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BIOTECHNOLOGICAL PROTOCOLS AND PHYSIOLOGICAL BASES OF *IN VITRO* MICROPROPAGATION OF *BERBERIS* L. SPECIES: A SYSTEMATIC LITERATURE REVIEW

Jamshid Temirov, Nargiza Jurayeva , Shuxratbek Inomjonov

Research Institute of Forestry, Tashkent, Uzbekistan

E-mail: jgtemirov@mail.ru, nargizajorayeva2124@.com

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Abstract: This article presents a systematic review of studies published between 2005 and 2023 on the *in vitro* micropropagation of representatives of the genus ***Berberis L.***, which are widely distributed across different regions of the world and constitute an important source of valuable bioactive alkaloids. The review comprehensively compares species, geographic regions, explant types, the ionic composition of culture media, plant growth regulators and their concentrations, as well as *ex vitro* acclimatization performance. The analysis demonstrated that **Murashige and Skoog (MS)** and **Wood Plant Medium (WPM)** media, together with **6-benzylaminopurine (BA/BAP)** and **indole-3-butyric acid (IBA)**, are the principal components ensuring high regeneration efficiency throughout all stages of micropropagation. Furthermore, current approaches for mitigating phenolic exudation from explants and the physiological mechanisms underlying adventitious root formation are critically synthesized. The findings provide a methodological and theoretical framework for establishing and optimizing *in vitro* propagation protocols and plant tissue culture systems for ***Berberis L.*** species.

Keywords: plant tissue culture, culture medium, plant growth regulators, organogenesis, berberine, phenolic oxidation, acclimatization.

Annotatsiya: Ushbu maqolada dunyo bo‘yicha turli mintaqalarda tarqalgan, qimmatli alkaloidlar manbai hisoblangan zirk (*Berberis L.*) turkumi vakillarini in vitro sharoitida mikroklonal ko‘paytirish bo‘yicha 2005–2023-yillar oralig‘ida chop etilgan ilmiy tadqiqotlar tizimli tahlil qilindi. Tadqiqotda turlar, geografik mintaqalar, eksplant tiplari, oziqa muhitlarining ion tarkibi, fitogormonlar va konsentratsiyalari hamda ex vitro akklimatizatsiya ko‘rsatkichlari kompleks qiyoslandi. Tahlil natijalariga ko‘ra, Murashige-Skoog (MS) va Wood Plant Medium (WPM) muhitlari, 6-benzilaminopurin (BA/BAP) hamda indolil-3-moy kislotasi (IBA) mikroko‘paytirishning barcha bosqichlarida yuqori regeneration faollikni ta’minlovchi asosiy komponentlar ekani tasdiqlandi. Shuningdek, eksplantlarning fenol eksudatsiyasini bartaraf etish usullari va adventiv ildizlanishning fiziologik mexanizmlari ilmiy umumlashtirildi. Maqola zirk turlarini in vitro ko‘paytirish va to‘qima kulturasini yo‘lga qo‘yishda metodik va nazariy ilmiy asos bo‘lib xizmat qiladi.

Kalit so‘zlar: To‘qima kulturasi, oziqa muhiti, fitogormonlar, organogenez, berberin, fenol oksidlanishi, akklimatizatsiya.

Аннотация: В данной статье представлен систематический анализ научных исследований, опубликованных в 2005–2023 годах, посвящённых микро-клональному размножению *in vitro* представителей рода ***Berberis L.***, широко распространённых в различных регионах мира и являющихся ценным источником биологически активных алкалоидов. В работе проведено комплексное сравнительное исследование видов барбариса, географических регионов их

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распространения, типов эксплантов, ионного состава питательных сред, используемых фитогормонов и их концентраций, а также показателей акклиматизации растений в условиях *ex vitro*. Результаты анализа показали, что питательные среды **Murashige–Skoog (MS)** и **Wood Plant Medium (WPM)**, а также **6-бензиламинопурин (BA/BAP)** и **индолил-3-масляная кислота (IBA)** являются ключевыми компонентами, обеспечивающими высокую регенерационную активность на всех этапах микроразмножения. Кроме того, обобщены современные подходы к предотвращению фенольной экссудации эксплантов и рассмотрены физиологические механизмы формирования адвентивных корней. Полученные результаты могут служить методологической и теоретической основой для разработки и совершенствования протоколов *in vitro* размножения представителей рода ***Berberis L.*** и внедрения технологий культуры тканей растений.

Ключевые слова: культура тканей, питательная среда, фитогормоны, органогенез, берберин, фенольное окисление, акклиматизация.

1. Introduction

The genus Berberis L., belonging to the family Berberidaceae, comprises more than 500 species worldwide and represents one of the most valuable groups of medicinal shrubs used in medicine and the pharmaceutical industry [1,2]. Species of Berberis are widely distributed throughout the temperate and subtropical regions of the Northern Hemisphere, including Asia, Europe, North Africa, and South America. In the flora of Uzbekistan, the genus is represented by nine species [3,4].

