5-бөлім
БИОТЕХНОЛОГИЯ

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БИОТЕХНОЛОГИЯ

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INFLUENCE OF REGULATORY FACTORS ON BIOMORPHOLOGICAL DEVELOPMENT *STEVIA REBAUDIANA IN VITRO*

The paper presents new data on the influence of regulatory factors on the biomorphological development of *Stevia rebaudiana* plants, which are widely cultivated worldwide for the production of natural sweeteners. Stevia has attracted attention due to its non-toxicity, biological activity and lack of harmful effects. In order to develop disease resistant stevia varieties with desirable traits along with traditional breeding, biotechnological approaches are being actively developed. Research on stevia cultivation is gaining importance in the context of its potential as a safer alternative to synthetic sweeteners derived from sugars with negative health effects and in the treatment of chronic diseases such as diabetes mellitus. The studies conducted revealed important factors determining the effectiveness of growth regulators on the morphogenesis of *S. rebaudiana*. The paper presents biotechnological methods, including in vitro micropropagation, which offer opportunities for large-scale production, development of new varieties and conservation of valuable genotypes. The dependence on both sucrose concentration in Murashige and Skoog medium and phytohormones was established. The optimum concentration of sucrose and growth hormones in the nutrient medium was selected during the experiment. After conducting studies on the effect of sucrose concentration on the growth and development of stevia, it was concluded that the optimum concentration of sucrose in the nutrient medium is 20 g/L. The authors conclude the importance of regulatory factors for the biomorphological development of *Stevia rebaudiana* plants *in vitro*.

Key words: *Stevia rebaudiana*, *in vitro*, regulatory factors, nutrient medium, plant biomorphology, sucrose, growth hormone, IBA.

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Реттеуші факторлардың *Stevia rebaudiana in vitro* өсімдіктерінің биоморфологиялық дамуына әсері

Мақалада реттеуші факторлардың бүкіл дүние жүзінде табиғи тәттілендіргіштерді өндіру үшін кеңінен өсірілетін *Stevia rebaudiana* (бал стевиясы) өсімдіктерінің биоморфологиялық дамуына әсері туралы жаңа мәліметтер ұсынылған. Стевия өзінің уытсыздығының, биологиялық белсенділігінің және зиянды әсерлері болмауының арқасында қызығушылық тудырады. Стевияның ауруларға төзімді қалаулы белгілерімен сұрыптарын өсіру үшін дәстүрлі селекциямен қатар биотехнологиялық тәсілдер де белсенді дамуда. Стевия өсіру бойынша зерттеулер оның қанттан алынатын және денсаулыққа, сондай-ақ қант диабеті тәрізді созылмалы ауруларды емдеуге жағымсыз әсер ететін синтетикалық тәттілендіргіштердің қауіпсіз баламасы ретіндегі потенциалының мәнмәтінінде көбірек маңызға ие болып келеді. Жасалған зерттеулер *S. rebaudiana* морфогенезіне өсу реттеуіштерінің тиімділігін көрсететін маңызды факторларды анықтады. Мақалада биотехнологиялық әдістер соның ішінде in vitro микрокөбею ұсынылған, олар ірі масштабты өндіріс, жаңа сұрыптар шығару және бағалы генотиптерді сақтап қалу үшін мүмкіндіктер ашады. Мурасиге и Скоуга ортасында сахарозаның қойырлығынан және де фитогормондардан тәуелділік бар екендігі анықталды. Эксперимент барысында құнарлы ортада гормондарының оңтайлы қойырлығы таңдалды. Сахароза қойырлығының стевияның өсуі мен дамуына әсері бойынша зерттеулер жасалғаннан кейін құнарлы ортада сахарозаның оңтайлы қойырлығы 20 г/л құрайтындығы туралы қорытынды жасалды. Авторлар реттеуші факторлардың *Stevia rebaudiana in vitro* өсімдіктерінің биоморфологиялық дамуы үшін маңызды екені туралы қорытынды жасайды.

Түйін сөздер: *Stevia rebaudiana, in vitro*, реттеуші факторлар, құнарлы орта, өсімдіктердің биоморфологиясы, сахароза, өсу гормоны, IBA.

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Влияние регуляторных факторов на биоморфологическое развитие *Stevia rebaudiana in vitro*

В статье представлены новые данные о влиянии регуляторных факторов на биоморфологическое развитие растений *Stevia rebaudiana*, которые широко культивируются во всем мире для производства натуральных подсластителей. Стевия привлекает внимание благодаря своей нетоксичности, биологической активности и отсутствию вредных эффектов. Для выведения устойчивых к болезням сортов стевии с желаемыми признаками наряду с традиционной селекцией активно развиваются биотехнологические подходы. Исследования по выращиванию стевии приобретают все большее значение в контексте ее потенциала как более безопасной альтернативы синтетическим подсластителям, получаемым из сахаров и оказывающим негативное воздействие на здоровье, а также в лечении хронических заболеваний, таких как сахарный диабет. Проведенные исследования выявили важные факторы, определяющие эффективность регуляторов роста на морфогенез *S. rebaudiana*. В статье представлены биотехнологические методы, в том числе микроразмножение in vitro, которые открывают возможности для крупномасштабного производства, выведения новых сортов и сохранения ценных генотипов. Была установлена зависимость как от концентрации сахарозы в среде Мурасиге и Скоуга, так и от фитогормонов. В ходе эксперимента была подобрана оптимальная концентрация сахарозы и гормонов роста в питательной среде. После проведения исследований по влиянию концентрации сахарозы на рост и развитие стевии был сделан вывод, что оптимальная концентрация сахарозы в питательной среде составляет 20 г/л. Авторы делают вывод о важности регуляторных факторов для биоморфологического развития растений *Stevia rebaudiana in vitro*.

Ключевые слова: *Stevia rebaudiana, in vitro*, регуляторные факторы, питательная среда, биоморфология растений, сахароза, регулятор роста, IBA.

