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Short communication

Effect of peptidomimetics nanofibers, as a carrier of silver on steviol glycoside content of micropropagated *Stevia*

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Introduction

The food industry is increasingly interested in replacing artificial sweeteners with other natural sugars to offer consumers more choice and to meet the demands of the part of the population that does not want or cannot eat sucrose. The *Stevia rebaudiana* Bertoni. leaves have been used as a low-calorie sweetener for centuries. The sweetness is due to the accumulation of diterpenoid steviol glycosides (Geuns, 2003). The steviosides are 300 times sweeter than table sugar, with the additional advantages of having: zero calories, zero carbohydrates, not causing spikes in blood sugar levels, having a non-fermenting ability, and maintaining thermal stability at 100 °C.

The amino acids are another important factor influencing plant growth because most of the N is bound up to them. During the *in vitro* propagation, the addition of amino acids to the MS medium provides a primary fast source of nitrogen to plants. An increase in formation and elongation of the cell wall and cell division due to the addition of amino acids was observed. To control the withering of shoots during *Rosa centifoliais* micropropagation, glutamine, asparagine, and proline have been added to the MS medium (Akhtar et al. 2016).

Several studies have represented data that $AgNO_3$ possessed a beneficial effect on various plant species regeneration (Hyde and Phillips 1996; Tamimi 2015). Castro-González et al. (2019) evaluate the response of *S. rebaudiana* to different concentrations of silver nanoparticles (0; 12.5; 25; 50; 100 200 mg L^{-1} AgNPs) added to the nutrient medium. The lowest AgNP concentrations (12.5 mg L^{-1}) promoted the greatest shoot production and length per explant. An increase in chlorophyll a, b, and total contents was observed after all treatments with AgNps. The dry matter gradually increases from 25 mg L^{-1} of AgNPs, obtaining the highest values with the 100 and 200 mgL^{-1} AgNPs concentrations.

There are several studies in the literature about the effect of amino acids on the plant *in vitro* propagation, but there is no information about their effect if they are bound in a polypeptide chain with a diameter in the nanoscale, which is a carrier of biologically active agents. The aim of the present study describes the influence of nanofibers, formed by low molecular weight peptidomimetics carriers of the silver ion (NF-Ag) on stevioside and rebaudioside A of *in vitro* propagated *Stevia rebaudiana* Bert.

Materials and methods

Plant materials

The seeds were surface sterilized by soaking in 70% ethanol and then treated with 15% bleach solution and washed with sterilized distilled water. For *in vitro* seed germination of *Stevia rebaudiana* Bert. seeds were cultured on an MS medium supplemented with 3.0% sucrose, 7.0 g L^{-1} agar and 0.4 mg L^{-1} GA, and 1.0 mg L^{-1} $CaCl_2$ for three weeks of culture. Nodal segments were aseptically excised and cultured on MS media with vitamins containing 1, 10, 50, and 100 mg L^{-1} NF-2%Ag for shoot multiplication. There were two controls - plants, *in vitro* propagated without 6-benzylaminopurine (BAP) added to the MS

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medium, and plants, *in vitro* propagated with BAP added to the MS medium.

Soluble sugar analyses

Reducing sugars were analyzed by the phenol-sulphuric acid procedure by Ashwell (1966).

Stevioside and rebaudioside A analyses

For sample preparation for HPLC analysis 50 mg of dried and powdered leaves were extracted with 5 ml of water at 40 °C in an ultrasonic bath for 30 min. The obtained extracts were centrifuged, filtered, and transferred to a volumetric flask, and methanol was added up to 5 ml. The extracts were treated with solid phase extraction (SPE) cartridges filled with C18 sorbents according to the procedure described by Bergs et al. (2012).

The HPLC analysis was performed on Shimadzu Nexera-i LC-2040C 3D Plus liquid chromatograph equipped with a photodiode array detector (Shimadzu, Japan), analytical column Intersil NH2 (3µm x 4.0 x 150 mm) (GL Sciences, Japan), wavelength 210 nm, mobile phase CH_3CN:H_2O in gradient mode, the oven temperature at 40 °C, the flow rate of 0.8 mL/min and injection volume of 4 µl.

Stevioside and rebaudioside A (Phytolab GmbH & Co. KG) were used as external standards. The quantification was performed by means of analytical standard curves prepared by mixing authentic standards at concentrations from 0.075 to 1.0 mg/ml.

Results and discussion

The obtained results showed that adding to the nutrient medium of a new type of nanofibers, formed by newly synthesized low molecular weight peptidomimetics carriers of the biologically active agent silver ion 2 % at different concentrations, has a significant effect on the growth parameters and stevioside and rebaudioside A content of micro propagated *Stevia* plantlets. Clonal propagation by direct organogenesis in the presence of the NFs riched with 2% Ag at all studied concentrations, increasing the fresh biomass, the average length of branches, and plant length, compared to the control plants. The level of the growth parameters increased until 50 mg L^{-1} NF-Ag, then at 100 mg L^{-1} NF-Ag decreased. Consequently, when applied at low doses, NF may favor the occurrence of a hormetic effect, stimulating growth characteristics of plants, however with increasing the concentration the growth was inhibited. At both control micro plantlets, no roots were recorded, but when the NF-Ag were added to the MS were recorded roots. This demonstrates that nanofibers promote rooting initiation.

There was a significant decrease in the stevioside and rebaudioside A content in stevia plantlets as well as total sugar content when adding BAP or NF-Ag at MS nutrient media compere with free of BAP media.

Conclusion

The results obtained from these analyzes make it possible to conclude that NF-Ag added to the MS media possessed a hormetic effect. At low concentrations, they have a beneficial impact on plant growth, but at high concentrations had a harmful effect. This has been also confirmed by the established reduced amount of stevioside and rebaudioside A.

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