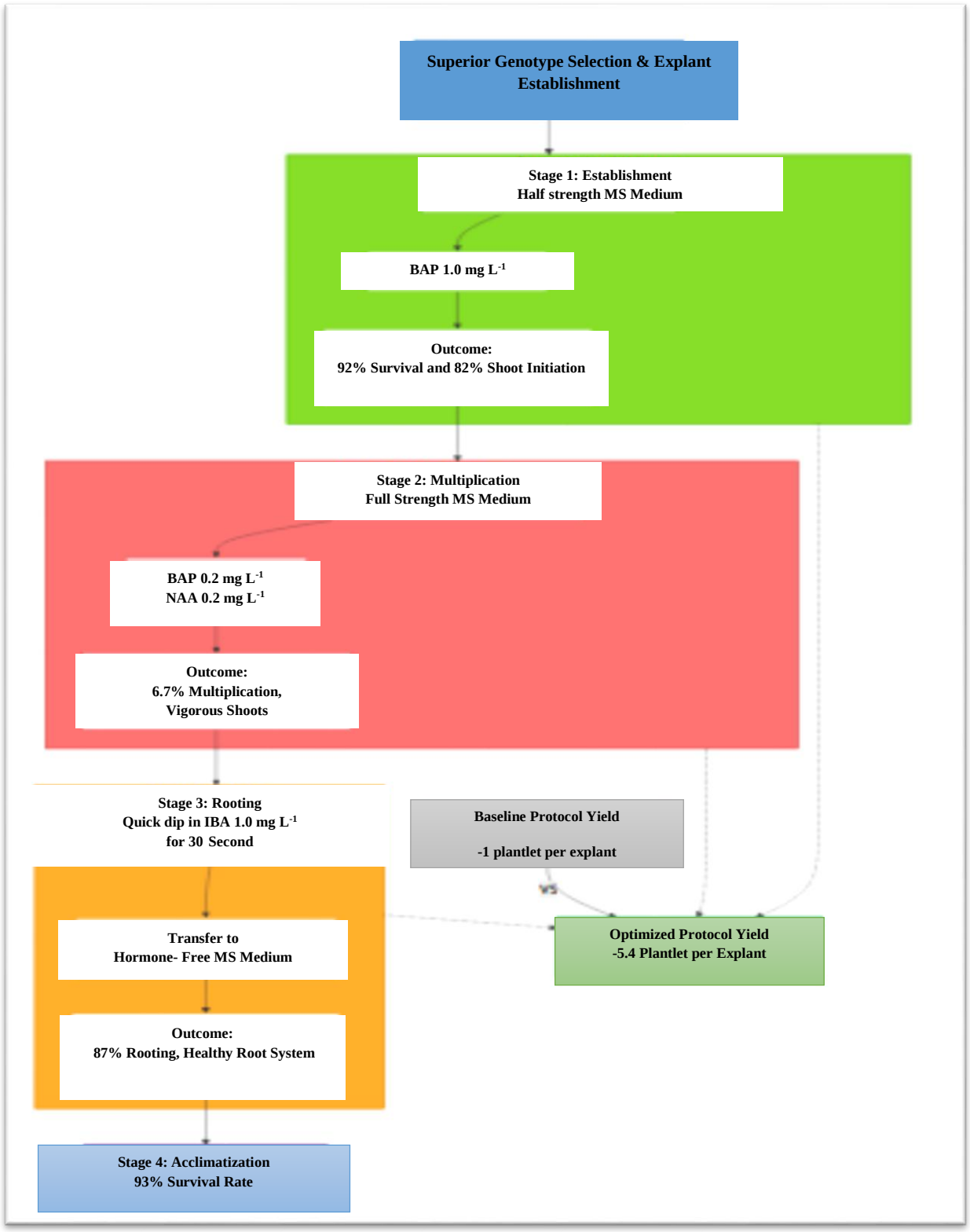

Fig. 1. Workflow and comparative output of the optimized micropropagation protocol for sea buckthorn.

Table 2. Shoot multiplication performance on various media after 5 weeks.