Introduction

Stevia rebaudiana Bertoni, belonging to the Asteraceae family, is a perennial plant grown commercially around the world for the natural sweetener steviol. The leaves of the stevia plant are mainly used as a sweetener and flavor enhancer in the food and beverage industry [1].

The widely popular natural sweetener steviol derived from stevia leaves is available in the market worldwide. The popularity of this sweetener is attributed to its non-toxicity and physiological harmlessness in addition to its biological activities including antioxidant, antimicrobial and antifungal activities [2].

Stevia is a nutrient-rich herb containing significant amounts of other nutrients such as 80-85% water, protein, fiber, amino acids, free sugars, lipids, essential oils, vitamins, macro- and micronutrients [3, 4].

The plant is rich in carbohydrates (62% dry matter), protein (11% dry matter), crude fiber (16% dry matter), minerals (K, Ca, Na, Mg, Cu, Mn, Fe, Zn) and essential amino acids [5].

Also, toxicological studies have shown that consumption of stevia sweetener does not lead to allergic, mutagenic or carcinogenic effects. In addition, the sweetener has a zero glycemic index and contains no calories [2, 6].

Based on the above, a very relevant issue in the use of this culture in practice, is the study of the growth and development of stevia in laboratory conditions. The relevance lies in the fact that by adding a variety of growth regulators to the nutrient medium for stevia cultivation, a large amount of culture can be obtained in the laboratory in a short period of time.

The study aimed to investigate the impact of regulatory factors on the biomorphological development of *Stevia rebaudiana* (honey stevia) plants in vitro.

Material and methods of research

Standard methods of cultivation of plant tissues of higher plants were used in the study [7]. The object of the study was regenerant plants of *Stevia rebaudiana*. During the study, two laboratory experiments with *Stevia rebaudiana* plants were laid down.

In the in vitro experiments were conducted to study the effect of sucrose on the growth and development of *S. Rebaudiana*. Stevia microcuttings were used for the study and grown in test tubes on Murashige and Skoog (MS) [8] nutrient medium with the addition of sucrose at different concentrations: 10 g/l, 20 g/l, 30 g/l. e first experiment, to study the effect of sucrose on growth and development of *S. rebaudiana*, the following variants of sucrose concentration in nutrient medium were taken:

- 1 expertise – MS + IBA (β-indole-3-butyric acid) (1 mg/l) + sucrose 10 g/l;
- 2 expertise – MS + IBA (1 mg/l) + sucrose 20 g/l;
- 3 expertise – MS + IBA (1 mg/l) + sucrose 30 g/l.

PS with sucrose concentration of 20 g/L, which is mainly used for cultivation of plant explants, was taken as a control.

In the 2nd experiment to study the effect of regulatory factors on stevia morphogenesis, the following variants of PS with addition of PP were taken:

- 1 expertise – MS without addition of hormonal preparations (control);
- 2 expertise – MS + IBA (2 mg/l);
- 3 expertise – MS + GA (gibberellic acid) (1 mg/l);
- 4 expertise – MS + Epin Extra (0.5 mg/l).

Sucrose with a concentration of 20 g/L was taken as a source of carbohydrate in PS for the 2nd experiment. Standard methods of cultivation of plant tissues of higher plants were used in the study.

To assess the significance of differences in plant growth parameters under different experimental conditions, statistical analysis was used to ensure the reliability of the results obtained.

Results and discussion

Effect of sucrose in nutrient medium on growth and development of *S. rebaudiana*

The laboratory experiment to study the effect of sucrose on growth and development of *S. rebaudiana* was carried out on 20 plants in each variant, in 2-fold repetition.

The experiment showed that growth and development of *S. rebaudiana* cuttings were not equal in the three variants and depended on the concentration of sucrose in the nutrient medium. Plants of the control variant (sucrose concentration of 20 g/l), as expected, had a higher percentage of rooting of explants in nutrient medium (hereinafter referred to as NM). The average rooting rate of microcuttings was observed on PS with sucrose concentration of 30 g/l. The lowest survival rate of honey stevia explants was observed in 1 variant – MS + IBA (1 mg/L) + sucrose 10 g/L. Experimental data on the survival of in vitro plants of *S. rebaudiana* during 16 days of cultivation are shown in Figure 1. The data obtained are in agreement with the results of modern researchers such as Wu et al. and Singh et al. [9-12], which showed that carbohydrate content in the nutrient medium has a direct effect on the processes of root system formation and shoot growth *in vitro.*

Throughout the whole cycle of cultivation of cultured plants, optimal development of the root system was observed at a sucrose concentration of 20 g/litre. At minimum (10 g/l) and maximum (30 g/l) concentrations, deterioration of root system formation of explants was detected. During the experiment, the number of roots in each plant was counted. The detailed data are shown in Figure 2. This effect can be explained by the fact that high sugar concentration causes osmotic stress that prevents normal uptake of water and nutrients, as also reported by the studies of Abdi et al. and Lata et al. [13, 14].

The study showed that explants with control sucrose concentration in MS medium had the highest number of leaves in two replicates. Lowered and increased carbohydrate content in the MS medium resulted in a decrease in the number of leaves in plants. The leaves of the control variant were larger and slightly elongated in shape compared to the others. Detailed results of the experiment are shown in Figure 3.

