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Protocol for medium-term *in vitro* conservation of *Humulus lupulus* using encapsulation technology

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Summary. Growing demand for hops with new organoleptic characteristics and improved agronomic performance provides a powerful impetus for the breeding of new varieties, which determines the need to create and maintain gene banks of the crop. Field gene banks are at great risk due to the threat of biotic and abiotic stress. The problem of preserving valuable germplasm can be solved by duplicating the gene pool in *in vitro* collections, one of the management tasks of which is to find ways to slow down the growth of crops. A protocol for the medium-term storage of *Humulus lupulus* has been developed, including all stages of propagule encapsulation technology: from the preparation of initial explants to their subsequent rehabilitation and restoration of regenerative capacity after deposition. The protocol has been successfully tested on four genotypes of varietal hops. For the first time, conditions for encapsulation of microcuttings have been selected that ensure their viability of at least 80 % after *in vitro* conservation at 4 °C. Treatment of the material with 3 % sodium alginate during the encapsulation stage, combined with 3 % calcium chloride during the polymerization stage, ensured high biocapsule survival. The optimal exposure time in the polymerizing solution did not exceed 10–15 minutes. It has been shown for the first time that 1 mg L^{-1} of abscisic acid in the artificial endosperm (AE) of biocapsules reduces the impact of cold stress during medium-term low-temperature storage of the material. Supplementing the Murashige-Skoog medium, which forms the basis of AE, with 2 mg L^{-1} 6-Benzylaminopurine (BAP) and 1 mg L^{-1} Gibberellic acid (GA3) allows explants to form up to 3–4 internodes when cultivated immediately after deposition. Subsequent subculturing of hop microcuttings on MS nutrient medium containing 0.5 mg L^{-1} of kinetin promotes the restoration of photosynthetic activity in the regenerated plants. This approach ensures high regenerative and root-forming potential of emerging shoots over four successive cloning generations. The multiplication rate reaches 8–9 microcuttings per explant.

Протокол среднесрочного сохранения *in vitro Humulus lupulus* с использованием технологии инкапсуляции

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Аннотация. Растущий спрос на хмель с новыми органолептическими характеристиками и улучшенными агрономическими показателями даёт мощный импульс для селекции новых сортов, что определяет необходимость создания и поддержания генетических банков культуры. Полевые генные банки подвергаются большому риску из-за угрозы биотических и абиотических стрессов. Решение проблемы сохранности ценной зародышевой плазмы возможно путём дублирования генофонда в *in vitro* коллекциях, одной из задач управления которыми является поиск способов замедления роста культур. Разработан протокол среднесрочного хранения *Humulus lupulus*, включающий все этапы технологии инкапсуляции пропагул: от подготовки исходных эксплантов и их хранения до последующего восстановления регенерационной способности после депонирования. Протокол успешно апробирован на четырёх генотипах сортового хмеля. Подобраны условия инкапсуляции микрочеренков, обеспечивающие их жизнеспособность не ниже 80 % после *in vitro* консервации при температуре 4 °C. Обработка материала 3 % альгинатом натрия на этапе инкапсуляции в сочетании с 3 % хлоридом кальция на этапе полимеризации обеспечила высокую сохранность биокапсул. Оптимальное время экспозиции в полимеризующем растворе не превышало 10–15 минут. Впервые показано, что 1 мг/л абсцизовой кислоты в составе искусственного эндосперма (ИЭ) биокапсул снижает влияние холодового стресса в процессе среднесрочного низкотемпературного хранения материала. Обогащение питательной среды Мурасиге-Скуга, составляющей основу ИЭ, 2 мг/л БАП и 1 мг/л ГК3 позволяет эксплантам формировать до 3–4 междоузлий при культивировании непосредственно после депонирования. Последующее субкультивирование микрочеренков хмеля на питательной среде МС, содержащей 0,5 мг/ л кинетина, способствует восстановлению фотосинтетической деятельности регенерантов. Такой подход обеспечивает высокий регенерационный и корнеобразующий потенциал формирующихся побегов в течение 4 последовательных поколений клонирования. Коэффициент размножения достигает 8–9 микрочеренков / эксплант.

Introduction

The conservation and sustainable use of the genetic diversity of cultivated plant species and their wild relatives is the foundation of food security for any country and determines its sustainable development (Khlestkina, Chukhina, 2020; Dunaeva et al., 2022). To conserve plant genetic resources (PGR), two basic strategies are used: *in situ* (in the natural habitat) and *ex situ* (outside the original habitat) (Gavrilenko et al., 2007; Dunaeva et al., 2012). *Ex situ* conservation of PGR is carried out under controlled environmental conditions in collections and plant gene banks. The integrated use of different types of collections (field, *in vitro*, cryo-, seed collections) increases the reliability of PGR conservation (Bamberg et al., 2016; Panis et al., 2020; Oves et al., 2022). The most common methods of *ex situ* storage of *Humulus lupulus* L. (Cannabaceae), an indispensable source of raw material for brewing, are field gene banks (Nesvadba et al., 2020; Osipova et al., 2022). However, valuable genotypes are at great risk in the field due to environmental stresses, pests, phytopathogens, viruses, and viroids (Postman et al., 2005; Pethybridge et al., 2008, 2009; Danilova et al., 2013; Gargani et al., 2017; Sastry et al., 2019; Davis, Gomez, 2021; Khlebova et al., 2024).