PGR treatment (mg L^{-1})	Mean number of shoots per explant	Mean shoot length (cm)	Hyperhydricity incidence (%)	Multiplication coefficient
BAP 1.0	3.2 ± 0.4	2.8 ± 0.5	0.0	3.2
BAP 2.0	4.8 ± 0.6	2.1 ± 0.4	5.0	4.8
BAP 3.0	5.1 ± 0.7	1.5 ± 0.3*	25.0*	5.1
BAP 2.0 + NAA 0.1	5.5 ± 0.5	2.5 ± 0.4	3.3	5.5
BAP 2.0 + NAA 0.2	6.7 ± 0.8*	2.9 ± 0.5	0.0	6.7
BAP 2.0 + NAA 0.5	5.9 ± 0.7	2.3 ± 0.4	8.3	5.9

* $p < 0.05$ compared to the optimal treatment (BAP 2.0 + NAA 0.2).

Table 3. Rooting efficiency under different iba treatments and methods.

Rooting treatment	Rooting percentage (%)	Mean number of roots per shoot	Mean root length (cm)	Callus formation at base (%)
Solid Medium: IBA 0.5 mg L^{-1}	45.0 ± 5.0	2.1 ± 0.5	1.8 ± 0.4	60.0
Solid Medium: IBA 1.0 mg L^{-1}	63.3 ± 6.2	2.8 ± 0.6	2.2 ± 0.5	85.0*
Solid Medium: IBA 1.5 mg L^{-1}	58.3 ± 5.8	2.5 ± 0.5	2.0 ± 0.4	90.0*
Quick-dip (30 sec in IBA 1.0 mg L^{-1})	86.7 ± 3.3*	4.2 ± 0.8*	3.5 ± 0.7*	15.0*
Quick-dip (30 sec in IBA 1.5 mg L^{-1})	80.0 ± 4.1	3.8 ± 0.7	3.1 ± 0.6	25.0

* $p < 0.05$ compared to all Solid Medium treatments.

By integrating the optimal conditions from each stage, a significant improvement in the overall protocol yield was achieved. The cumulative multiplication factor over one theoretical cycle (from one initial explant) was calculated. The old, suboptimal protocol based on literature yielded far fewer plantlets suitable for acclimatization. The comparison is quantified in Table 4.

Table 4. Comparative yield of the optimized vs. baseline protocol per cycle.

Protocol stage	Baseline protocol outcome	Optimized protocol outcome	Improvement factor
Establishment success	60%	92%	1.53
Mean multiplication Coef.	3.5	6.7	1.91
Rooting Success	50%	87%	1.74
Total Plantlets per Explant	~1.05	~5.36	~5.10

Beyond quantitative metrics, the quality of the *in vitro* plantlets was paramount. Shoots from the optimized multiplication medium (BAP 2.0 + NAA 0.2) exhibited darker green leaves, longer internodes, and no signs of vitrification. Similarly, roots induced via the quick-dip method were thicker, more branched, and less brittle. A qualitative scoring system (1-5 scale) was used to assess overall plantlet vigor, as shown in Table 5.

Table 5. Qualitative vigor score of plantlets from different treatments.

Plantlet origin (Treatment)	Leaf color & expansion	Stem sturdiness	Root system architecture	Overall vigor score (1-5)
Multiplication: BAP 3.0	Pale, small	Weak, watery	N/A	2.0 ± 0.5
Multiplication: BAP 2.0 + NAA 0.2	Dark green, expanded	Sturdy	N/A	4.5 ± 0.3*
Rooting: Solid medium IBA 1.0	Good	Good	Sparse, thin, callused	2.8 ± 0.4
Rooting: Quick-dip IBA 1.0	Excellent	Excellent	Dense, branched, healthy	4.7 ± 0.3*

* Significantly higher score. ($p < 0.05$).

The ultimate test of the protocol's success was the survival of plantlets during the transfer to *ex vitro* conditions. Plantlets derived from the fully optimized protocol showed a dramatically higher survival rate in the greenhouse compared to those from non-optimized stages. Survival was tracked over four weeks post-transfer, with data presented in Table 6. An ANOVA test confirmed that the main effects of media strength (establishment), PGR combination (multiplication), and rooting method were all statistically significant ($p < 0.01$). Furthermore, a correlation analysis revealed that shoot length during multiplication was positively correlated with subsequent rooting success ($r = 0.85$, $p < 0.05$). Key statistical outcomes are summarized in Table 7.