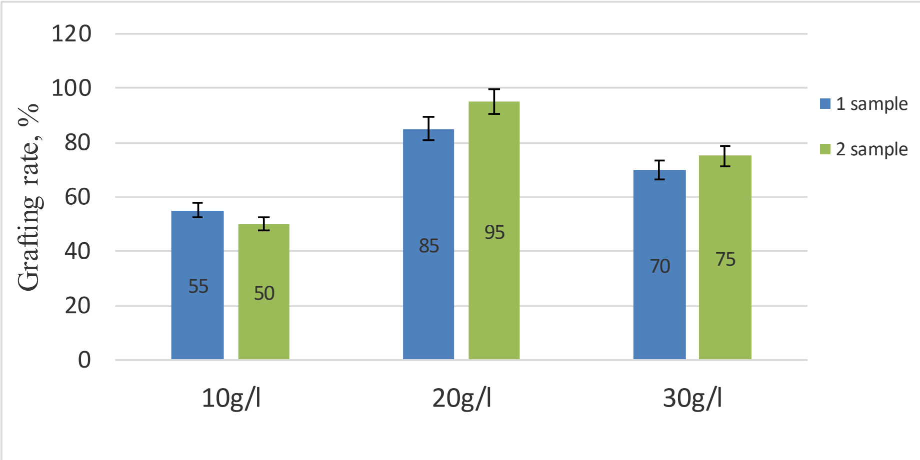

Figure 1 – Effect of sucrose concentration on plant rooting behaviour

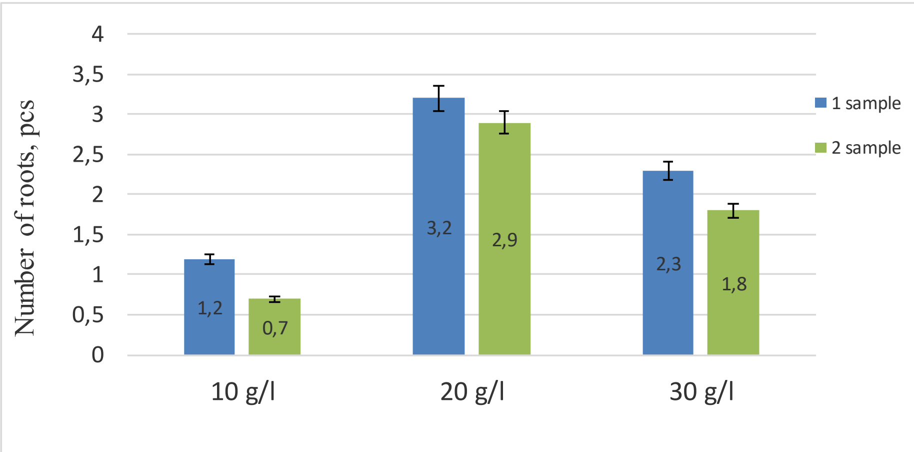

Figure 2 – Effect of sucrose concentration on root formation

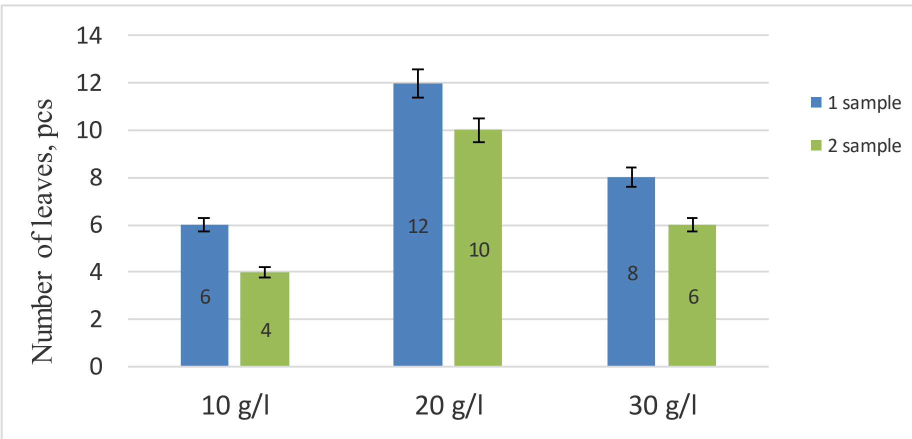

Figure 3 – Effect of sucrose concentration on leaf formation

Comparison of shoot height at different sucrose concentrations showed that at 20 g/l in the medium, plants reached an average height of 7-8.5 cm and had a thin, elongated stem (Fig. 4). At lower concentration (10 g/l), the stems were short, and at higher concentration (30 g/l), the stems slightly increased in height but did not reach the level of the control variant, which is associated with carbohydrate overload in the medium and reduced efficiency of carbohydrate utilisation. These results indicate a positive effect of medium sucrose concentration on shoot growth of *S. rebaudiana*.

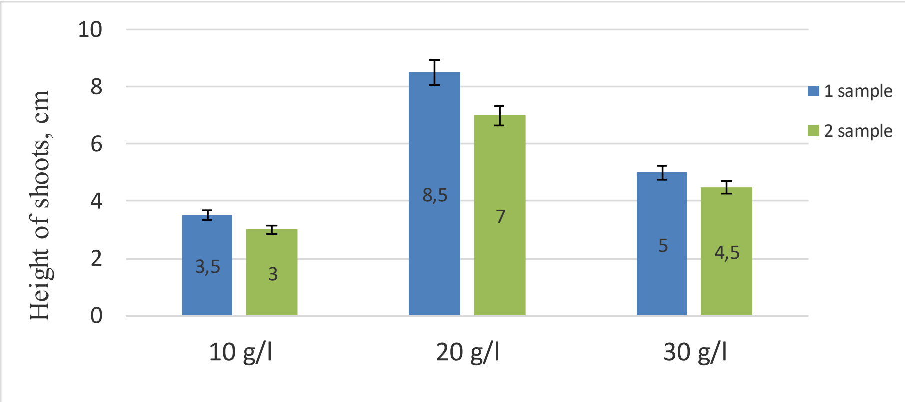

Figure 4 – Effect of sucrose concentration on shoot height

It should be noted that the growth and development of *S. rebaudiana* was the most successful at a sucrose concentration of 20 g/l in Murashige-Skooga nutrient medium. The plants had well developed root system, tall thin stem with larger and elongated leaves. There was also high rooting of explants in cultivation compared to other variants of sucrose content in MS. Repeated cuttings of stevia on NM resulted in a 10% increase in the percentage of explants rooting (1 plant out of 20 did not survive). Honey stevia grown on MS + IBA (1 mg/l) medium with sucrose content of 20 g/l in NM is shown in Figure 5.