Preservation of hop collections in tissue culture is an alternative approach to depositing large volumes of material. A promising method for long-term preservation of hops *in vitro* is cryoconservation. However, the implementation of this method requires expensive equipment, complex protocols and often leads to tissue degeneration during the freezing / thawing process. To date, only a few examples of successful cryoconservation of this crop are known (Reed et al., 2003; Reed, 2005; Faltus et al., 2011; Jenderek, Reed, 2017; Malhotra et al., 2024).

Short-term storage of microplants *in vitro* under optimal growth conditions requires frequent subculturing of explants in fresh nutrient medium. As a result, the cost of storing samples and the risk of their infection by various microorganisms increase. Frequent use of phytohormones stimulates active cell division, which can lead to the induction of somaclonal variants (Bairu et al., 2011). To extend the interval between passages, various methods and techniques based on slowing down the growth of regenerants are used: osmotic stress, low temperatures, reduced levels of nutrients in the medium, etc. (Mitrofanova et al., 2018; Alzubi et al., 2019).

An innovative method for the medium-term *in vitro* deposit of valuable germplasm is the use of artificial, or synthetic, seeds. Synseeds (biocapsules) are micropropagules encapsulated in an alginate matrix that have the ability to regenerate into a whole plant *in vitro* (Magray et al., 2017; Rihan et al., 2017). Encapsulation technology can combine clonal micropropagation and gene pool storage. The use of unipolar propagules (microcuttings, root segments, shoot apices) for deposition makes it possible to overcome or significantly to reduce the possibility of somaclonal variability, ensuring the homogeneity of the genetic material. This technology is most developed for orchids, hybrid agricultural crops with high seed production costs, and some ornamental, food, and industrial crops with low seed and vegetative propagation rates (Micheli et al., 2019; Manokari et al., 2021; Khoddamzadeh, Dunn, 2022). In our country, in recent years, this technology has begun to be applied to potatoes and some medicinal plants (Asanakunov, 2011; Oves et al., 2022). *In vitro* cloning of hops is widely used in various laboratories around the world (Mafakheri, Hamidoghli, 2019; Hirakawa, Tanno, 2022; Mironenko et al., 2024). However, there is no information on the successful use of encapsulated hop explants for medium-term storage. There are only a few reports of attempts to produce hop biocapsules and evaluate their viability immediately after production (Liberatore et al., 2020) or after three weeks of storage (Di Sario et al., 2025), which cannot be considered medium-term preservation. Due to the dependence of the success of the method on the plant genotype, the selection of conditions for encapsulation and subsequent storage of biocapsules, as well as an assessment of the morphogenetic potential of encapsulated explants for each individual species, is relevant (Kikowska et al., 2020; Liberatore et al., 2020; Shilpha et al., 2021).

The aim of the study was to evaluate the potential of using artificial seeds for *in vitro* storage of valuable hop germplasm and to develop a protocol for medium-term conservation of the crop using explant encapsulation.

Materials

Plant material

Aseptic regenerants of *Humulus lupulus* cultivars ‘Magnum’, ‘Flagman’, ‘Bryanskij’ and ‘Taurus’ cultivated in an *in vitro* collection for 6–8 months, according to the standard clonal micropropagation protocol developed at the Altai Center for Applied Biotechnology of the Altai State University (Russia) (Mironenko et al., 2024). For introduction into *in vitro* culture, samples obtained from the field collection of the Chuvash Scientific Research Institute of Agriculture, a branch of the Federal Agricultural Research Center of the North-East named N.V. Rudnitsky, Russia, Chuvashia Republic (cultivar ‘Flagman’) and “Magnum LLC”, Russia, Altai Republic (cultivars ‘Magnum’, ‘Bryanskij’ and ‘Taurus’) were used.

Reagents

1. Major elements: ammonium nitrate, calcium chloride, magnesium sulfate, potassium hydroorthophosphate, potassium nitrate (Russia).
2. Minor elements: Boric acid, Cobalt chloride, Copper (II) Sulfate, Manganese (II) sulfate, Potassium iodide, Sodium Molybdate, Zinc Sulfate (India).
3. Iron Chelate: Ethylenediaminetetraacetic acid (India), Iron (II) sulfate (Russia).
4. Organic elements: mesoinositol, Nicotinic acid, Pyridoxine HCL, Thiamin HCL (India), Glucose (Russia).
5. Plant growth regulators: 6-Benzylaminopurine (BAP), Gibberellic acid (GA3), Indole-3-butyric acid (IBA), Abscisic acid (ABA), Kinetin (N6-furfuryladenine) (India).
6. Agar-agar 900 (Italy).
7. Sodium alginate (organic sodium salt of alginic acid $(C_6H_7O_6Na)_n$, medium viscosity) (Russia).

Equipment

1. Laminar flow box BAVnp-01-“Laminar-C”-1.2 (Lamsystems, Russia).
2. Autoclave MLS-3020U (Panasonic, Japan).
3. Refrigerating chambers Liebherr FKvsl 4113 (LIEBHERR, Germany).
4. pH meter Econix Export (Econix, Russia).
5. Magnetic stirrer Heidolph RZR 2020 (Heidolph, Germany).
6. Water distillation unit GFL-30938 (GFL, Germany).
7. Dispensers Ekros PE 0.25-2.5 HF (EkrosChim, Russia).
8. Analytical scales AX224/E (Ohaus, USA).
9. Culture vessels – glass tubes (size 200 × 21 mm), medium volume is 10 ml.
10. Disposable plastic Petri dishes, sterile (diameter 90 mm).

