

Chlorella as the source of biologically active compounds for *in vitro* plant propagation

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Abstract. The use of nutrient media with an organic composition is a potentially commercially effective way to avoid the use of expensive components of nutrient media while maintaining and increasing the biosynthetic potential of cell cultures of higher plants *in vitro*. A method has been developed for the *in vitro* propagating of various taxonomic groups plants. It has been shown that the medium after cultivating chlorella at ¼ MS is optimal for the *in vitro* plant propagation. The method is intended for *in vitro* cultivation of seeds, microcuttings and microshoots of plants of different taxonomic groups and can be used for accelerated propagation of valuable plant varieties and hybrids.

1 Introduction

As a rule, when cultivating cell cultures *in vitro*, various nutrient media differing in mineral composition are used. Moreover, the mineral composition of the nutrient medium may differ for plants of different taxonomic groups. For example, a nutrient medium containing mineral salts according to Murashiga and Skoog's recipes is used for *in vitro* cultivation of agricultural, medicinal, and some berry plants; WPM (Woody Plant Medium) nutrient medium is used for *in vitro* cultivation of tree species [1], and Hamburg or Nitsch nutrient medium is used to obtain haploid crop plants *in vitro* [2]. However, the proposed nutrient media are not optimal for growing *in vitro* cell cultures of some plant species, and their modification is required for use in cell engineering.

Currently, there is searching for alternative nutrient media and organic biostimulants that reduce the cost of the biotechnological process and promote satisfactory growth of *in vitro* cell cultures of higher plants. The use of nutrient media with an organic composition is a potentially commercially effective method that allows one to abandon the use of expensive components of nutrient media while maintaining and increasing the biosynthetic potential of *in vitro* cell cultures of higher plants [2].

A unique organic product is chlorella (*Chlorella vulgaris*) – a green microalgae containing more than 650 substances. *Chlorella vulgaris* is a spherical microscopic cell with a diameter of 2–10 µm. The microalgae chlorella contains a pool of biologically active compounds: about 50% protein, consisting of 18 essential amino acids; vitamins B1, B2, B3, B5, B6, A, E; a full complex of essential unsaturated fatty acids; 13 macro- and micronutrients important for health in organic form.

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The rich composition creates the prerequisites for the widespread use of chlorella in various areas of human activity, which allows us to call chlorella the multifunctional element of the economic environment [3].

An important industry for the use of chlorella is a food production. Traditionally, chlorella is consumed in the form of powders or tablets, and there is also evidence that a live suspension of chlorella is commercially available.

Scientists have found that the complex of biologically active compounds of chlorella biomass has anti-inflammatory, antioxidant, antimicrobial and wound-healing effects [4].

Chlorella is cultivated in ponds or bioreactors, which differ in the degree of control and the ability to create special conditions for the growth of chlorella biomass. This microalgae has a relatively fast growth rate and responds to a set of conditions, and therefore it is possible to control the release of specific components, for example, lipids [5].

The rich composition of microalgae and, in particular, chlorella creates the prerequisites for their effective use as a substrate for the *in vitro* propagation of cultures [6,7]

One study assessed the *in vitro* propagation and establishment of *S. crispa* (*Schomburgkia crispa* Lindley (*Orchidaceae*), an epiphytic species native to the Brazilian Cerrado) in a modified growth medium containing an extract of the microalgae *Chlorella sorokiniana*. Supplementation of WPM (Woody Plant Medium) with microalgae suspended in NPK medium, or as the supernatant resulting from the centrifugation of a culture in NPK medium, was analyzed. The extracts were added to WPM instead of distilled water. The compounds 6-benzylaminopurine (BAP) and indolebutyric acid (IBA) were used as reference in the *in vitro* multiplication and rooting of *S. crispa*, respectively. Both growth regulators were tested at 0, 2.5, and 5.0 mg L⁻¹. During *in vitro* multiplication of *S. crispa*, WPM supplemented with 5.0 mg L⁻¹ BAP favored the formation of more sprouts, whereas WPM containing 2.5 mg L⁻¹ IBA supplemented with microalgae extract stimulated *in vitro* rooting. *Schomburgkia crispa* explants cultivated in medium supplemented with microalgae suspension or the supernatant of *C. sorokiniana* showed growth similar to explants cultivated in WPM alone. Therefore, it is possible to use the microalga *C. sorokiniana* as a supplement and/or alternative to WPM for the *in vitro* cultivation of *S. crispa* [6].