The control variant with a sucrose concentration of 20 g/l showed the highest grafting of explants and optimal development of the root system, which coincides with the studies of Abdi et al. [13], where it is indicated that the concentration of 20-30 g/l sucrose favours the maintenance of active root and leaf growth. When the concentration was increased up to 30 g/l, deterioration in rooting was observed, which is in agreement with the works of Lata et al. [14], where it is described that increased levels of sugar can cause osmotic stress, reducing the efficiency of nutrient uptake.

Figure 5 – Stevia plant development with 20 g/l sucrose concentration in nutrient medium

Effect of growth regulators on morphogenesis of *S. rebaudiana*

The laboratory experiment on studying the influence of regulatory factors on the biomorphological development of *S. rebaudiana* was carried out on 10 plants of each variant. Rebaudiana was conducted on 10 plants in each variant, in 3-fold repetition. After the arithmetic mean was deduced. The experiment showed that the growth and development of cuttings of *S. Rebaudiana* did not proceed equally in the four variants and depended on the effect of growth regulators in NM. Sucrose with a concentration of 20 g/l was taken as a source of carbohydrate in NM.

Plants of the control variant (MS without addition of GA) had the lowest percentage of explants rooting in PS, which was 46 %. The average rooting of microcuttings was observed on MS + IBA (2 mg/l) and MS + Epin Extra (0.5 mg/l) media, which was 56 and 50 %, respectively. The highest survival percentage of honey stevia explants, 80 %, was in 3 variants – MS + GA (1 mg/l). Experimental data on the survival of in vitro plants of *S. rebaudiana* during 16 days of cultivation are shown in Figure 6.

Repeated cultivation of explants on MS medium without growth regulator resulted in stunted shoot growth and formation of weak and thin stems, reduced number of leaves, and the percentage of plant engraftment decreased each time [15-19]. This indicated that in the long absence of growth regulator, plants under in-vitro conditions use up their biosynthetic reserves and subsequently cannot synthesise phytohormones of natural origin on their own.

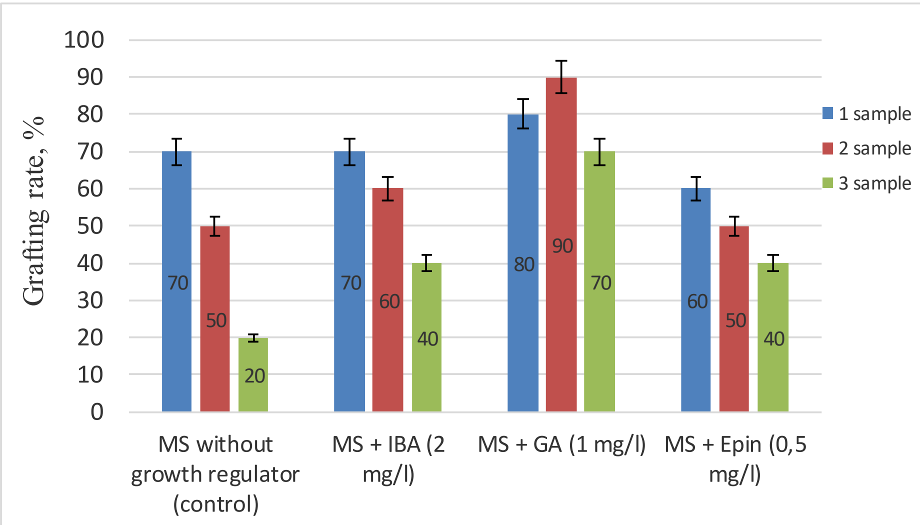

Figure 6 – Influence of growth regulators on plant rooting behaviour

The highest survival percentage (80%) was achieved when GA was added (Figure 6). This is in agreement with the data of Sahai et al. and Erden et al. [20, 21], where it was shown that HA stimulates shoot growth and root formation in-vitro. In the control variant (without PP), the rooting rate was only 46 %, which is in agreement with the results of Moyo et al. [22], emphasising the necessity of hormonal support for normal development of *S. rebaudiana* under artificial cultivation conditions. The use of IBA resulted in moderate growth and root development, which is in agreement with the findings of Javed et al. [23] on the stimulating effect of IBA on rhizogenesis.

The efficiency of root formation depended on the type and concentration of growth regulator. When GA was added to the nutrient medium, roots were developed most intensively, when IBA was added – moderately, and in plants cultivated without addition of PP, the root system practically did not develop (Fig. 7). The presence of Epin Extra provided root formation only in 10% of microcuttings, which is probably due to the inhibitory effect of the biostimulant on root formation under in vitro conditions.

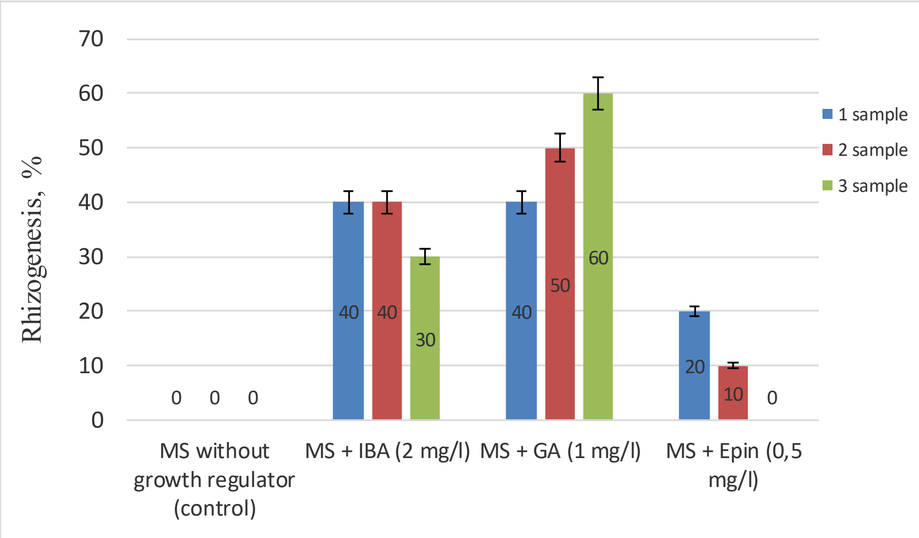

Figure 7 – Effect of growth regulators on plant rhizogenesis

During the study, the number of leaves for all variants of growth regulators did not differ significantly. The average value of nutrient medium without hormones and with IBA was 11 and 10 leaves, respectively. The highest number of leaves was in the medium with addition of GA which was 17 and the lowest with growth regulator Epin Extra which was 8. The details are shown in Figure 8.

