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PRODUCTIVITY AND SELENIUM ENRICHMENT OF STEVIA IN HYDROPONIC AND SOIL CULTIVATION SYSTEMS IN THE ARARAT VALLEY


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Abstract.

Hydroponics offers a viable solution for obtaining plant products that are rich in micronutrients and ultramicronutrients, such as selenium. Selenium plays a crucial role in strengthening the body's immune defense and acts as a potent antioxidant. Low levels of selenium have been associated with an increased risk of heart attacks, strokes, and cancer. Stevia rebaudiana Bertoni (SrB), a relatively new crop in plant cultivation, was the focus of the present study. SrB has numerous medicinal and prophylactic properties, and its leaves are rich in macro- and microelements, vitamins, and diterpene glycosides. These diterpene glycosides, when extracted from the plant material, exhibit a sweetness that is 200-300 times greater than that of sugar. The study aimed to investigate the effects of exogenous application of varying amounts of selenium to the nutrient solution and foliar feeding of SrB plants with a selenium aqueous solution. The productivity of SrB and selenium accumulation in the leaves were assessed under both hydroponic and soil cultivation conditions in the Ararat Valley. Comparative analyses were also conducted on biometric and biochemical indicators, as well as the productivity of SrB cultivated using different fillers, including black volcanic slag, red volcanic slag, and gravel, in hydroponic and soil environments. The findings of this research hold practical significance as they serve as a foundation for the development of biotechnological approaches to enhance selenium enrichment in various crops. By applying these strategies, crop cultivation methods can be improved, and the selenium content in plant products can be enhanced. This optimization of crop production techniques can increase the nutritional value and potential health benefits of selenium-enriched plant products.

Key words. Stevia rebaudiana Bertoni, Se, hydroponics, soil.

Introduction.

The development of biotechnologies that enrich plant raw materials with organonimeral microelements and ultramicronutrients is an important current problem. Hydroponics is a solution that allows for the control of the chemical composition of plant material through a nutrient solution. The ultramicronutrient selenium (Se) performs many protective functions in the human body, such as strengthening the immune system, acting as an antioxidant, and helping increase lifespan. However, many studies have shown that 60-80% of the population has an increased incidence of heart attacks, strokes, and cancer due to low Se levels, as their daily intake is often less than optimal. The World Health Organization recommends an average daily intake of 55-70 μg of Se for adults. The lack of Se in food chains, and consequently in the human body, is attributed to the low content of this element in the soil. Therefore, it is necessary to develop measures to increase the Se content in the soil-plant system. One way to address Se deficiency is through the production of Se-enriched crop products. Various countries have accumulated experience in fortifying crops such as parsley, radish, dill, lettuce, garlic, and wheat with Se. The Se content in agricultural crops ranges from 78 to 166 μg/kg, depending on the geochemical conditions. On average, the Se content in plants belonging to different families is up to 100 μg/kg [1-5,6-12,13-20].

We conducted a study to investigate the impact of exogenously adding varying amounts of Se to the nutrient solution and applying Se solution through foliar nutrition on Se accumulation in the leaves of Stevia plants.

The study aimed to enrich the plant material with Se and assess the efficiency of hydroponics and soil cultivation in the Ararat Valley. The research was based on the widespread use of Stevia as a technical crop and considering the significant medical and biological importance of Se.

Materials and methods.