Berberis species are distinguished by their high content of biologically active isoquinoline alkaloids, including berberine, palmatine, jatrorrhizine, and magnoflorine, as well as numerous antioxidant compounds. Among these metabolites, berberine has attracted considerable pharmacological interest because of its antibacterial, anti-inflammatory, hypoglycemic, hypolipidemic, and anticancer properties [5,6]. Consequently, Berberis species have long been used in traditional medicine for the treatment of liver and biliary disorders, inflammatory skin diseases, diarrhea, and various other ailments [7,8].

Under natural conditions, seed propagation of Berberis species is constrained by deep physiological seed dormancy, resulting in relatively low germination rates that rarely exceed 30–40% [9,10]. Vegetative propagation by stem cuttings is also inefficient because of its slow multiplication rate, seasonal dependence, and limited rooting capacity [11,12]. Furthermore, increasing anthropogenic pressure, habitat degradation, deforestation, and excessive harvesting for medicinal purposes have led to a substantial decline in natural populations of many Berberis species [13,14]. In particular, Berberis aristata and Berberis asiatica are currently recognized among the threatened medicinal plant species of the Indian subcontinent [15,16].

Among the available propagation techniques, in vitro micropropagation has emerged as the most effective biotechnological approach for the year-round production of genetically uniform, disease-free, and high-quality planting material while preserving valuable germplasm resources [17,18]. This technique enables the rapid multiplication of thousands of genetically identical and healthy plantlets from a single explant within a relatively short period.

Over the past two decades, efficient in vitro propagation protocols have been developed for several Berberis species, including B. asiatica [19], B. aristata [20], B. chitria [21], B. vulgaris [22], and B. buxifolia [23]. These studies have evaluated the effectiveness of different culture media and combinations of plant growth regulators for shoot regeneration and plant development. The primary

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objective of the present review is to systematically synthesize existing biotechnological approaches, critically analyze the physiological effects of culture media and plant growth regulators on organogenesis, and propose an optimized micropropagation strategy for Berberis species based on the available scientific evidence.

2. Research methodology

This review is based on scientific publications published between 2005 and 2023, including both international and regional studies conducted in India, Iran, Kazakhstan, and Argentina. The literature was selected according to the following inclusion criteria:

Studies describing in vitro regeneration of Berberis species with detailed information on explant types and culture media composition;

Studies reporting the concentrations of plant growth regulators (cytokinins and auxins) and evaluating their effects on morphogenesis;

Studies providing quantitative data on rooting efficiency and ex vitro acclimatization performance.

3. Results and physiological discussion

3.1. Ionic Composition of Culture Media and Explant Selection

The analysis revealed that Murashige and Skoog (MS) medium was employed in 62.5% of the studies reviewed, making it the most widely used basal medium for in vitro propagation of *Berberis species*. Woody Plant Medium (WPM) and Woody Plant (WP) media, specifically developed for woody plant species, were utilized in 25% of the studies (Table 1).

Table 1. Methodological and geographical characteristics of in vitro micropropagation studies on Berberis species.

No.	Author(s), Year	Species	Region	Basal Culture Medium	Explant Type
1	Bisht A. *et al*., 2022	*B. asiatica*	India	MS	Nodal shoot segments
2	Brijwal L. *et al*., 2015	*B. aristata*	India	WP	Leaf-derived callus
3	Pandey A. *et al*., 2013	*B. chitria*	India	WPM	Apical and axillary buds
4	Tiwari S., Lata C., 2023	*Berberis* spp.	Review	Various media	Meristems and nodal explants
5	Sazmand M. *et al*., 2016	*B. vulgaris*	Iran	MS	Nodal and leaf explants
6	Romadanova N.V. *et al*., 2017	*Berberis* spp.	Kazakhstan	Modified MS	Meristems and axillary buds

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7	Arena M.E. *et al*., 2005	*B. buxifolia*	Argentina	MS	Nodal segments
8	Miriam E. *et al*.	*B. buxifolia*	Argentina	MS	Shoot segments

Physiological interpretation. The high concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) ions in Murashige and Skoog (MS) medium promote rapid protein synthesis and enhance cellular metabolic activity, thereby supporting vigorous shoot proliferation. However, in certain Berberis species, the elevated salt concentration may induce hyperhydricity (vitrification), a physiological disorder characterized by excessive water accumulation in tissues, resulting in translucent, brittle, and morphologically abnormal shoots. Consequently, for woody Berberis species, Woody Plant Medium (WPM), which contains lower total nitrogen and salt concentrations, or half-strength MS (½ MS) medium is generally recommended to improve shoot quality and reduce the incidence of hyperhydricity.

3.2. Plant Growth Regulator Balance and Shoot Proliferation

Plant growth regulators (PGRs) and the balance between cytokinins and auxins represent the principal regulatory factors governing in vitro organogenesis. Their concentrations and interactions directly influence shoot induction, multiplication efficiency, and subsequent morphogenetic development (Table 2).