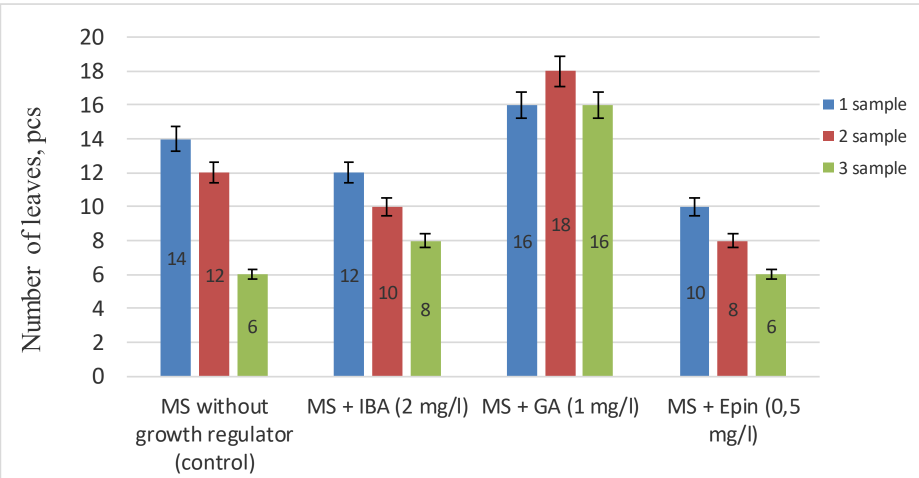

Figure 8 – Effect of growth regulators on leaf formation

The same number of internodes was observed in explants with SAR without growth regulators and with IBA – 5. The maximum number of internodes, 8, was observed using MS + GA, and the minimum number was 3 using MS + Epin Extra. The detailed number of internodes in *S. rebaudiana* plants, in three counts is presented in Figure 9. The findings of the study are in agreement with the findings of Gupta et al. [24] about the inhibitory effect of biostimulants on shoot growth.

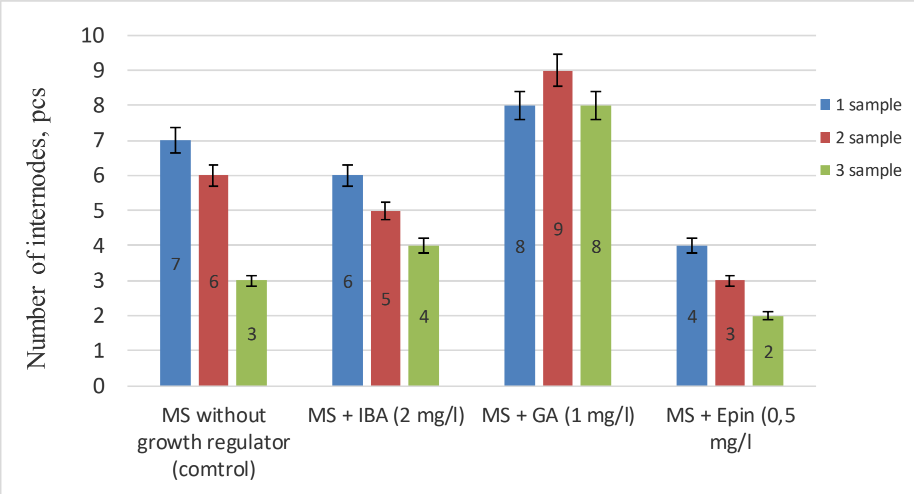

Figure 9 – Effect of growth regulators on the number of internodes

Based on the results of the study, the highest internode length was found in plants with hormone-free medium, and the lowest length was observed in MS + Epin Extra. The average values belong to the media with the addition of growth regulator IBA and GA – 1.2 and 1 cm, respectively. The effect of growth regulator on internode length is shown in Figure 10.

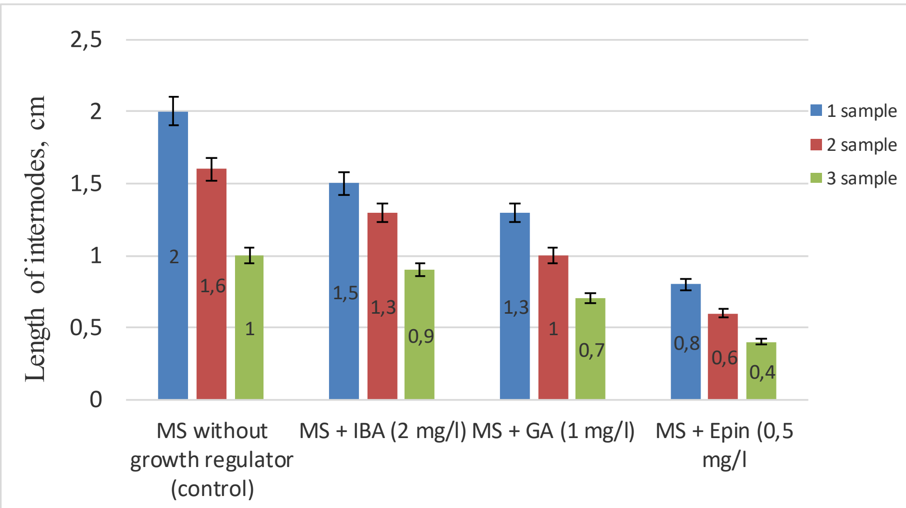

Figure 10 – Effect of growth regulators on internode length

Plants of the control variant had the tallest shoots compared to the others, the average shoot height was 8.3 cm. Explants grown on MS + Epin Extra medium had the shortest shoots; the average shoot length did not exceed 3 cm. With a slight difference were plants grown on MS + IBA and MS + GA media, their length reached 7 and 7.8 cm, respectively.

