



Microclonal propagation of the species from the collection of the botanical garden: *Ungernia sewerzowii* (Regel) B. Fedtsch (Amaryllidaceae J. St.-Hil.)

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Abstract. The main problem with conserving introduced plants in botanical gardens is that more than 45 % of species exist as isolated specimens, many of which cannot be propagated by seed or cuttings. Microclonal propagation offers a promising solution that allows rapid propagation of rare and valuable plant material.

Ungernia sewerzowii (US) is a medicinal plant rich in alkaloids, particularly lycorine (0.04–0.46 % in leaves, 0.15–0.38 % in bulbs), used for bronchitis treatment. Due to overharvesting, natural populations are at risk, necessitating in vitro propagation as an alternative source of raw material. Callus induction was achieved on Murashige and Skoog (MS) medium with 2,4-D (0.5 mg/l) combined with BAP, Kin, or Zea (0.5 mg/l each). Organogenesis, both direct and indirect, was observed on MS with BAP or Kin (0.5 mg/l) supplemented with IAA or NAA (0.5 mg/l). These protocols enable sustainable production of bioactive compounds while conserving wild populations.

Key words: *Ungernia sewerzowii*, plants, selection medium, nutrient media, biologically active compounds, *in vitro*.

Мікроклональне розмноження виду з колекції ботанічного саду: Унгернія Сєверцова (*Ungernia sewerzowii* (Regel) B. Fedtsch) (Amaryllidaceae J. St.-Hil.)

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Резюме. Основною проблемою збереження інтродукованих рослин у ботанічних садах є те, що понад 45 % видів представлені окремими ізольованими екземплярами, багато з яких неможливо розмножити насінням або живцями. Мікроклональне розмноження пропонує перспективне рішення, що дозволяє швидко розмножувати рідкісний та цінний рослинний матеріал. *Ungernia sewerzowii* (US) – лікарська рослина, багата на алкалоїди, зокрема лікорин (0,04–0,46 % у листках, 0,15–0,38 % у цибулинах), що застосовується для лікування бронхіту. Через надмірний збір природні популяції перебувають під загрозою, що зумовлює необхідність впровадження *in vitro* розмноження як альтернативного джерела сировини. Ініціація калюсу досягнута на середовищі Мурасіге і

Скуга (MS) з 2,4-Д (0,5 мг/л) у поєднанні з ВАР, кінетином або зеатином (по 0,5 мг/л кожен). Органогенез, як прямий, так і непрямий, спостерігався на середовищі MS з ВАР або кінетином (0,5 мг/л), доповненому IAA або NAA (0,5 мг/л). Запропоновані протоколи дозволяють забезпечити сталий синтез біологічно активних сполук та зберегти дикорослі популяції.

Ключові слова: *Ungernia sewertzowii*, рослини, селекційне середовище, поживні середовища, біологічно активні сполуки, *in vitro*.

INTRODUCTION

Founded in 1950, the Tashkent Botanical Garden originally covered 80 hectares and now occupies 66 hectares in the north-east of the city. It houses a large ex situ collection of approximately 6,000 species, forms and varieties of plants. This includes over 2,000 species of trees, shrubs and subshrubs, over 900 species of herbs, over 2,100 ornamental species and over 900 tropical and subtropical species. In recent years, the collection has expanded significantly from approximately 4,500 species in 2013. The garden's 40-hectare arboretum presents dendroflora from East Asia, North America, Central Asia, the Far East, Europe, the Crimea and the Caucasus.