The research was conducted between 2019 and 2022 at the hydroponic Ararat Valley's experimental station of the National Academy of Sciences of the Republic of Armenia. The study involved both soil and hydroponic vegetation vessels, each with an area of 1m² and three replicates. The Ararat Valley is situated at an altitude of approximately 850-900 meters above sea level and experiences a very dry climate, with an average annual temperature of 11.0-11.8°C, a relative humidity of 40%, and an average annual precipitation of 200-300 mm [21]. The surrounding soils of the hydroponic institute are semi-desert, loamy, and carbonate-based, with a humus content of 1.5-2.5%. These soils are rich in phosphorus and potassium. In soil culture, standard agricultural practices were followed, including soil tillage, fertilization, loosening, regular watering, and weed removal. In the hydroponic system, the plants were provided with the nutrient solution recommended by Davtyan (Table 1) [22]. Red and black volcanic slags, along with gravel particles ranging from 3 to 15 mm in diameter, were used as the growing medium. Prior to use, the filler material was disinfected with a 0.05% solution of KMnO4. The experimental crop chosen for the study was Stevia rebaudiana Bertoni (Figure 1-2).
Table 1. The nutrient solution recommended by Davtyan consisted of various essential nutrients necessary for plant growth and development, with their respective concentrations in g/m³ of water.

<table>
<thead>
<tr>
<th>Amount of nutrients</th>
<th>The beginning of the vegetation period</th>
<th>Vegetative growth period</th>
<th>Fertility period</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-80-200</td>
<td>80</td>
<td>175</td>
<td>200</td>
</tr>
<tr>
<td>KNO₃</td>
<td>580</td>
<td>580</td>
<td>580</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>-</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>-</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>CO(NH₂)₂</td>
<td>-</td>
<td>-</td>
<td>56</td>
</tr>
<tr>
<td>P-45-65</td>
<td>45</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>250</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>K-310-350</td>
<td>310</td>
<td>310</td>
<td>350</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>S-100-150</td>
<td>100</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Ca-150</td>
<td>150</td>
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<td>CaSO₄·2H₂O</td>
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<td>Mg-30-50</td>
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<tr>
<td>MgSO₄·7H₂O</td>
<td>300</td>
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<td>FeCl₃·6H₂O or Fe₂(SO₄)₃·9H₂O</td>
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<tr>
<td>H₂BO₂</td>
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<td>KMnO₄ or MnSO₄·4H₂O</td>
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<tr>
<td>ZnCl₂ or ZnSO₄·7H₂O</td>
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<tr>
<td>Na₂MoO₄</td>
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<tr>
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<td>KI</td>
<td>0.2-1.0</td>
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Stevia rebaudiana Bertoni (SrB) is one of the youngest agricultural crops in modern plant breeding and was introduced to Armenia in 2009 as a new technical crop. It is a perennial herb native to South and Central America, as well as northern Mexico. SrB possesses numerous healing and preventive properties. The leaves of SrB are abundant in vitamins (A, E, C, beta-carotene, etc.), macro- and micro-elements, as well as diterpene glycosides such as stevioside, rebaudiosides A, B, C, D, E, steviolbioside, and dulcoside A. Notably, the dry leaves of SrB are 10-15 times sweeter than sugar, and the diterpene glycosides isolated from the plant material are 200-300 times sweeter than sugar [14,19,23].

Several biochemical analyses were conducted on vegetation in the study. The vitamin C content in the vegetable raw material was determined using the method described by Yermakov [24]. The organic acids were analyzed using the “Water Separations Modul 2695” (USA) according to Shomod-Nelson [25,26]. The protein nitrogen content was determined using the Kjeldahl method [27,28]. The analysis of extractives and tannins was conducted according to the pharmacopoeia [29]. The quantification of photosynthetic pigments followed the method developed by Vetstein [30,31]. Carotene content was determined according to the method described by Sapozhnikov [32], while flavonoids were analyzed using the method outlined by Georgievsky [33].

Sampling, sample pretreatment, and determination of Se concentrations in the soil and plant samples were carried out following the state standard method EASC-19413-89. This method relies on the reaction between selenite ions and 2,3-diaminonaphthalene in an acidic environment, leading to the formation of 4,5-benzopiazoselenol [34,35]. Selenite ions were utilized in the form of Se. The obtained data were subjected to statistical analysis using Excel and following the guidelines outlined by Dospechov [36].

Results and Discussion.