The effect of microalgae *Messastrum gracile* and *Chlorella vulgaris* on the *in vitro* reproduction of *Cattleya labiata* orchids was studied in another research [7]. The aim of this study was to evaluate the effect of two green microalgae (*Messastrum gracile* and *Chlorella vulgaris*) in comparison with plant growth regulators (6-benzylaminopurine, BAP; thidiazuron, TDZ; zeatin, ZEA) on the *in vitro* propagation of *Cattleya labiata*, an endangered orchid, using the thin cell layer (TCL) technique from protocorms. TCL sections were cultivated in MS/2 medium containing *M. gracile* extract (EM) and biomass (BM); *C. vulgaris* extract (EC) and biomass (BC); and BAP, TDZ, and ZEA in different concentrations. Subsequently, the explants were grown in MS/2 medium, with 2 g L⁻¹ of activated charcoal, to induce elongation and roots formation. For acclimatization, plants were transplanted in trays using *Sphagnum sp.* as substrate. TCL explants showed a higher formation of protocorm-like bodies (PLBs) than entire protocorms. Explants cultivated in media supplemented with BM showed a high rate of PLB regeneration (59%) and high mean number of PLBs (4 per explant) and 85% survival after acclimatization of the plants. Supplementation with BAP stimulated similar morphogenic responses to those observed with BM and superior results obtained with ZEA and TDZ. Mass propagation of *C. labiata* plants was successfully achieved using TCL, and it is recommended to supplement the MS/2 medium with 4 g L⁻¹ of BM or 4 mg L⁻¹ of BAP. Microalgae extracts and biomasses are effective alternatives for *in vitro* propagation of *C. labiata* that can replace plant growth regulators, as they favored the formation of PLBs and plants.

To fully exploit the commercial potential of microalgae biotechnology, bottlenecks in biological, agronomic, economic and technological fields need to be addressed [8]. Recently, many companies in Spain (Agroplasma, AlgaEnergy, Agrialgae, Allgrow and

Biorizon biotech), Turkey (Mikroalg Inc. and MCT Tarim Ltd.), USA (AgroValley Inc.), Hungary (Natur Agro) and India (Soley Biotech, Hindustan bioenergy Ltd.) have advanced their research and increased investment in the commercialization of biostimulants and biofertilizers from microalgae. Extracts of microalgae such as *Chlorella*, *Arthrospira*, *Scenedesmus*, *Haematococcus* and *Nannochloropsis* are mainly studied [9-13].

2 Materials and methods

Chlorella was grown on a nutrient medium containing different concentrations of compounds according to the Murashige-Skoog recipe (¼ MS, ½ MS, 1 MS, 1.5 MS). After sowing *chlorella* into the nutrient medium, the optical density of the solution was determined (optical density was measured on a Cary-50 spectrophotometer, Varian, USA). *Chlorella* was grown at a temperature of 24±1°C, a 16-hour photoperiod, and illuminated with white fluorescent lamps with a lighting intensity of 3 thousand lux for 5 days. After 5 days, the optical density of the suspension was measured again to determine the increase in biomass, and the growth index (I) and specific growth rate (μ) were calculated using the formulas:

$$I = \frac{X_{\max} - X_0}{X_0}, \quad \mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1},$$

where X_{\max} and X_0 are the maximum and initial values of optical density, units, X_2 and X_1 are the value of optical density (mm) at time t_2 and t_1 , days, respectively.

The lowest growth index of the *chlorella* suspension was noted on the ¼ MS medium, and the highest - on the 1.5 MS medium, as well as in the control variant with distilled water. These variants of nutrient media on which *chlorella* was grown were used in further studies as a basis. 6-benzylaminopurine (BAP) 1.0 mg/L and Indole-3-acetic acid (IAA) 0.5 mg/L were added to all media. As a control option, we used the MS nutrient medium, on which the microalgae suspension had not previously been grown, as well as pure distilled water, to which similar hormones were added (BAP 1.0 mg/l and IAA 0.5 mg/l).

Microcuttings of aseptically plants of various taxonomic groups (*Ipomoea batatas* – varieties Purple and Vinnitsky rozovyy, *Mentha ×piperita* – variety Simferopolskaya, *Hyssopus officinalis* – variety Accord, *Alternanthera reineckii roseafolia* and *Staurogyne repens*) were used as the object of study.

3 Results and discussion

The results of measurements of optical density, growth index (I) and specific growth rate (μ) of a *chlorella* suspension using various concentrations of nutrients in the MS medium are presented in Table 1.