1. Since no country in the world can technically and economically afford to keep the entire existing world assortment of ornamental crops in a preserved or vegetative state, and all this material is very scattered across the botanical gardens of different countries, the problem of concentrating and systematizing data from existing funds and bringing them into publicly accessible formats is acute at the international level. Much attention is paid to the issue of unification of plant documentation systems, which is one of the guarantees of biodiversity conservation and the creation of an international information base of collection funds of botanical institutions of the world [16, 22, 23, 24, 28]. *Ungernia oligostroma* Popov & Vved. ex Popov & Androssov was identified in the territory of the Kyiv Botanical Garden named after Hryshko [25, 26, 27, 28]. The genus *Ungernia* (Amaryllidaceae) includes six species native to Central Asia, four of which grow in Uzbekistan. *Ungernia sewertzowii* (US), endemic to the Western Tien Shan, is found in the Parkent region at elevations of 600–2800 m, thriving on rocky and steppe slopes [13, 18, 19]. US contains biologically active alkaloids, with alkaloid content varying by region. Leaves contain 0.29–0.81 %, bulbs 0.25–1.86 %, and roots 1.44–2.15 %. *Lycorine*, a key compound (0.04–0.46 % in leaves, 0.15–0.38 % in bulbs), is used for bronchitis treatment and exhibits antiviral, antifungal,

and anti-inflammatory properties [11, 12, 20]. Other alkaloids include galanthamine, pancratine, and ungerine. Lycorine hydrochloride, extracted from dried US leaves, is used medicinally and in anticancer research [1, 2]. Leaves are harvested in April, dried within 4–5 days, and used as raw material. Traditionally, baked bulbs treat wounds and boils [7, 9, 10, 21]. Despite the species' ability to reproduce by seeds and bulbs, wild populations face threats due to overharvesting for economic gain. Protocols for lycorine extraction, developed at Uzbekistan's Institute of Plants' Chemical Substances, aim to support pharmaceutical needs while reducing reliance on natural populations.

2. According to the Ukrainian customs legislation, *Ungernia Victor*, *u. Severtseva*; leaves, corms *Ungernia victoris* Vved. Ex Artjuschenko, *u. sewertzowii*, contain substances, and seeds: galantamine, lycorine, pancratin, tazcetin and others (in much smaller quantities), which are included in the list of the necessity of referral for laboratory research, regarding the presence of alkaloids in plant raw materials used in the manufacture of medicines [6, 7, 28].

Therefore, experience in microclonal cultivation is necessary for the implementation of technologies for the preservation of valuable medicinal plants. In Ukraine, technologies for microclonal breeding of *Victor's Ungernia* were first developed both by direct regeneration from fragments of bulb scales and by inducing regeneration from callus tissues that were grown *in vitro* for a long time, as described in the works of V. A. Kunakh, and O. M. Bublyk [5].

MATERIALS

Ungernia sewertzowii (Regel) B. Fedtsch, commonly known as *Sewertzow's ungernia* (US), is a perennial species endemic to the Western Tien Shan. It thrives on rocky and steppe slopes at elevations ranging from 600 to 2800 m (Figure 1).



Figure 1. *Ungernia sewertzowii*. Adult plants. Western Tien Shan, Pskem range, Aksarsay river, vicinity of Nanay village. 6 May 2022. Photo by Mustafina F.U.

US bears 4–6 two-ranked, glaucous leaves that are linear, smooth, and slightly crisped, measuring 1.5–2 cm in width and approximately 20 cm in length. Leaf emergence begins in early February, reaching full development by mid-April, coinciding with peak lycorine accumulation. By late May to early July, the leaves senesce, leaving behind grayish scales on the soil surface.

Flowering occurs from late July to early August. The plant produces an umbel of (5)7–12 brick-red flowers on a single stalk measuring 20–40 cm in height. The flowers possess unequal pedicels (2–8 cm, extending up to 10 cm during fruiting), and the perianth leaflets, which dry to a purple hue, are 20–25 mm long and 4–5 mm wide—approximately three times the length of the floral tube. The fruits are spherical, measuring 2–2.5 cm in diameter, and ripen between September and early October.

Lycorine, the primary bioactive compound, is predominantly concentrated in the leaves and plays a key role in pharmaceutical applications. The seasonal growth cycle of US is closely aligned with its alkaloid biosynthesis, making April the optimal period for harvesting the leaves as raw material for medicinal use.