Procedures

Nutrient media and cultivation conditions. Nutrient media were prepared and sterilized according to standard procedures. Murashige-Skoog medium (MS) supplemented with 20 $g \cdot L^{-1}$ glucose was used as the base one. *In vitro* cultivation of hop mother plants (donors of microcuttings for encapsulation), micropropagation, and rooting of shoots in Passages II–V after deposition were performed on MS medium supplemented with 0.5 $mg \cdot L^{-1}$ IBA and 7.3 $g \cdot L^{-1}$ agar-agar (MS1). For encapsulation of microcuttings, artificial endosperm (AE) of the following composition was used: MS + 2 $mg \cdot L^{-1}$ BAP + 1 $mg \cdot L^{-1}$ GA3 + 1 $mg \cdot L^{-1}$ ABA + 3 % sodium alginate (MS2). The regrowth of encapsulated microcuttings after cold storage (Passage 0) was carried out on MS medium supplemented with 2 $mg \cdot L^{-1}$ BAP, 1 $mg \cdot L^{-1}$ GA3 and 7.3 $g \cdot L^{-1}$ agar-agar

(MS3). Micropropagation during Passage I was performed on MS medium supplemented 0.5 mg·L^{-1} kinetin and 7.3 g·L^{1} agar-agar (MS4). The nutrient media were sterilized by autoclaving at 2.0 atm and 121 °C for 20 minutes. The pH of the nutrient media was adjusted to 5.8–5.9 before autoclaving.

Cultivation of the initial hop regenerants, encapsulated explants after deposition and subsequent micropropagation were carried out in a culture room at a temperature of 21–23 °C, a photoperiod of 16 / 8 hours (day / night), a light intensity of at least 2500 lux, and a relative humidity of 55–60 %.

Preparation of explants for conservation.

1. Hop microcuttings 3–4 mm long, containing dormant axillary buds at the nodes, were separated from *in vitro* proliferated shoots.

2. The explants were encapsulated in AE, polymerized in calcium chloride solution and placed in a refrigerator at a temperature of 4 ± 1 °C for cold storage for 12 months.

All stages of preparation and encapsulation of explants, as well as micropropagation of shoots, were carried out in a laminar flow hood under aseptic conditions.

Morphometric and reproductive parameters of encapsulated microcuttings (EM) after 12 months of storage under cold conditions. Variability was defined as the number of green (non-necrotic) microcuttings to the total number of EM × 100 %.

Regrowth was defined as the number of microcuttings at least 4–5 mm long to the total number of EM × 100 %.

Reproduction rate is the number of proliferating microcuttings obtained from one shoot of the previous passage.

Shoot length, mm.

Statistical data analysis. The method was performed in three biological replicates, with 50 explants in each replicate. Microsoft Excel 2010 was used for statistical data analysis. The mean and standard error of the mean were calculated for each parameter. The significance of differences was assessed using a two-way analysis of variance (ANOVA). To compare statistically significant differences between the mean values of the variants, the least significant difference (LSD) test at $P < 0.05$ was used.

Method implementation

Selection of explants for encapsulation

Hop mother plants – the donors of explants for encapsulation – were cultured *in vitro* in test tubes containing 10 ml of MS1 nutrient medium. Regenerants in the active growth stage, which had formed at least 5–6 stem nodes and had showed no signs of chlorosis or leaf yellowing, were selected for storage (Fig. 1a). Microcuttings were prepared by dividing the shoot into segments with axillary buds at the nodes and separating the leaves.

Production of hop biocapsules

To obtain biocapsules, a three-step procedure was carried out.

1. Encapsulation was achieved by briefly (5–10 s) immersing the microcuttings in an aseptic encapsulating solution (MS2) – artificial endosperm supplemented with 3 % sodium alginate.

2. For polymerization, microcuttings coated with sodium alginate were added dropwise into an aseptic 3 % calcium chloride solution using an automatic pipette with a tip diameter of about 3 mm. Polymerization was carried out for 10–15 minutes with gentle stirring of the polymerization solution (Fig. 1b).

3. The calcium chloride solution was removed using a sterile filter. The biocapsules were then rinsed in sterile distilled water (5 min × 3) and lightly blotted on sterile filter paper (Fig. 1c).

Cold storage conditions

1. Hop biocapsules were placed in disposable plastic Petri dishes (90 mm in diameter) on filter paper (2 layers) moistened with sterile distilled water (20 ml). The Petri dishes were secured with parafilm and labeled with the genotype and original storage date (Fig. 1d). The biocapsules were stored in a refrigerator at 4 ± 1 °C in the dark for 12 months.

2. Every 3 months, the moisture level was checked, and sterile distilled water was added if the filter paper dried out.

3. After 4, 8 and 24 months of deposition, the morphometric and reproductive parameters of the explants were assessed. Biocapsules containing AE without ABA were used as a control.

