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Short communication

Evaluation of the antioxidant activity of micropropagated stevia affected by peptidomimetic nanofibers as an Ag carrier

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Introduction

The presence of biologically active secondary metabolites in medicinal and aromatic plants makes them a good raw material for the food, cosmetic and pharmaceutical industries. Of great importance is the collection of knowledge about the level of antioxidant activity and accumulation of secondary metabolites in plants, produced by *in vitro* micropropagation using the genetically determined biosynthetic potential of species, without applying genetic manipulations.

Due to the increasing number of people who cannot or do not want to consume sucrose, the food industry is focusing on replacing artificial sweeteners with natural sugars. *Stevia rebaudiana* Bertoni leaves accumulate diterpenoid steviol glycosides, which are 300 times sweeter than ordinary table sugar and has zero calories and carbohydrates, does not cause spikes in blood sugar levels, and maintains thermal stability at 100 ° C (Geuns, 2003).

Several studies have represented data that $AgNO_3$ possessed a beneficial effect on various plant species regeneration (Hyde and Phillips 1996; Tamimi 2015). Because the ethylene and the polyamines use the same precursor for their biosynthesis inhibiting the ethylene action by $AgNO_3$ will lead to enhancing polyamine biosynthesis, which has been shown to enhance plant growth and development (Bais and Ravishankar 2002; Kumar and Rajam 2004). On the other hand, there are hypotheses that $AgNO_3$ inhibits ethylene action because the Ag ions replace Cu ions, located in the ethylene receptors leading to the reducing the capacity to bind ethylene (Jakubowicz et al. 2010).

Nanomaterials have a range of specific chemical, physical, and optical properties, which are not found in macromolecules (Tsekova et al., 2009). Silver nanoparticles have a highly developed surface area, which leads to some special characteristics and properties like high catalytic activity, reactivity, adsorption ability, and antimicrobial activity (Anchev et al., 2019). There are a number of studies in the literature about the effect of amino acids on plant propagation *in vitro*, but there is no information about their effect if they are bound in a polypeptide chain with a diameter in the nano scale, which to be a carrier of biologically active agents.

The objective of the present study was to use nanofibers, formed from a derivative of amino acid valine as a carrier of the biologically active agent silver particles (NF-2%Ag) and their impact on the antioxidant activity of *S. rebaudiana* plantlets propagated by direct organogenesis.

Materials and methods

Plant materials

For *in vitro* seed germination of *Stevia rebaudiana* Bert. surface-sterilized 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

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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 medium and plants, *in vitro* propagated with BAP.

Antioxidant capacity

The extraction and determination of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities were described by Zayova et al. (2019). Total phenolic compounds, total flavonoid content, free radical-scavenging activity (DPPH method), the ferric reducing antioxidant power (FRAP method), water-soluble (WS-AOM) and lipid-soluble (LS-AOM) metabolites with antioxidant capacity, expressed as equivalents of ascorbate and α-tocopherol were determined by Zayova et al. (2019).

Results and discussion

The level of the activity of the enzymes possessed antioxidant capacity (SOD, CAT, APX, GPX) increased when plants, were *in vitro* propagated at MS nutrient media with BAP adding, in comparison with control plants, cultured without BAP. The presence of nanofibers enriched with Ag, formed by low molecular weight peptidomimetics in the MS medium causes the amplification of the level of the activity of the enzymes with antioxidant capacity. Therefore, this activity increase to 50 mg L^{-1} NF-Ag concentration was observed. At the higher tested concentration of 100 mg $L^{-1,}$ the enzymes activities decreased. A similar trend was observed in the content of the metabolites with antioxidant activity total phenols, total flavonoids, water soluble and lipide soluble metabolites with antioxidant power in the *S. rebaudiana* plantlets extracts. Fazal et al. (2016), also reported enhanced total protein content and the activity of superoxide dismutase and peroxidase in callus cultures of *Prunella vulgaris* L when MS has been supplied with AgAu (1:3) or Au nanoparticles.

Conclusion

This study provides the first evidence of the hermetic effect of the nanofibers enriched with Ag on the culture by direct organogenesis on the development and production of natural antioxidants in *S. rebaudiana*. The activity of enzymes with antioxidant capacity increased from 0 to 50 mg L^{-1}, concentration, after that it decreased.

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