METHODS

Sterilization of Explant. Various sterilizing agents were tested to develop an optimal protocol, including sodium hypochlorite (4–18 %), hydrogen peroxide (2–15 %), silver nitrate (0.01 %), ethanol (70 %), and commercial disinfectants such as “Belizna” and “Domestos.” In addition, fungicides—difenoconazole, mancozeb, metalaxyl, fludioxonil, and propiconazole—and antibiotics such as streptomycin, amoxicillin, and gentamicin were evaluated. Over 30 sterilization protocols were assessed to minimize fungal contamination [3, 4, 7, 8].

Fungal analysis identified *Penicillium* and *Aspergillus* as the primary contaminants, frequently associated with internal infections. The most effective sterilization method involved treating plant material and seeds with a 0.2 % solution of propiconazole (Agrotilt) and fludioxonil (Maxim). Furthermore, Murashige and Skoog (MS) and Vch nutrient media supplemented with 0.2% streptomycin achieved up to 80% sterility. In contrast, amoxicillin and gentamicin demonstrated lower efficacy. Petri dishes and jars were autoclaved at 2 atm and 126 °C for 20 minutes prior to use.

The optimized sterilization protocol—combining propiconazole, fludioxonil, and streptomycin—significantly reduced fungal contamination, with streptomycin-supplemented media reaching up to 80 % sterility.

Explant Sources. Two types of explants were used for in vitro propagation:

1. **Bulb Scales and Bases.** Bulbs were cleaned, washed, and separated into individual scales. The inner scales underwent sterilization, including ethanol (70 %), sodium hypochlorite (4 %), and hydrogen peroxide (4 %) treatments. The sterilized 5 × 5 mm scale segments were cultured on a solid nutrient medium

supplemented with sucrose (30 g/L), agar (7 g/L), streptomycin (0.2%), and phytohormones.

2. **Seeds.** Freshly collected seeds were stratified at –10°C for 30 days, then sterilized using ethanol (70%), sodium hypochlorite (4 %), and sterilizing soap. They were initially cultured on 25 % MS medium with sucrose (7.5 g/l) and agar (7 g/l) at +5 °C for 1–4 weeks, then transferred to 50% MS medium with sucrose (15 g/l) at +24 ± 2 °C under a 16/8 photoperiod. Germinated cotyledon, hypocotyl, and radicle segments were used as explants.

Nutrient Media. The ready-made nutrient media from Duchefa Biochemie B.V (<https://www.duchefa-biochemie.com>) according to Murashige and Skoog (1962) (MS), Chu et al. (1975) (N6), Gamborg et al. (1968), and McCown Woody Plant Medium (Lloyd G. and McCown, 1980) (WPM) were used. The nutrient medium by Vollosovich (1979) (Vch) was prepared with reagents and biochemicals from Duchefa Biochemie B.V (<https://www.duchefa-biochemie.com>). Streptomycin (0.2 % v/v) was added to the nutrient media only during the initial stages of in vitro cultivation when bulb scales were used as explants and in 25 % MS media for seed stratification. Once callus formation and plant regeneration were established, antibiotics were no longer included, as the cultures remained contamination-free.

To optimize the nutrient composition, sucrose concentrations ranging from 10 % to 35 % were tested, with the best results observed at 30 %. The final nutrient medium contained 7 g/L agar and 30 g/L sucrose. At the initial stages, streptomycin (0.2% v/v) was incorporated as necessary. All media were autoclaved at 2 atm. and 126°C for 20 minutes before use. Petri dishes, also autoclaved under the same conditions, were filled with 25 mL of the prepared nutrient medium.

Phytohormones and Compositions. Stock solutions of auxins and cytokinins were prepared at a concentration of 1 mg/ml in a volume of 10 mL. The auxins 2,4-dichlorophenoxyacetic acid (2,4D), indole-3-acetic acid (IAA), α -naphthyl acetic acid (NAA), and indole-3-butyric acid (IBA) were dissolved in 10 ml of 70% ethyl alcohol. Cytokinins 6-benzylaminopurine (BAP) and kinetin (Kin) were dissolved in a small amount of 1 N alkali or acid solution and then diluted with distilled water up to 10 ml.