In vitro regrowth after storage

1. After cold storage, hop biocapsules were placed in test tubes on MS3 nutrient medium only slightly deepening into the substrate, to avoid asphyxiation of living tissue (Fig. 2a). The tubes were transferred to the cultivation room and incubated for 6 weeks (Passage 0).

2. After 10–35 days, axillary bud activation (Fig. 2b), proliferation (Fig. 2c, d), and the formation of shoots with 2–4 pairs of leaves occurred (Fig. 2e, f). Shoots formed in Passage 0 were divided into microcuttings with 2 stem nodes and subcultured vertically in test tubes on MS4 nutrient medium for 4 weeks (Passage I). The microcuttings were at least 10 mm in size.

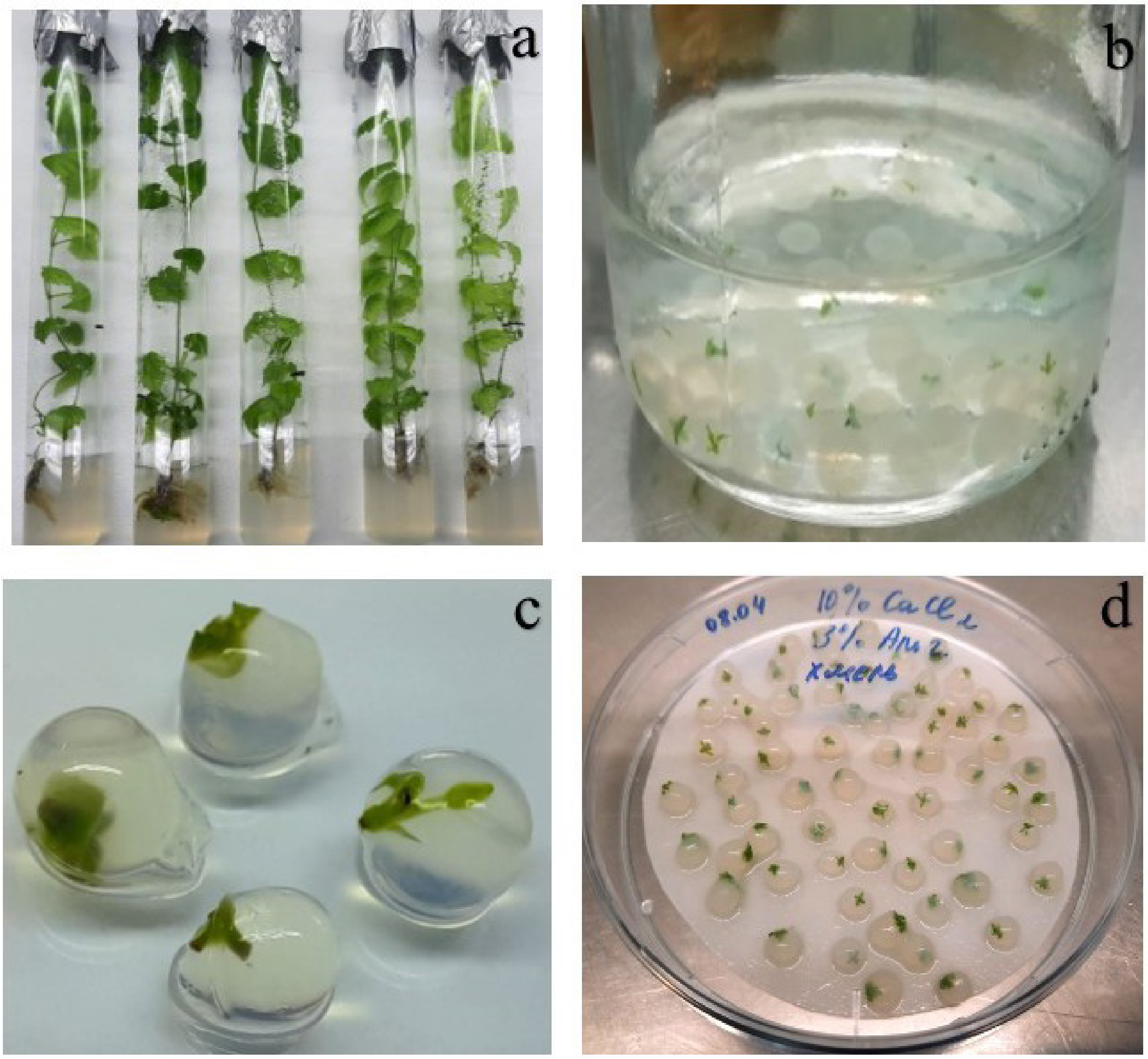

Fig. 1. Stages of encapsulation of *Humulus lupulus* explants for medium-term deposition of *in vitro* collections: a – regenerants – donors of microcuttings for encapsulation; b – formation of a polymeric shell of a biocapsule in a polymerizing solution of calcium chloride; c – biocapsules with encapsulated microcuttings; d – biocapsules in Petri dishes on filter paper, prepared for cold storage.

Micropropagation and in vitro rooting

The plants regenerated in Passage I were then cloned sequentially by microcutting during Passages II–V on MS1 nutrient medium. Thus, within 3–4 subcultures of explants, the *in vitro* hop collection was restored after conservation of microcuttings in biocapsules under cold conditions.