Table 1. Results of measurements of optical density, growth index (I) and specific growth rate (μ) of a *chlorella* suspension using different concentrations of nutrients in the MS medium

Type of culture medium	At wavelength 440 nm					At wavelength 690 nm				
	D ₀	D	D-D ₀	I	μ	D ₀	D	D-D ₀	I	μ
¼ MS	0.30	0.49	0.19	0.64	0.12	0.28	0.39	0.11	0.40	0.08

½ MS	0.29	0.88	0.59	2.08	0.28	0.26	0.77	0.51	1.95	0.27
1 MS	0.35	1.01	0.65	1.86	0.26	0.32	0.87	0.55	1.70	0.25
1,5 MS	0.34	1.44	1.10	3.23	0.36	0.31	1.23	0.93	3.01	0.35
Control (distilled water)	0.30	0.78	0.49	1.64	0.24	0.27	0.73	0.46	1.72	0.25

The initial appearance of chlorella suspensions before cultivation and after cultivation are presented in Figures 1 and 2.



Fig. 1. Chlorella suspension for the first day of cultivation, from left to right: ¼ MS, ½ MS, 1 MS, 1.5 MS and control (distilled water)

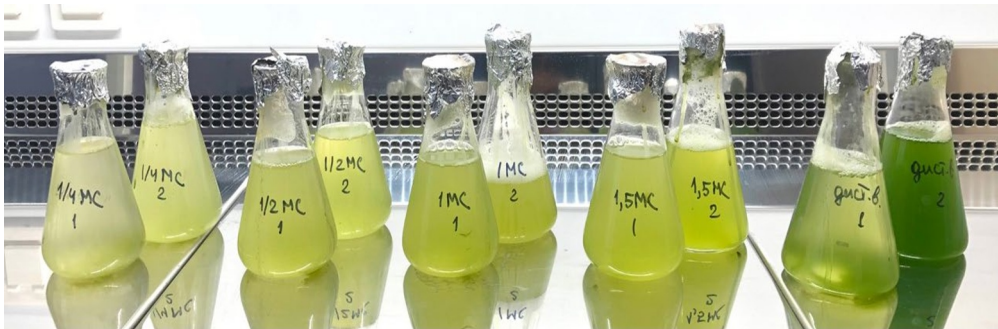


Fig. 2. Chlorella suspension for fifth day of cultivation, from left to right: ¼ MS, ½ MS, 1 MS, 1.5 MS and control (distilled water)

As a result of the studies, it was established that when the studied objects were cultivated on nutrient media in which a suspension of chlorella was previously grown, active growth of the main and lateral shoots was observed. The data obtained were similar to the results noted when growing microcuttings on MS medium (Figures 3-8). Moreover, the best results for the growth of microshoots were obtained when using ¼ MS, on which chlorella was previously grown. When using a nutrient medium based only on distilled water, yellowing of the microcuttings and further death of the explants were observed.

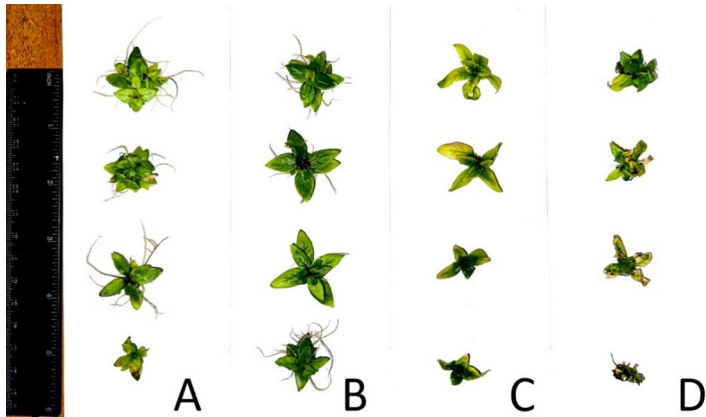


Fig. 3. Microcuttings of *Staurogyne repens*

A – on a medium based on distilled water after cultivating chlorella, B – on 1/4 MS medium after cultivating chlorella, C – on 1,5 MS medium after cultivating chlorella, D – on MS medium without cultivating chlorella

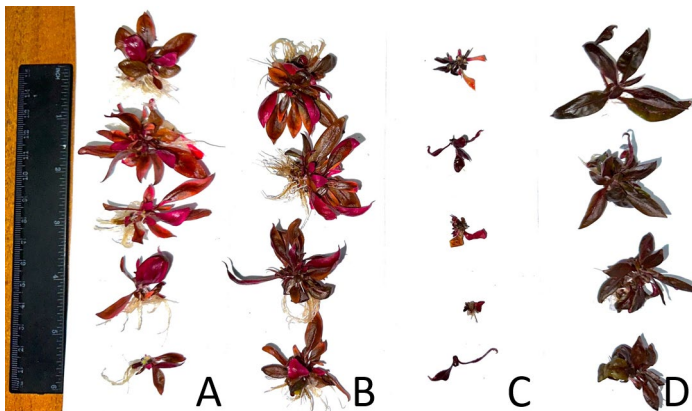


Fig. 4. Microcuttings of *Alternanthera reineckii roseafolia*

A – on a medium based on distilled water after cultivating chlorella, B – on 1/4 MS medium after cultivating chlorella, C – on 1,5 MS medium after cultivating chlorella, D – on MS medium without cultivating chlorella