RESULT AND DISCUSSION

No structural changes were observed in explants cultured on N₆, B5, and WPM media [8, 14, 15]. Nutrient media MS, N₆, B5, WPM, and Vch were tested using bulb scales of *U. sewerzowii* as explants. Callusogenesis, indirect organogenesis, and direct/indirect somatic embryogenesis were induced on MS and Vch media using up to 183 phytohormone combinations; however, none of these processes resulted in the development of mature plants. Callus formation was observed in bulb scale segments of *U. sewerzowii* on MS media, while somatic embryos were identified on Vch media [14, 15, 17].

This study revealed that seeds of *Ungernia* species require cold stratification for successful germination. Stratification was essential to break dormancy and promote seed germination in *U. sewertzowii*. Germinated seed segments, including the hypocotyl, cotyledon, and radicle, were used as explants for in vitro propagation.

Almost 90% of the explants exhibited structural changes on MS media with 30% sucrose. In contrast, no changes were observed on N₆, B5, and WPM media. Sucrose concentration played a crucial role: lower concentrations did not induce callus formation, whereas higher concentrations led to abnormal callus development in *U. sewertzowii*.

In vitro propagation of *U. sewertzowii* was successfully achieved through two approaches: direct and indirect organogenesis.

Callusogenesis. Callusogenesis in *U. sewertzowii* was induced on MS media with M5 (2.4D 0.5 mg/l + BAP 0.5 mg/l), M56 (2.4D 0.5 mg/l + Kin 0.5 mg/l), and M87 (2.4D 0.5 mg/l + Zea 0.5 mg/l) (Figure 2). The first subculture used the same media, while the second subculture for proliferation was on M56. Intensive callus formation (95 ± 2 % of explants) occurred on the basal hypocotyl and cotyledon, initially bright yellow, later turning green. After the first and second subcultures, bud development was observed, leading to microbulb formation within 1–2 weeks.

Direct Organogenesis. Direct organogenesis in *U. sewertzowii* was induced on MS media with M17 (IAA 0.5 mg/l + BAP 0.5 mg/l), M32 (NAA 0.5 mg/l + BAP 0.5 mg/l), M44 (NAA 0.5 mg/l + Kin 0.5 mg/l), and M68 (IAA 0.5 mg/l + Kin 0.5 mg/l). Explants (cotyledon, hypocotyl, and radicle) placed on these media developed directly into microbulbs without callus formation, whereas callus-derived explants underwent indirect organogenesis.

Subculturing followed the same media for the first stage, while the second subculture used a 5:1 auxin-to-cytokinin ratio (M17, M32, M44, M68 with IAA/NAA

2.5 mg/l + cytokinin 0.5 mg/l). Root development was stimulated in the third subculture on 50 % MS with NAA 2.5 mg/l + BAP 0.5 mg/l + TDZ 0.3 mg/l, followed by additional subcultures on 50 % MS with NAA 0.5 mg/l for soil adaptation.

Direct organogenesis produced 3–5 bulbs per explant, while indirect organogenesis yielded 100–150 well-developed plants ready for soil adaptation within 6 months. The response to phytohormones was consistent across both methods. The full cycle from initiation to soil adaptation took 5–6 months.

Among the tested combinations, M5 (2.4D 0.5 mg/l + BAP 0.5 mg/l), M56 (2.4D 0.5 mg/l + Kin 0.5 mg/l), and M87 (2.4D 0.5 mg/l + Zea 0.5 mg/l) were most effective for callusogenesis. M17, M32, and M68 promoted bud development, while M44 induced microbulb formation but in low numbers.