Features of the technology of encapsulation, cold storage and in vitro regrowth of H. lupulus

1. Optimization of encapsulation conditions was performed after 6 months of incubation of biocapsules with microcuttings under cold conditions. The regenerative capacity of explants on MS3 medium under standard cultivation conditions depended on the concentration of the encapsulating and polymerizing solutions, as well as the exposure time. The maximum result was obtained with a combination of 3 % sodium alginate and 3 % calcium chloride (Table 1). Polymerization for 10–15 minutes ensured 100 % preservation of the ‘Magnum’ cultivar propagules. Increasing the exposure period to 25 min resulted in a decrease in explant viability by 19.0–27.0 %, and to 35 min – by 49.0–79.3 %. After a short immersion of the forming biocapsules in the polymerizing solution for 5 min, the regeneration success rate was less than 50 %. Thus, subsequent manipulations to obtain artificial seeds were carried out using optimized time intervals and concentrations of encapsulating and polymerizing solutions.

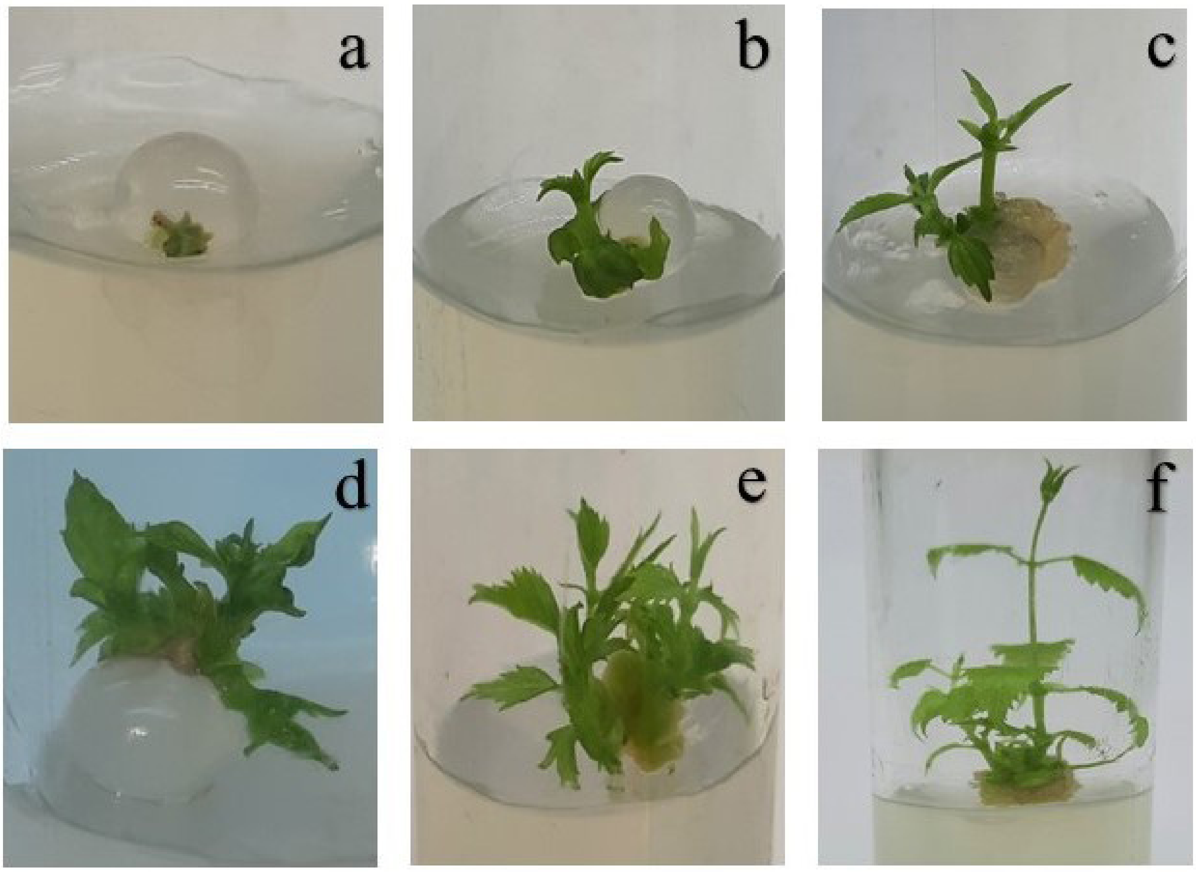

Fig. 2. Development of axillary buds of encapsulated explants of *Humulus lupulus* on MS nutrient medium after deposition for 12 months at a temperature of 4 °C (Passage 0): a – 3-day-old explant in the dormant stage; b – 10-day-old explant in the activation stage; c, d – 20-day-old explants in the initial proliferation stage; e, f – 25-35-day-old explants forming shoots.