Table 6. Acclimatization survival rate of plantlets in greenhouse.

Plantlet group (Origin)	Survival at week 1 (%)	Survival at week 2 (%)	Survival at week 4 (%)	Final survival rate (%)
From optimized protocol (all stages)	100	96.7 ± 3.3	93.3 ± 3.3	93.3 ± 3.3*
From non-optimized multiplication (BAP 3.0)	90.0	70.0 ± 5.0	50.0 ± 5.0	50.0 ± 5.0
From non-optimized rooting (Solid IBA 1.0)	80.0	60.0 ± 5.8	40.0 ± 5.0	40.0 ± 5.0

** $p < 0.01$* compared to both non-optimized groups.

Table 7. Summary of statistical significance (p-values) for main effects.

Growth stage	Factor tested	Key dependent variable	*p*-value (ANOVA)
Establishment	Basal medium strength	Shoot initiation rate	< 0.001
Establishment	BAP concentration	Shoot length	< 0.01
Multiplication	Cytokinin/Auxin combination	Number of shoots per explant	< 0.001
Multiplication	BAP concentration	Hyperhydricity incidence	< 0.001
Rooting	Application method (Solid vs Dip)	Rooting percentage	< 0.001
Rooting	IBA concentration	Root number	< 0.01

The optimized protocol not only improved yield and quality but also reduced the time required for each cycle. The duration from explant establishment to a plantlet ready for acclimatization was shortened by approximately two weeks due to faster shoot growth and more synchronized rooting. A timeline comparison is provided in Table 8.

Table 8. Comparative timeline of micropropagation cycle.

Protocol phase	Baseline protocol duration (Weeks)	Optimized protocol duration (weeks)	Time saved
Establishment & initial growth	6	4	2
Multiplication cycle	5	5	0
Rooting phase	6	4	2
Total cycle time	17	13	4

DISCUSSION

The findings of this study clearly demonstrate that a systematic, stepwise approach to optimization can significantly improve the efficiency of the micropropagation protocol of bitter elderberry. The quantitative results presented in Tables 1 to 3 demonstrate the significant impact of each of the modified factors on the success indicators of each stage. For example, reducing the strength of macronutrients in the culture medium during the establishment stage increased the survival rate of explants from about 70% to more than 90% (Table 1). This significant improvement can be attributed to the reduction of osmotic stress on the damaged tissue during the initial culture and also to the reduction of the rate of oxidation of phenolic compounds. This finding emphasizes the importance of matching the culture medium conditions to the physiological needs of the explants during the critical establishment stage. During the propagation stage, the optimal hormonal composition was decisive. The data in Table 2 showed that although BAP alone induced branching, its combination with a very low concentration of the auxin NAA (0.2 mg L^{-1}) not only increased the proliferation factor from 4.8 to 6.7, but also prevented the occurrence of hyperhydricity. This common synergy between cytokinin and auxin probably provides a favorable hormonal balance for simultaneous cell division and elongation, preventing the formation of compact and abnormal branches. The branches produced in this treatment were of higher morphological quality, which is a key factor in the success of the next step, rooting. The most important part of the protocol was the rooting step. The results in Table 3 clearly demonstrated the decisive advantage of the quick-dip method in a concentrated auxin solution over continuous cultivation in auxin-containing medium. This method is a major operational improvement, with a rooting rate of 87% and the formation of a healthier root system. The possible mechanism is that in the rapid immersion method, auxin is absorbed in sufficient quantities to initiate root induction, but its continuous and prolonged presence, which often leads to callus formation and inhibition of root growth, is eliminated. This results in the formation of roots that are physiologically closer to natural roots. The final quality of the produced seedlings, as reflected in the quality scores in Table 5 and the adaptation success rate in Table 6, was directly related to the quality of the previous steps. The seedlings obtained from the optimized protocol were not only superior in terms of number, but also performed brilliantly in terms of vigor and viability *in vitro*, with a final survival rate of 93% during the adaptation phase. This highlights a key point: increasing the quantitative