Indirect Organogenesis. Transferring callus to 100 % MS media with M17 (IAA 0.5 mg/l + BAP 0.5 mg/l), M32 (NAA 0.5 mg/l + BAP 0.5 mg/l), M44 (NAA 0.5 mg/l + Kin 0.5 mg/l), and M68 (IAA 0.5 mg/l + Kin 0.5 mg/l) induced microbulb formation. Direct organogenesis from cotyledon, hypocotyl, and radicle was observed but yielded fewer bulbs than indirect organogenesis.

The first subculture used the same media, while the second subculture applied a 5:1 auxin-to-cytokinin ratio (M17, M32, M68 with IAA/NAA 2.5 mg/l + cytokinin 0.5 mg/l). Root development was promoted on 100 % MS with M44 (NAA 2.5 mg/l + Kin 0.5 mg/l) and later on 50 % MS with IAA 2.5 mg/l + BAP 0.5 mg/l + TDZ 0.3 mg/l. Final subcultures on 50 % MS with NAA 0.5 mg/l prepared plants for soil adaptation.

Indirect organogenesis produced up to 100–150 plants per explant, while direct organogenesis resulted in 3–5 bulbs. Both methods showed similar morphogenic responses. Well-developed plants were successfully transferred to soil, completing the process in six months.

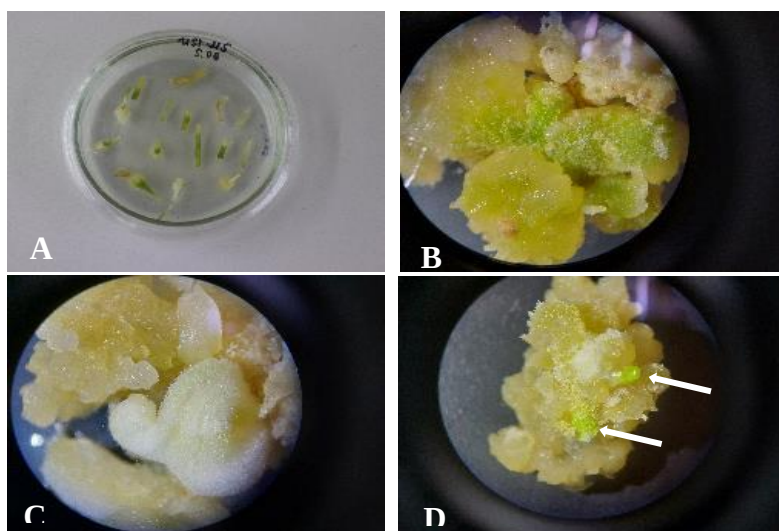


Figure 2. *Ungernia sewertzowii*. Callusogenesis on nutrient media by Murashige and Skoog (1962) with phytohormones M5 2.4D 0.5 mg/l + BAP 0.5 mg/l (A). Three weeks after placing the explants on nutrient media (B, C). Callus after first subculture with green spots—rudiments of buds (D). Callus after second subcultures with green buds (showed with arrows)

CONCLUSIONS

The *in vitro* regeneration of *U. sewerzowii* offers a promising strategy for both the conservation of its natural populations and the sustainable production of biologically active compounds. Callus cultures and regenerated plants provide alternative sources for the extraction of valuable secondary metabolites, thereby reducing dependence on wild populations. The use of elicitors, controlled light conditions, and exogenous phytohormones has been shown to enhance the biosynthesis of target compounds, presenting an effective approach for optimizing their yield.

The established *in vitro* propagation protocols offer a scalable and controlled platform for the mass production of *U. sewerzowii*, with potential applications in the pharmaceutical, agricultural, and biotechnological industries. By substituting raw materials traditionally sourced from natural habitats, these protocols support biodiversity conservation and promote the sustainable utilization of plant resources.

Future research should aim to refine cultivation conditions to maximize secondary metabolite production,

evaluate the genetic stability of *in vitro* regenerated plants, and develop effective acclimatization strategies for reintroduction into natural or semi-natural environments. Additionally, further investigation into the pharmacological properties of bioactive compounds derived from *U. sewerzowii* will broaden its potential applications in medicine and biotechnology.

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