Table 1. The effect of calcium chloride concentration at the stage of biocapsule polymerization on the viability of *Humulus lupulus* (‘Magnum’ cultivar) explants, %

Exposure time, min	Calcium chloride concentration, %					
	1.0	1.5	3.0	5.0	10.0	The mean
5	33.7 ± 1.4	27.7 ± 1.9	48.3 ± 2.7	62.0 ± 2.2	71.3 ± 2.9	48.6
10	35.7 ± 1.8	43.0 ± 2.6	100.0	91.7 ± 3.9	75.7 ± 2.2	69.2
15	46.7 ± 2.2	52.0 ± 1.6	100.0	73.0 ± 2.6	48.0 ± 1.6	63.9
20	49.0 ± 1.6	47.0 ± 1.8	81.0 ± 2.4	20.0 ± 1,0	46.0 ± 1.0	48.6
25	62.0 ± 2.3	49.0 ± 1.8	73.0 ± 1.9	8.7 ± 0.7	32.0 ± 2.2	44.9
30	67.0 ± 1.9	62.7 ± 1.9	51.0 ± 2.2	10.0 ± 1.1	4.7 ± 0.5	39.1
35	70.0 ± 2.6	68.0 ± 1.2	20.7 ± 1.2	12.0 ± 0.6	8.7 ± 0.7	35.9
The mean	52.0	49.9	67.7	39.6	40.9	
$LSD_{0,05}$	7,0					

Note: $LSD_{0.5}$ for exposure factor – 3.1; $LSD_{0.5}$ for concentration factor – 2.5.

2. Addition of 1 mg L^{-1} ABA in combination with 2 mg·L^{-1} BAP and 1 mg·L^{-1} GK3 to the artificial endosperm (MS2) of biocapsules reduced the negative impact of cold stress on explants during medium-term storage. Viability and post-storage regrowth of ‘Magnum’ microcuttings transferred to MS3 nutrient medium reached 85 % and 80 %, respectively, exceeding the control by almost three times (Table 2). Shoots grew to 3.8 cm, forming 1–3 pairs of leaves, which ensured the maximum reproduction rate in Passage 0 (Fig. 3a, b). Higher ABA concentrations inhibited the growth and development of microshoots (Fig. 3c, d).

3. Activation of axillary buds in biocapsules transferred to a nutrient medium after cold storage (Passage 0) was uneven. Shoot length varied between 0.2 and 6 cm (Fig. 4 a, b). Individual microcuttings initiated the formation of 5–6 adventitious buds, from which very small shoots developed (Fig. 4c), making their subsequent use in microcloning difficult.

4. During shoot regrowth in Passage 0, chlorophyll loss occurred, leading to yellowing of the leaves (Fig. 5a). These shoots were divided into microcuttings and transferred to test tubes on a nutrient medium containing cytokinins. The concentration of cytokinins determined the efficiency of reproduction in Passage I (Table 3). The addition of 0.5–1.0 mg·L^{-1} kinetin promoted the restoration of photosynthetic activity. Regenerants formed 1.4–2.8 internodes with bright green leaves (Fig. 5c, d). The effect of BAP at this stage of propagation was less effective (Fig. 5 b), resulting in a lower reproduction rate.

5. The efficiency of *in vitro* hop propagation after 12 months of conservation of encapsulated microcuttings at 4 °C was determined by genotype and the number of passages. Restoration of reproductive capacity in regenerated plants of all studied cultivars occurred after four consecutive subcultivations (Table 4). The reproduction rate in Passage IV was 8.0–9.1 and remained at this level in the next

Table 2. Characteristics of encapsulated microshoots of *Humulus lupulus* (‘Magnum’ cultivar) after 12 months of storage under cold conditions (MS3, Passage 0) depending on the concentration of ABA in the AE of biocapsules

ABA, mg L^{-1}	Parameter			
	Viability, %	Regrowth, %	Shoot length, cm	Reproduction rate
0 (control)	30.4 ± 3.4	24.0 ± 2.4	1.2 ± 0.2	1.0 ± 0.3
1	85.0 ± 5.1	80.0 ± 5.5	3.8 ± 0.5	1.4 ± 0.3
2	78.1 ± 6.0	48.2 ± 3.2	1.0 ± 0.2	0.5 ± 0.1
3	74.6 ± 4.7	18.6 ± 2.7	0.5 ± 0.1	0.5 ± 0.1
4	75.1 ± 4.1	20.1 ± 3.2	0.5 ± 0.1	0.2 ± 0.1
5	62.3 ± 3.9	13.3 ± 1.9	0.4 ± 0.1	0.2 ± 0.1
$LSD_{0.05}$	16.1	14.7	0.8	0.3

Table 3. *In vitro* reproduction rate of *Humulus lupulus* after deposition of encapsulated microcuttings for 12 months under cold conditions (Passage 1) depending on the concentration of cytokinins in the MS nutrient medium

Cultivar	Kinetin, mg L^{-1}			BAP, mg L^{-1}		
	0.5	1.0	2.0	0.5	1.0	2.0
‘Magnum’	1.6 ± 0.2	1.4 ± 0.1	1.0 ± 0.2	0.6 ± 0.1	0.4 ± 0.1	1.0 ± 0.2
‘Flagman’	2.8 ± 0.1	2.5 ± 0.3	2.0 ± 0.3	2.2 ± 0.4	1.9 ± 0.1	2.4 ± 0.4
$LSD_{0.05}$	0.4	1.0	0.5	1.2	1.4	1.2