It is well known that Se accumulation in plants is influenced by various factors, including the plant species, Se content in the soil, soil properties (such as soil type, acidity, humus content, total Se supply, and interactions with other mineral elements), chemical form of Se, method of Se introduction (such as spraying plants with Se solution or soaking seeds), precipitation, and environmental temperature [2-3,8-11,15]. The bioaccumulation coefficient (BAC) is a measure of the ratio of Se content in plants to Se content in soil. According to the literature, the typical range of BAC for Se is from 0.2 to 0.6. For example, when the black soil had insufficient Se content (Se = 100 μg/kg), corn and sunflower plants accumulated Se at levels of 107 μg/kg and 104 μg/kg, respectively, resulting in BAC values of 1.07 and 1.04, respectively [1]. Despite the semi-desert irrigated gray soils surrounding HPI having a low Se content (Se = 40 μg/kg), the BAC values ranged from 1.3 to

Figure 3. Effect of Se on the accumulation coefficient of Se in the soil-plant system (a), and in the nutrient-plant system (b).
3.2, indicating Se enrichment (Figure 3a).

By employing foliar feeding of plants with Se solutions in both hydroponic and soil cultivation systems, as well as the exogenous addition of Se to the shoot nutrient solution, the accumulation of Se in plant leaves can be enhanced. In hydroponics, the Se accumulation ranges from 8 to 43 μg/kg, while in soil cultivation, it ranges from 52 to 130 μg/kg (Figure 4a). Furthermore, foliar feeding of plants in the soil with a 0.001% solution of Se leads to a 2.5-fold increase in Se accumulation in leaves compared to the control (variant 6). Similarly, in the case of foliar feeding of plants with a 0.001% solution of Se in hydroponics (variant 5), Se accumulation in leaves increases by 1.3 times.

The addition of 1.0 mg/L Se to the nutrient solution in hydroponics resulted in a 12% increase in yield compared to the control (Figure 4b). Additionally, foliar feeding of plants with a 0.001% Se solution (variant 5) led to a 22% higher yield. Table 3 provides data indicating that Se also had a positive impact on the content of diterpene glycosides in Stevia rebaudiana. When 1.0 mg/L Se was present in the hydroponic nutrient solution or when plants received extra-root nutrition with a 0.001% Se solution, the content of stevioside increased by 18%, rebaudioside A by 8%, rebaudioside B by 28%, rebaudioside C by 33%, and dulcoside A by 22%.

Hydroponic systems have been shown to outperform soil-based systems in terms of various biological and biochemical efficiency indicators. This superiority can be attributed to the regulation of certain environmental abiotic factors in hydroponics, which leads to improved nutrition and water-air balance in plants, resulting in higher biological efficiency. Consequently, hydroponic plants have exhibited better performance than soil plants in several aspects, including plant height (1.9), diameter (2.8), leaf and root length (1.4) (Fig. 5), as well as dry weight of leaves (3.9 times) (options 1 and 6) (Fig. 2 b). Furthermore, hydroponic plants showed higher levels of extractive substances, proteins, stevioside, nitrogen (Fig. 6 a), carotene, vitamin C, and chlorophyll content (a+b) (1.1-1.2) (Fig. 6 b). The only parameters in which hydroponic plants were inferior to soil plants were the content of tannins (1.4 times) and flavonoids (1.1 times) (Fig. 6 a).

The levels of organic acids in Stevia rebaudiana (SrB) leaves did not significantly differ under different cultivation conditions. Both hydroponic and soil cultivation resulted in a similar decreasing order of organic acid content: malic acid > sorbic acid > ascorbic acid > genic acid > fumaric acid. However, in the case of soil cultivation, SrB leaves exhibited a lower content of malic acid (1.3 times), while showing higher levels of ascorbic acid, tartaric acid, citric acid, and fumaric acid (1.8; 2.6; 1.4; 17; 3.3 times) compared to hydroponically grown plants.