The use of Epin Extra at a concentration of 0.5 mg/l resulted in the formation of short, thick shoots and undeveloped root system, which was also observed in the study of Gupta et al. [24], which reported a possible inhibitory effect of biostimulants at high concentrations or in the presence of other active ingredients in the nutrient medium. The effect of htuekznznjhj on the height growth of stevia honeydew shoots is shown in detail in Figure 11.

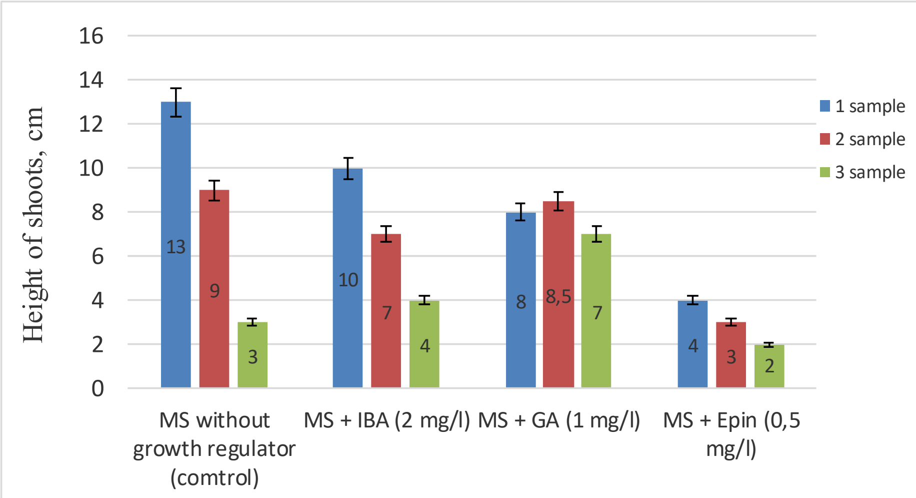

Figure 11 – Effect of growth regulators on shoot height

Stevia honey cultivated on MS + GA medium (1 mg/l) had a well-developed root system. In 43 % of cases explants formed 2 shoots, of medium thickness and length. The cuttings had medium length internodes with large, sometimes medium-sized, slightly rounded leaves. Honey stevia plants grown on medium supplemented with GA are shown in Figure 12.

Plants of *S. rebaudiana* cultivated on medium with MS + Epin Extra (0.5 mg/litre) had poorly developed root system. Explants formed only one short and thick shoot. Leaves were large and rounded, with short internode length. Honey stevia plants grown on the medium with Epin Extra are shown in Figure 13.

Figure 12 – *S. rebaudiana* on MS + GA medium

Figure 13 – *S. rebaudiana* on MS + Epin Extra medium

Thus, the optimal sucrose concentration (20 g/l) and the use of gibberellic acid as a growth regulator can improve the parameters of microclonal propagation of *S. rebaudiana* and increase the percentage of engraftment, which is especially important for further agro-technological applications and commercial cultivation [25].

The results of this study confirm that the optimum medium for cultivation of *S. rebaudiana* is a nutrient medium with a sucrose concentration of 20 g/litre and the addition of gibberellic acid at a concentration of 1 mg/litre. This combination ensures high rooting of explants, active growth of the root system, formation of large leaves and improved branching. The medium with 20 g/litre sucrose and HA favours microclonal propagation and preparation of plants for subsequent transplantation into natural conditions, which is particularly important for commercial cultivation of *S. rebaudiana* as a source of natural sweetener.

Conclusion

In the course of this study, data on the effect of sucrose and growth regulators on the morphogenetic development of *Stevia rebaudiana* Bertoni under in vitro conditions were obtained. Optimal results of rooting, growth and root system formation were achieved when using nutrient medium with sucrose concentration of 20 g/l, which promoted the formation of healthy shoots and large leaves. It was found that decrease or increase of sucrose concentration causes deterioration of root formation and decrease of total rooting ability, confirming the importance of correct selection of carbohydrate composition of nutrient medium for successful cultivation of stevia.

When growth regulators were added, gibberellic acid (GA) at a concentration of 1 mg/l showed the highest efficiency, which provided the best development of the root system, increased the number of leaves and promoted optimal shoot growth. Nutrient media without phytohormone addition or with Epin Extra were less effective, indicating limited resources for growth and morphogenesis in the absence of exogenous hormonal support under in vitro conditions.

Thus, the results of this study confirm that optimisation of nutrient medium composition, including sucrose concentration and selection of growth regulators, plays a key role in the successful cultivation of honey stevia. The findings may be useful for further improvement of stevia microclonal propagation methods and its application in agro-biotechnological projects. Prospects for research include further development of cultivation techniques using different phytohormones and adaptation of biotechnological approaches to increase the commercial productivity of stevia as a source of natural sweetener.

References

1 Gentry A.H. A Field Guide of the Families and Genera of Woody Plants of Northwest South America (Colombia, Ecuador, Peru) with Supplementary Notes on Herbaceous Taxa. – Chicago: The University of Chicago Press, 1996. – 895 p. DOI: 10.1017/S0266467400009895.

2 Lemus-Mondaca R., Vega-Gálvez A., Zura-Bravo L., Ah-Hen K. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects // Food Chemistry. – 2012. – Vol. 132. – P. 1121–1132. DOI: 10.1016/j.foodchem.2011.11.140.

3 Chatsudthipong V., Muanprasat C. Stevioside and related compounds: Therapeutic benefits beyond sweetness // Pharmacology & Therapeutics. – 2009. – Vol. 121. – P. 41–54. DOI: 10.1016/j.pharmthera.2008.09.007.

4 Soejarto D.D. Botany of Stevia and Stevia rebaudiana // In: Kinghorn A.D. (Ed.) Stevia. The Genus Stevia. – New York: Taylor & Francis Inc., 2002. – P. 18–39. DOI: 10.4324/9780203165942-10.