Table 2. Morphogenetic Roles of Plant Growth Regulators Used in *Berberis In Vitro* Micropropagation

Plant Growth Regulator	Frequency of Use	Proportion	Physiological Mechanism of Action
Cytokinins (BA, BAP, TDZ, Kinetin)	7	87.5%	Suppress apical dominance and stimulate the growth and proliferation of axillary shoots.
Auxins (NAA, 2,4-D)	3	37.5%	Promote cell dedifferentiation and induce callus formation.
Auxins (IBA, IAA)	6	75.0%	Activate cambial tissues at the basal region of shoots, thereby inducing adventitious root formation.
Gibberellins (GA_3)	3	37.5%	Promote cell elongation and increase internode length by enhancing cell wall extensibility.

The results indicate that 6-benzylaminopurine (BA/BAP) was the most frequently used and effective cytokinin, being applied in 87.5% of the studies reviewed. BA/BAP is relatively resistant to enzymatic degradation, allowing it to maintain biological activity within plant tissues for an extended period and thereby promoting sustained shoot proliferation. In contrast, thidiazuron (TDZ) exhibits exceptionally high cytokinin-like activity and is capable of inducing high multiplication rates even at very low concentrations.

3.3. Explant Sterilization and Reduction of Phenolic Oxidation (Browning)

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When wounded, Berberis tissues release phenolic compounds, including tannins and alkaloids, into the culture medium. These compounds are oxidized by the enzyme polyphenol oxidase (PPO), resulting in tissue browning, explant necrosis, and deterioration of the culture medium. Sterilization. The most commonly employed sterilizing agents were 3% sodium hypochlorite (NaOCl) for 15 min and 0.1% mercuric chloride ($HgCl_2$) for 8 min, providing sterilization efficiencies ranging from 46.4% to 85%.

Strategies for controlling phenolic exudation. According to the reviewed studies, supplementation of the culture medium with activated charcoal (0.1–0.5%) and ascorbic acid (50–100 mg L^{-1}), together with maintaining explants in darkness during the first 3–5 days of culture, reduced phenolic exudation by approximately 70–80%, thereby improving explant survival and regeneration.

3.4. Root Induction and Ex Vitro Acclimatization

Root induction represents a critical stage in the successful establishment and subsequent acclimatization of regenerated plantlets under ex vitro conditions (Table 3).

Table 3. Rooting and *Ex Vitro* Acclimatization Efficiency of *Berberis* Species

Species	Rooting Hormone	Rooting (%)	Ex Vitro Acclimatization (%) / Substrate
B. asiatica	IAA (0.05 µM)	70%	65% (Peat : Perlite = 1:1)
B. aristata	IBA	55.6 ± 2.1%	61.1% (Soil : Vermiculite)
B. chitria	IBA (100 µM, pulse treatment)	100%	Successful (Peat)
B. vulgaris	IBA (0.5 mg L^{-1})	High	Successful
B. buxifolia	IBA (1.25 µM)	80%	Successfully acclimatized

Among the auxins evaluated, indole-3-butyric acid (IBA) demonstrated the highest rooting efficiency and was employed in 75% of the studies reviewed. A short-term pulse treatment with a high concentration of IBA (100 µM) resulted in 100% rooting in Berberis chitria. During the ex vitro acclimatization phase, the incomplete functionality of the stomatal apparatus and the underdeveloped cuticular layer increase plantlet susceptibility to water loss. Therefore, the gradual reduction of relative humidity from approximately 90% to 60%, combined with the use of well-aerated substrates such as a peat: perlite (1:1, v/v) mixture, substantially improves plant survival and acclimatization success.

4. Conclusions

The findings of this review demonstrate that the ionic composition of the culture medium, particularly Murashige and Skoog (MS) and Woody Plant Medium (WPM), together with the balance of plant growth regulators, especially 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA), plays a decisive role in the successful in vitro micropropagation of *Berberis L.* species.

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To overcome the problem of phenolic oxidation (browning), which is commonly observed in woody explants, the incorporation of activated charcoal and antioxidant compounds into the culture medium, together with the use of reduced-salt media such as half-strength MS (½ MS), is recommended to improve explant viability and regeneration efficiency.

For root induction, pulse treatment with IBA provides superior rooting performance. At the same time, the use of a peat: perlite (1:1, v/v) substrate during the ex vitro acclimatization stage ensures high plant survival and successful establishment under greenhouse conditions.

Recommendation. The methodological conclusions presented in this review provide a scientific basis for future biotechnological studies aimed at developing efficient in vitro propagation protocols for native and threatened Uzbek Berberis species, particularly Berberis integerrima and Berberis nummularia, as well as for the large-scale production of the pharmaceutically important alkaloid berberine through plant biotechnology.

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