The application of different fillers (black, red volcanic slag, boulder) in hydroponic cultivation of SrB revealed that SrB exhibited superior biometric characteristics (plant height: 1.6-1.8; diameter: 1.8-2.3; leaf length: 2.3-3.7; stem length: 1.7-3.3; root length: 1.2-1.3 times) (Fig. 8a) and higher efficiency (dry weight of leaf, stem, root: 1.9-3.1; 2.9-4.3; 1.9-3.1 times) (Fig. 8b) compared to SrB cultivated in soil.

Moreover, black volcanic slag yielded the best results in terms of SrB's biometric performance indices.

**Conclusion.**

Stevia rebaudiana (SrB) grown in hydroponics has been shown to exhibit superiority in terms of yield, extractive substances, proteins, stevioside, and malic acid content compared to plants grown in soil. Hydroponic systems have been shown to outperform soil-based systems in terms of various biological and biochemical efficiency indicators, providing high crop yield and high-quality products, even in areas with adverse conditions.
Growing conditions. Hydroponic systems allow flexibility and intensification, providing high crop yield and high-quality products, even in areas with adverse growing conditions. The addition of exogenous Se to the hydroponic nutrient solution results in an increase in Se content in the SrB plant material. The highest Se content in SrB plant material was observed in hydroponics and soil when plants were foliar-fed with a 0.001% solution of Se, supplemented with 1 mg/L Se in the nutrient solution, and when the soil contained 40 μg/kg of Se. The introduction of 1.0 mg/L Se in the nutrient solution and foliar feeding of plants with a 0.001% Se solution resulted in a 22% additional yield compared to the control.
Based on the provided search results, here are some practical suggestions for enriching Stevia rebaudiana (SrB) plant material with selenium and improving its efficiency:

1. Hydroponic cultivation: To ensure high efficiency and enrich the plant material with Se, it is recommended to prefer the hydroponic method of cultivation. Maintain a nutrient solution with a concentration of 1 mg/L Se and provide additional foliar feeding of plants with a 0.001% Se solution. Black volcanic slag can be used as a filler in hydroponic systems, as it has been shown to yield the best results in terms of SrB’s biometric performance indices.

2. Soil cultivation: To enrich the plant material with Se in soil cultures of SrB, it is suggested to apply exfoliating foliar feeding of plants with a 0.001% solution of Se.

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Продуктивность и обогащение селеном медовой травы в условиях гидропоники и почвы в Араратской долине

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Гидропоника дает возможность решить проблему получения растительных продуктов, богатых микроэлементами и ультрамикроэлементами. Ультрамикроэлемент Se (селен) укрепляет иммунную защиту организма, является сильным антиоксидантом. Установлено, что увеличение частоты инфарктов, инсультов и онкологических заболеваний у 60-80% населения связано с низким содержанием селена. Объектом исследований служила Stevia rebaudiana Bertoni (SrB) — самая молодая культура в растениеводстве. В Армению интродуцирована в 2009 г., обладает многими лечебными и профилактическими свойствами. Листья SrB богаты макро- и микроэлементами, витаминами (A, E, C, бета-каротином и др.), дитерпеновыми гликозидами (стевиозид, ребаудиозиды A, B, C, D, E, стевиолбиозид и дулкозид-А). Дитерпеновые гликозиды, выделенные из растительного сырья, в 200-300 раз слаще сахара. Исследовано влияние экзогенного внесения различных количеств Se в питательный раствор и внекорневой подкормки растений водным раствором Se на продуктивность SrB и накопление Se в листьях в условиях гидропоники и почвы в Араратской долине. Также были проведены сравнительные исследования по биометрическим, биохимическим показателям и по продуктивности SrB, культивируемого в различных наполнителях (черный, красный вулканический шлак, гравий) в условиях гидропоники и почвы. Полученные данные имеют практическое значение, так как могут быть положены в основу разработки биотехнологии обогащения сельскохозяйственных культур селеном.

Ключевые слова: медовая трава, Se, гидропоника, почва.