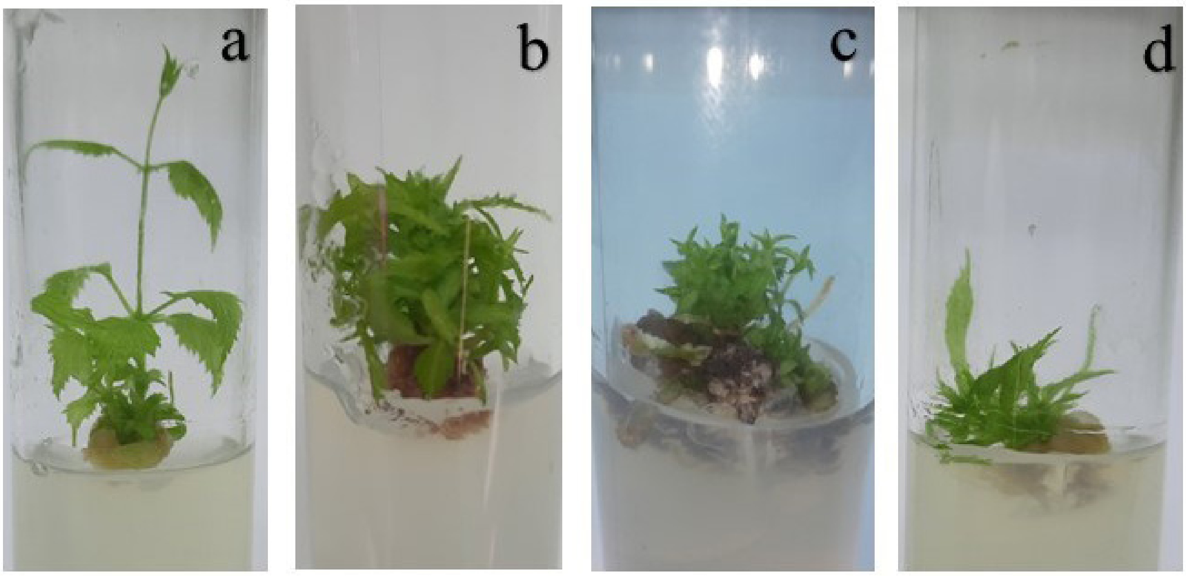

Fig. 3. The effect of ABA contained in biocapsules on the post-storage regrowth of encapsulated *Humulus lupulus* microcuttings (Passage 0): a, b – 1 mg L^{-1} ABA; c – 2 mg L^{-1} ABA; d – 3 mg L^{-1} ABA.

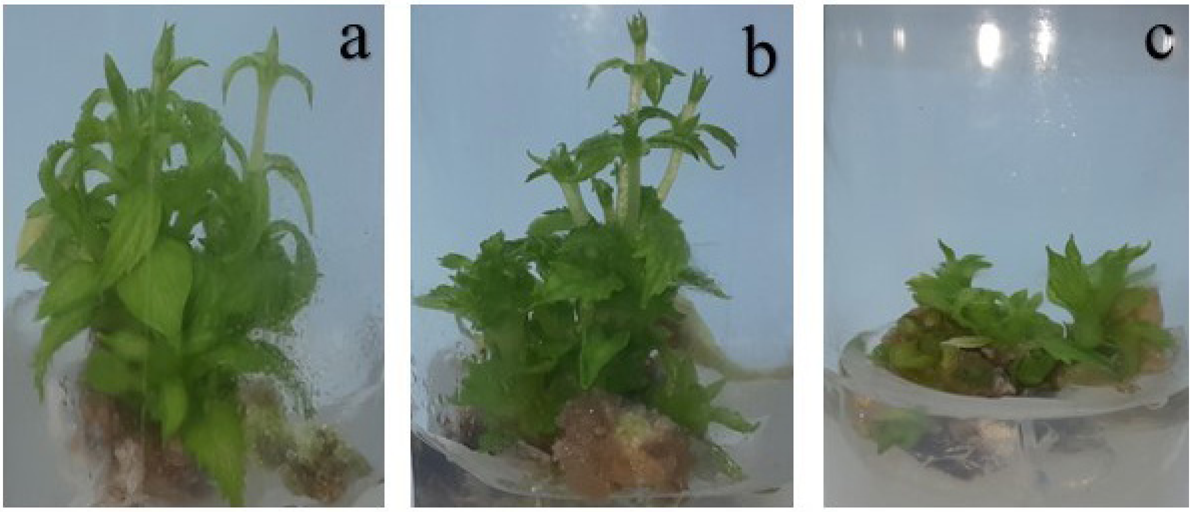

Fig. 4. Uneven development of *Humulus lupulus* microshoots and formation of numerous adventitious buds (Passage 0): a, b – regenerants at least 5 cm long; c – regenerants with shortened stems.

Table 4. *In vitro* reproduction rate of *Humulus lupulus* (MS1) after deposition of encapsulated microcuttings for 12 months under cold conditions

Cultivar	Passage			
	II	III	IV	V
‘Flagman’	2.6 ± 0.4	7.9 ± 1.2	8.6 ± 0.9	8.9 ± 0.7
‘Magnum’	1.5 ± 0.4	4.8 ± 0.6	8.0 ± 0.6	8.0 ± 0.9
‘Bryanskij’	2.5 ± 0.5	6.0 ± 0.3	9.1 ± 1.2	9.2 ± 1.2
‘Taurus’	1.5 ± 0.1	7.0 ± 0.7	8.5 ± 0.6	8.8 ± 0.4
$LSD_{0.05}$	0.7	2.2	0.4	0.5