5 Aminha S., Soumya A.N., Raju V.G., Goud B.M., Irfath M., Quadri S.A.P. Isolation and extraction of artificial sweetener (Stevia) // World Journal of Pharmaceutical Research. – 2014. – Vol. 3. – P. 481–486.

6 Singh S.D., Rao G.P. Stevia: The herbal sugar of 21st century // Sugar Tech. – 2005. – Vol. 7. – P. 17–24. DOI: 10.1007/BF02942413.

7 Ditchenko T. Culture of cells, tissues and organs of plants. – Minsk: BSU, 2007. – C. 21-26.

8 Murashige T., Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures // Physiologia Plantarum. – 1962. – Vol. 15, No. 3. – P. 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

9 Wu H., Yamaguchi J., Chen W., Kikuchi S. Influence of sugar concentration in culture media on in vitro growth and development of Stevia rebaudiana // Plant Cell, Tissue and Organ Culture. – 2017. – Vol. 130, No. 1. – P. 111–119. DOI: 10.1007/s11240-017-1205-2.

10 Singh R., Khan R., Mirza H. Effects of sucrose levels on micropropagation and biochemical profile of Stevia rebaudiana // Journal of Plant Biochemistry and Biotechnology. – 2020. – Vol. 29, No. 4. – P. 691–698. DOI: 10.1007/s13562-019-00530-8.

11 Bhingradiya V., Mankad A., Patel R., Mathur Sh. In vitro shoot multiplication of Stevia rebaudiana (Bert.) through plant tissue culture // International Journal of Advanced Research. – 2016. – Vol. 4, No. 11. – P. 2300-2307.

12 Emara H.A., Nower A.A., Hamza E.M., El Shaib F. Evaluation of photomixotrophic technique and several carbohydrate sources as affecting banana micropropagation // International Journal of Current Microbiology and Applied Sciences. – 2018. – Vol. 7, No. 10. – P. 788-804.

13 Abdi G., Salehi H., Khosh-Khui M., Khalighi A. Effect of sucrose on the growth and physiology of in vitro grown Stevia rebaudiana // In Vitro Cellular & Developmental Biology – Plant. – 2018. – Vol. 54, No. 2. – P. 234–242. DOI: 10.1007/s11627-018-9908-7.

14 Lata N., Gupta M., Chandra S. The impact of different sucrose concentrations on Stevia shoot regeneration in vitro // Plant Science Today. – 2021. – Vol. 8, No. 1. – P. 25–32. DOI: 10.14719/pst.2021.8.1.1072.

15 Debnath M. Clonal propagation and antimicrobial activity of an endemic medicinal plant Stevia rebaudiana // Journal of Medicinal Plant Research. – 2008. – Vol. 2. – P. 45-51.

16 Ghorbani T., Kahrizi D., Saeidi M., Arji I. Effect of sucrose concentrations on Stevia rebaudiana Bertoni tissue culture and gene expression // Cell and Molecular Biology (Noisy-le-Grand). – 2017. – Vol. 63, No. 8. – P. 32-36.

17 Hoang N.N., Kitaya Y., Shibuya T., Endo R. Development of an in vitro hydroponic culture system for wasabi nursery plant production – Effects of nutrient concentration and supporting material on plantlet growth // Scientia Horticulturae. – 2019. – Vol. 245. – P. 237-243.

18 Huh Y.S., Lee J.K., Nam S.Y. Improvement of ex vitro acclimatization of mulberry plantlets by supplement of abscisic acid to the last subculture medium // Journal of Plant Biotechnology. – 2017. – Vol. 44, No. 4. – P. 431-437. DOI: 10.5010/JPB.2017.44.4.431.

19 Jain P., Kachhwaha S., Kothari S.L. Biotechnology and metabolic engineering of Stevia rebaudiana (Bert.) Bertoni: Perspective and possibilities // International Journal of Life Sciences Biotechnology & Pharmaceutical Research. – 2014. – Vol. 3, No. 3. – P. 15-37.

20 Sahai V., Kumar P., Kumar M. Influence of gibberellic acid and cytokinin on shoot multiplication and rooting of Stevia rebaudiana // Journal of Medicinal Plants Studies. – 2019. – Vol. 7, No. 3. – P. 101–106.

21 Erden N., Gurel A., Turker M. Gibberellic acid-induced morphogenesis in Stevia rebaudiana and its potential in vitro applications // International Journal of Plant Growth and Development. – 2022. – Vol. 41, No. 2. – P. 89–97.

22 Moyo M., Aremu A.O., Van Staden J. Effects of plant growth regulators on in vitro propagation of selected medicinal plants // Plant Growth Regulation. – 2016. – Vol. 78, No. 1. – P. 97–108. DOI: 10.1007/s10725-015-0078-3.

23 Javed F., Shafique S., Aslam M. Impact of indole-3-butyric acid and sucrose concentrations on the rooting and growth of Stevia rebaudiana // Plant Tissue Culture & Biotechnology. – 2021. – Vol. 31, No. 1. – P. 15–24. DOI: 10.3329/ptcb.v31i1.55567.

24 Gupta A., Choudhary P., Patel S. Effects of Epin-Extra and other plant growth regulators on growth and development of Stevia rebaudiana Bertoni in vitro // Journal of Applied Research on Medicinal and Aromatic Plants. – 2019. – Vol. 12. – P. 40–46. DOI: 10.1016/j.jarmap.2018.11.002.

25 Khlebova L.P., Orazov A., Titova A.M., Pirogova A.V. Adaptation to ex vitro conditions of Stevia rebaudiana (Bertoni) Hemsl. regenerants // Ukrainian Journal of Ecology. – 2019. – Vol. 9, No. 3. – P. 376-380.

References

1 Gentry, A.H. (1996) A Field Guide of the Families and Genera of Woody Plants of Northwest South America (Colombia, Ecuador, Peru) with Supplementary Notes on Herbaceus Taxa; The University of Chicago Press: Chicago, IL, USA, 1996; p. 895. DOI: 10.1017/S0266467400009895.