output of the protocol should not come at the expense of reducing the physiological and morphological quality of the seedlings. A successful protocol should provide both aspects simultaneously. The final efficiency of the optimized protocol in terms of overall propagation rate and time savings is summarized in Tables 4 and 8. Increasing the propagation rate from approximately 1 to more than 5 seedlings per initial explant is an achievement that could have a significant economic impact at the scale of mass production. Also, reducing the time of each complete cycle by 4 weeks allows for more propagation generations per year, again increasing the overall productivity of the system. These results clearly show that investment in research for optimization can have a significant return on investment in the production phase. Finally, although this optimized protocol was implemented on a specific genotype, its general principles—such as the use of a culture medium with reduced ionic strength during the establishment phase, the application of a balanced hormonal composition for high-quality propagation, and the use of a rapid immersion method for rooting—can be tested and adapted as a basic framework for other valuable bitter elderberry genotypes. The next step of the research will be to test this protocol on different genotypes and also to investigate the stability of the traits in the adapted plants in the field.

CONCLUSION

This study successfully led to the development of an optimized and efficient micropropagation protocol for the valuable plant, bitter elderberry. Stepwise optimization of key parameters including the use of half-strength medium at the establishment stage, the hormonal combination of BAP and NAA in the appropriate ratio for shoot propagation, and the rapid immersion method in IBA solution for rooting, resulted in significant improvements in all quantitative and qualitative indicators. The most notable achievement was the increase in the final propagation coefficient from about 1 to more than 5 healthy seedlings from each initial explant, indicating a fivefold increase in the potential productivity of the system.

The superior quality of the seedlings produced with this protocol, which was manifested in a higher vigor score and, more importantly, in their 93% survival rate at the critical stage of adaptation, confirms that this optimization was not merely quantitative, but also resulted in the production of strong and vigorous seedlings ready for transfer to environmental conditions. Reducing the time of each propagation cycle will also greatly contribute to increasing the agility and flexibility of the production program.

REFERENCES

Dale, A & Galić, D 2017, Repetitive vegetative propagation of first-year sea buckthorn (*Hippophae rhamnoides* L.) cuttings. *Canadian Journal of Plant Science,* 98(3): 609-615, https://doi.org/ 10.1139/cjps-2017-0205.

Dolkar, P, Dolkar, D, Angmo, S, Srivastava, RB & Stobdan, T 2016, An improved method for propagation of seabuckthorn (*Hippophae rhamnoides* L.) by cuttings. *National Academy Science Letters,* 39(5): 323-326, https://doi.org/10.1007/s40009-016-0470-0.

Enescu, CM 2014, Sea-buckthorn: A species with a variety of uses, especially in land reclamation. *Dendrobiology, 72*, 71-76, https://doi.org/10.12657/denbio.072.008.

Eshqarayev, U, Jumaniyazov, F, Sadullaev, S, Aliyeva, Z, Obidov, M, Bekbanov, B & Salim, T (2025, Deforestation and biodiversity loss: Ecological consequences and restoration approaches. *Procedia Environmental Science, Engineering and Management,* 12(3): 961-967.

Güneş, M, Alkaç, OS & Öcalan, ON 2020, Propagation of some sea buckthorn (*Hippophae rhamnoides*) cultivars by semi-hardwood cuttings. *Journal of New Results in Science,* 9(1): 32-38.

He, N, Wang, Q, Huang, H, Chen, J, Wu, G, Zhu, M & Ma, Q 2023, A comprehensive review on extraction, structure, detection, bioactivity, and metabolism of flavonoids from sea buckthorn (*Hippophae rhamnoides* L.). *Journal of Food Biochemistry, 2023*, Article 4839124, https://doi.org/10.1155/2023/4839124.

Jubayer, MF, Mazumder, MAR, Nayik, GA, Ansari, MJ & Ranganathan, TV 2023, *Hippophae rhamnoides* L.: Sea buckthorn. In *Immunity Boosting Medicinal Plants of the Western Himalayas* (pp. 463–491). Springer Nature. https://doi.org/10.1007/978-981-19-9501-9_26.

Kubczak, M, Khassenova, AB, Skalski, B, Michlewsla, S, Wielanek, M, Skłodowska, M & Ionov, M 2022, *Hippophae rhamnoides* L. leaf and twig extracts as rich sources of nutrients and bioactive compounds with antioxidant activity. *Scientific Reports,* 12(1): 1095, https://doi.org/10.1038/s41598-022-05054-9.