Fig. 5. Microcuttings of *Ipomoea batatas* (Vinnitsky rozovyy variety)

A – on a medium based on distilled water after cultivating chlorella, B – on $\frac{1}{4}$ MS medium after cultivating chlorella, C – on 1,5 MS medium after cultivating chlorella, D – on MS medium without cultivating chlorella



Fig. 6. Microcuttings of *Ipomoea batatas* (Purple variety)

A – on a medium based on distilled water after cultivating chlorella, B – on $\frac{1}{4}$ MS medium after cultivating chlorella, C – on 1,5 MS medium after cultivating chlorella, D – on MS medium without cultivating chlorella

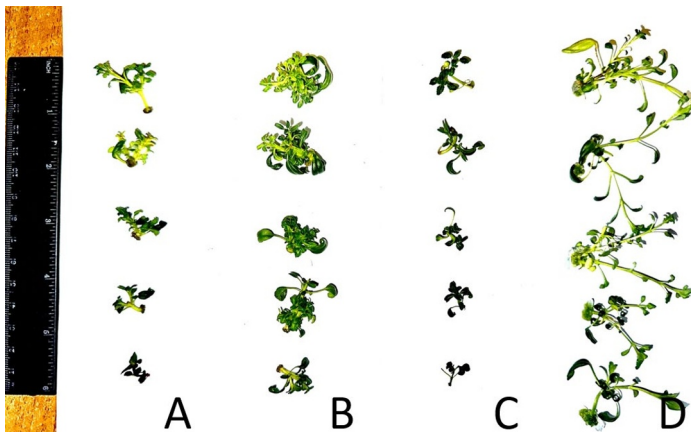


Fig. 7. Microcuttings of *Hyssopus officinalis* (Accord variety)

A – on a medium based on distilled water after cultivating chlorella, B – on $\frac{1}{4}$ MS medium after cultivating chlorella, C – on 1,5 MS medium after cultivating chlorella, D – on MS medium without cultivating chlorella

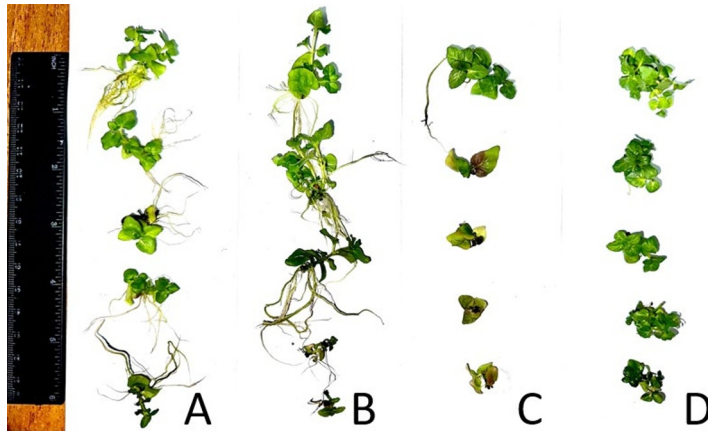


Fig. 8. Microcuttings of *Mentha × piperita* (Simferopolskaya variety)

A – on a medium based on distilled water after cultivating chlorella, B – on $\frac{1}{4}$ MS medium after cultivating chlorella, C – on 1,5 MS medium after cultivating chlorella, D – on MS medium without cultivating chlorella

4 Conclusion

The method of *in vitro* cultivating of different taxonomic groups plants by the growing plant explants (seeds, microcuttings, microshoots) on a nutrient medium containing a suspension of the microalgae *Chlorella vulgaris* can be used for accelerated *in vitro* propagation of valuable plant varieties and hybrids. This can be achieved by using a chlorella suspension as the basis of a nutrient medium, which is initially obtained on a hormone-free nutrient medium containing $\frac{1}{4}$ of the norm of macrosalts according to Murashiga and Skoog's prescription; after 5 days of cultivation, 20 g/l sucrose, 8 g/l agar, 6-benzylaminopurine at a concentration of 1 mg/l and indolyl-3-acetic acid at a concentration of 0.5 mg/l should be added to the suspension, and this plant medium can be used for cloning plants at the micropropagation stage.

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