2 Lemus-Mondaca, R.; Vega-Gálvez, A.; Zura-Bravo, L.; Ah-Hen, K. (2012). Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chem. 2012, 132, 1121–1132. https://doi.org/10.1016/j.foodchem.2011.11.140

3 Chatsudthipong, V.; Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacol. Ther. 2009, 121, 41–54. https://doi.org/10.1016/j.pharmthera.2008.09.007

4 Soejarto, D.D. Botany of Stevia and Stevia rebaudiana. In Stevia. The genus Stevia, 1st ed.; Kinghorn, A.D., Ed.; Taylor & Francis Inc.: New York, NY, USA, 2002; pp. 18–39. https://www.taylorfrancis.com/chapters/edit/10.4324/9780203165942-10/botany-stevia-stevia-rebaudiana-djaja-djendoel-soejarto

5 Aminha, S.; Soumya, A.N.; Raju, V.G.; Goud, B.M.; Irfath, M.; Quadri, S.A.P. (2014) Isolation and extraction of artificial sweetner (Stevia). World J. Pharm. Res. 2014, 3, 481–486.https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1412668922.pdf

6 Singh, S.D.; Rao, G.P. Stevia: The herbal sugar of 21st Century. Sugar Tech 2005, 7, 17–24. https://link.springer.com/article/10.1007/BF02942413

7 Ditchenko T. (2007). Culture of cells, tissues and organs of plants. Minsk: BSU, 2007. – C. 21-26. http://elib.bsu.by/handle/123456789/42225

8 Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15(3), 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x

9 Wu, H., Yamaguchi, J., Chen, W., & Kikuchi, S. (2017). Influence of sugar concentration in culture media on in vitro growth and development of Stevia rebaudiana. Plant Cell, Tissue and Organ Culture, 130(1), 111–119. doi:10.1007/s11240-017-1205-2

10 Singh, R., Khan, R., & Mirza, H. (2020). Effects of sucrose levels on micropropagation and biochemical profile of Stevia rebaudiana. Journal of Plant Biochemistry and Biotechnology, 29(4), 691–698. doi:10.1007/s13562-019-00530-8

11 Bhingradiya, V., Mankad, A., Patel, R., & Mathur, Sh. (2016) In vitro shoot multiplication of Stevia rebaudiana (Bert.) through plant tissue culture Int. J. Adv. Res., 4(11), 2300-2307.

12 Emara, H. A., Nower, A. A., Hamza, E. M., & El Shaib, F. (2018). Evaluation of photomixotrophic technique and several carbohydrate sources as affecting banana micropropagation. Int. J. Curr. Microbiol. App. Sci, 7(10), 788-804.

13 Abdi, G., Salehi, H., Khosh-Khui, M., & Khalighi, A. (2018). Effect of sucrose on the growth and physiology of in vitro grown Stevia rebaudiana. In Vitro Cellular & Developmental Biology – Plant, 54(2), 234–242. doi:10.1007/s11627-018-9908-7

14 Lata, N., Gupta, M., & Chandra, S. (2021). The impact of different sucrose concentrations on stevia shoot regeneration in vitro. Plant Science Today, 8(1), 25–32. doi:10.14719/pst.2021.8.1.1072

15 Debnath, M. (2008). Clonal propagation and antimicrobial activity of an endemic medicinal plant Stevia rebaudiana. Journal of Medicinal Plant Research, 2, 45-51.

16 Ghorbani, T., Kahrizi, D., Saeidi, M., & Arji, I. (2017). Effect of sucrose concentrations on Stevia rebaudiana Bertoni tissue culture and gene expression. Cell Mol Biol (Noisy le Grand), 63, 8, 32-36.

17 Hoang, N. N., Kitaya, Yo., Shibuya, T., & Endo R. (2019). Development of an in vitro hydroponic culture system for wasabi nursery plant production – Effects of nutrient concentration and supporting material on plantlet growth. Scientia Horticulturae, 245, 237-243.

18 Huh, Y. S., Lee, J. K., & Nam, S. Y. (2017). Improvement of ex vitro acclimatization of mulberry plantlets by supplement of abscisic acid to the last subculture medium. J Plant Biotechnol, 44(4), 431-437. DOI: https://doi.org/10.5010/JPB.2017.44.4.431/

19 Jain, P., Kachhwaha, S., & Kothari, S. L. (2014). Biotechnology and metabolic engineering of Stevia rebaudiana (Bert.) Bertoni: perspective and possibilities. Int. J. LifeSc. Bt & Pharm. Res., 3, 3, 15-37

20 Sahai, V., Kumar, P., & Kumar, M. (2019). Influence of gibberellic acid and cytokinin on shoot multiplication and rooting of Stevia rebaudiana. Journal of Medicinal Plants Studies, 7(3), 101–106.

21 Erden, N., Gurel, A., & Turker, M. (2022). Gibberellic acid-induced morphogenesis in Stevia rebaudiana and its potential in vitro applications. International Journal of Plant Growth and Development, 41(2), 89–97.

22 Moyo, M., Aremu, A. O., & Van Staden, J. (2016). Effects of plant growth regulators on in vitro propagation of selected medicinal plants. Plant Growth Regulation, 78(1), 97–108. doi:10.1007/s10725-015-0078-3

23 Javed, F., Shafique, S., & Aslam, M. (2021). Impact of indole-3-butyric acid and sucrose concentrations on the rooting and growth of Stevia rebaudiana. Plant Tissue Culture & Biotechnology, 31(1), 15–24. doi:10.3329/ptcb.v31i1.55567

24 Gupta, A., Choudhary, P., & Patel, S. (2019). Effects of Epin-Extra and other plant growth regulators on growth and development of Stevia rebaudiana Bertoni in vitro. Journal of Applied Research on Medicinal and Aromatic Plants, 12, 40–46. doi:10.1016/j.jarmap.2018.11.002

25 L.P. Khlebova, A. Orazov, A.M. Titova, and A.V. Pirogova. «Adaptation to ex vitro conditions of Stevia rebaudiana (Bertoni) Hemsl. Regenerants» Ukrainian Journal of Ecology, vol. 9, no. 3, 2019, pp. 376-380.

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