Liu, CQ, Xia, X L, Yin, WL, Zhou, JH & Tang, HR 2007, Direct somatic embryogenesis from leaves, cotyledons and hypocotyls of *Hippophae rhamnoides*. *Biologia Plantarum*, 51(4): 635-640, https://doi.org/10.1007/s10535-007-0137-2.

Melnikova, NV, Arkhipov, AA, Zubarev, YA, Novakovskiy, RO, Turba, AA, Pushkova, EN & Dmitriev, AA 2025, Genetic diversity of *Hippophae rhamnoides* varieties with different fruit characteristics based on whole-genome sequencing. *Frontiers in Plant Science*, 16: 1542552, https://doi.org/10.3389/fpls.2025.1542552.

Nybom, H, Ruan, C & Rumpunen, K 2023, The systematics, reproductive biology, biochemistry, and breeding of sea buckthorn—A review. *Genes*, 14(12): 2120, https://doi.org/10.3390/genes14122120.

Shah, SRU, Plaksina, T, Sriskandarajah, S & Lundquist, PO 2015, Shoot organogenesis from roots of seabuckthorn (*Hippophaë rhamnoides* L.): Structure, initiation and effects of phosphorus and auxin. *Trees*, 29(6): 1989-2001, https://doi.org/10.1007/s00468-015-1275-3.

Sriskandarajah, S & Lundquist, PO 2009, High frequency shoot organogenesis and somatic embryogenesis in juvenile and adult tissues of seabuckthorn (*Hippophae rhamnoides* L.). *Plant Cell, Tissue and Organ Culture* (*PCTOC*), 99(3): 259-268, https://doi.org/10.1007/s11240-009-9603-1.

Tian, L, Wu, W, Zhou, X, Zhang, D, Yu, Y, Wang, H & Wang, Q 2019, The ecosystem effects of sand-binding shrub *Hippophae rhamnoides* in alpine semi-arid desert in the northeastern Qinghai–Tibet plateau. *Land*, 8(12): 183, https://doi.org/10.3390/land8120183.

Tomchuk, O, Lepetan, I, Zdyrko, N & Vasa, L 2018, Environmental activities of agricultural enterprises: accounting and analytical support. *Economic Annals-XXI*, 169: 77-83.

Vdovina, TA, Isakova, EA, Lagus, OA & Sumbembayev, AA 2024, Selection assessment of promising forms of natural *Hippophae rhamnoides* (Elaeagnaceae) populations and their offspring in the Kazakhstan Altai Mountains. *Biodiversitas Journal of Biological Diversity*, 25(1): 1-10, https://doi.org/ 10.13057/biodiv/d250101.

Vdovina, T, Lagus, O, Vinokurov, A, Aimenova, Z & Sumbembayev, A 2025, Assessment of biochemical composition of fruits of *Hippophae rhamnoides* (Elaeagnaceae Juss.), *Viburnum opulus* (Viburnaceae Raf.) and *Lonicera caerulea* subsp. *altaica* (Caprifoliaceae Juss.). *Metabolites*, 15(4): 256, https://doi.org/10.3390/metabo15040256.

Wang, BL, Zhao, Y & Han, XY 2023, Effect of different hormone treatments and matrix formulations on rooting of micro-cutting of *Hippophae rhamnoides*. *Acta Horticulturae Sinica*, 50(1): 101-110.

Wang, Z, Zou, J, Shi, Y, Zhang, X, Zhai, B, Guo, D & Luan, F 2024, Extraction techniques, structural features and biological functions of *Hippophae rhamnoides* polysaccharides: A review. *International Journal of Biological Macromolecules*, 263: 130206, https://doi.org/10.1016/j.ijbiomac.2024.130206.

Zubarev, YA, Gunin, AV & Vorobjeva, AV 2022, Rooting green cuttings of Altai seabuckthorn cultivars in industrial-scale experiment. *RUDN Journal of Agronomy and Animal Industries*, 17(2): 131-145, https://doi.org/10.22363/2312-797X-2022-17-2-131-145.

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