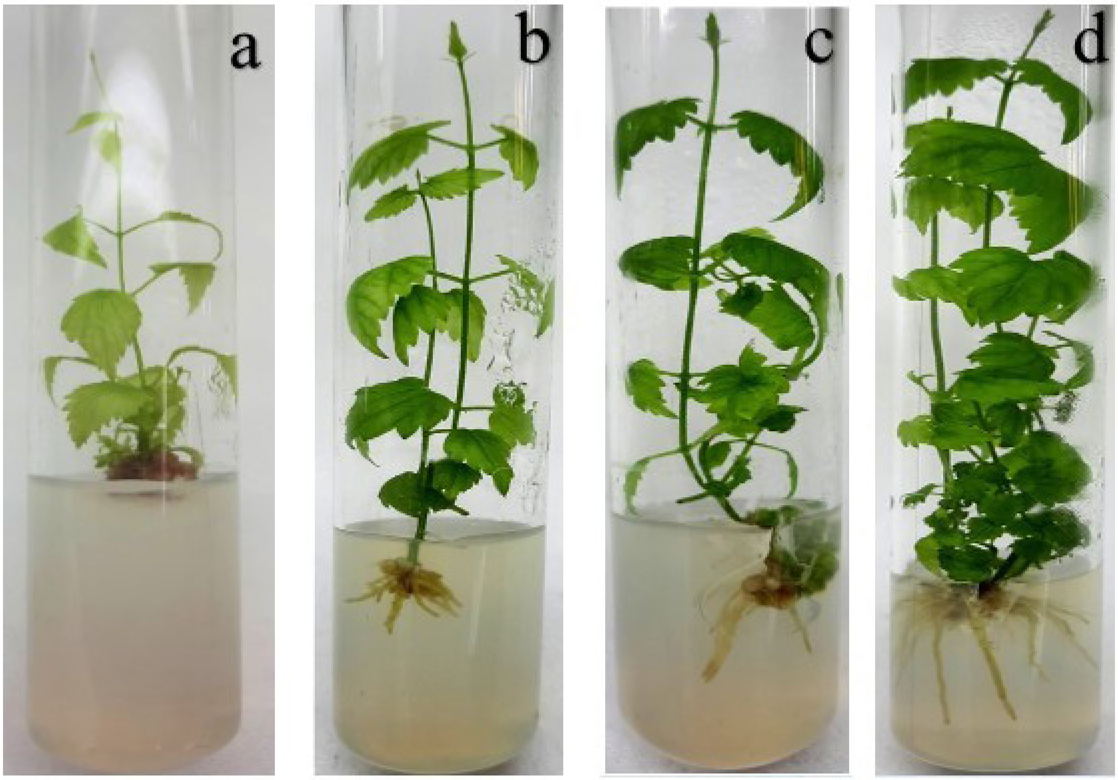

Fig. 5. The effect of kinetin on the post-storage regrowth of encapsulated *Humulus lupulus* microcuttings: a – regenerant with signs of chlorophyll loss (Passage 0); b – regenerant growing on a medium with 2 mg L^{-1} BAP (Passage I); c – regenerant growing on a medium with 1 mg L^{-1} kinetin (Passage I); d – regenerant growing on a medium with 0,5 mg L^{-1} kinetin (Passage I).

generation, reaching values typical of cultivars not subjected to encapsulation and conservation (Mironenko et al., 2024; Khlebova et al., 2025).

Conclusion

A protocol for medium-term storage of *H. lupulus* has been developed, including all stages of encapsulation technology from the preparation of initial explants to their subsequent rehabilitation and restoration of regenerative capacity after 12 months of storage at a low positive temperature (4 °C). The protocol was tested on four hop genotypes demonstrating high morphogenetic potential after deconservation. It has been shown that the viability and post storage regrowth of axillary hop buds depends on the concentration of the encapsulating and polymerizing solution, as well as the explant exposure time during biocapsule production. Treatment of propagules with 3 % sodium alginate during the encapsulation stage, combined with 3 % calcium chloride during the polymerization stage, ensures 100 % and 80 % survival of the synseeds after 6 and 12 months of storage. The optimal exposure time to the polymerizing solution does not exceed 10–15 minutes.

For the first time, it has been shown that 1 mg L^{-1} of abscisic acid (ABA) in artificial endosperm of hop biocapsules reduces the impact of cold stress during medium-term low-temperature storage of the material. Supplementing the MS nutrient medium, which forms the basis of the AE, with 2 mg L^{-1} of BAP and 1 mg L^{-1} of GA3 allows explants to form up to 4 internodes when cultured immediately after storage (Passage 0). Subsequent subcultivation of hop microcuttings on MS nutrient medium containing 0.5 mg L^{-1} kinetin promotes the restoration of photosynthetic activity in the regenerants, alleviating chlorosis symptoms. This approach ensures high regenerative and root-forming potential of the emerging shoots over four consecutive clonal generations. The reproduction rate reaches 8–9 nodes by Passages IV–V.

The practical implementation of an innovative technological method for depositing an active *in vitro* collection of hops in the form of biocapsules allows for a significant reduction in the annual routine volume of work; increases the use of cloned lines in tissue culture by reducing the frequency of shoot dissection; maintains the typicality of the cloned material; and ensures the preservation of healthy samples over a long period of time through medium-term storage at low positive temperatures.

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