Antioxidant properties of *in vitro* cultivated *Stevia rebaudiana* Bert. treated with Valin Ag-nanofibers

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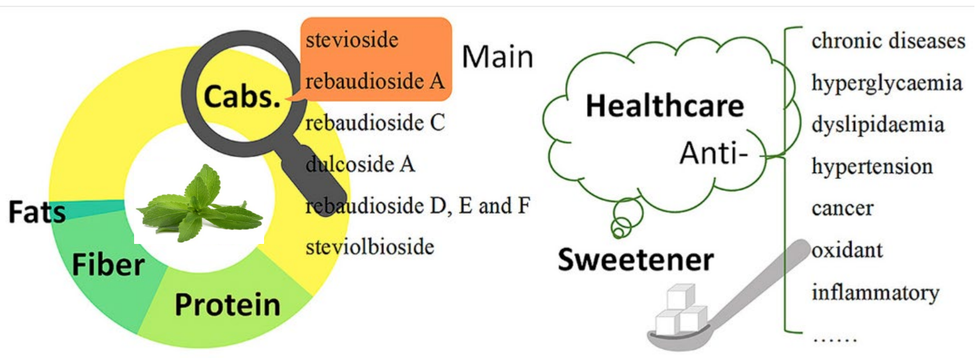
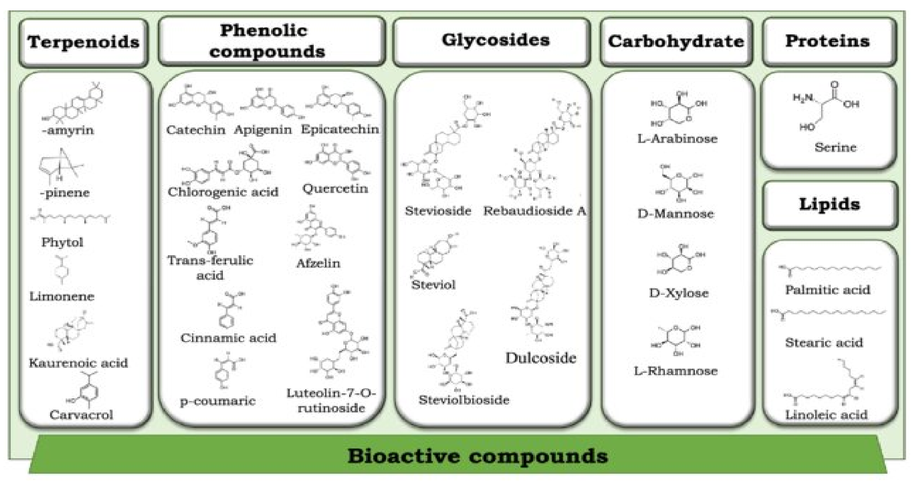


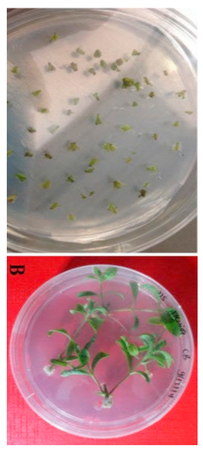


OBJECTIVE: The objective of the research was to study the impact of two low molecular weight peptidomimetics (synthesized from the amino acid valine with pyridine residue, which also has a hydrophobic spacer of six carbon atoms between two valine residues) self-regulated at nanofibers on the plant growth parameters and the antioxidant activity of *S. rebaudiana* plantlets grown by direct organogenesis and their use as carriers of the biologically active agent silver ion (NM6-1% Ag and NM6-2% Ag) in concentrations 1, 10, 50, 100 mg L^{-1}.

METHODS: The synthesis of nanofibers derived from valine loaded with colloidal silver was carried out at the Department of Organic Chemistry at the University of Chemical Technology and Metallurgy. A valine derivative containing valine residues linked to pyridine residues with a hexane linker between them was used. The resulting compound (M6) was dissolved in ethanol, then 1% and 2% colloidal silver solution were added to obtain the desired NM6 1%Ag and NM6 2%Ag nanofibers. By evaporation the solvent is removed and the resulting solid is used in the preparation of the nutrient medium in the subsequent treatment of the plants.

Initiation of *in vitro* cultures of *Stevia rebaudiana* Bert.

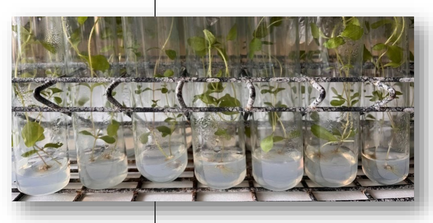
- ✓sterilized with 70% ethanol and 15% bleach.
- ✓washing 3 times with sterile distilled water in 15 min
- ✓Murashige and Skoog medium:
  30 g L^{-1} sucrose; 0.4 mg L^{-1} GA_3;
- ✓7 g L^{-1} plant agar.



MS media variations:

Controls
1 mg L^{-1} BAP
1 mg L^{-1} NM6-1%Ag
10 mg L^{-1} NM6-1%Ag
50 mg L^{-1} NM6-1%Ag
100 mg L^{-1} NM6-1%Ag

- carbon source : 20 g L^{-1} sucrose
- pH 5.7
- 0.7% agar
- Autoclaving (1.2atm, 121°C, 25 min)
- 22 ± 2 °C, 16 h daylight (4 weeks)



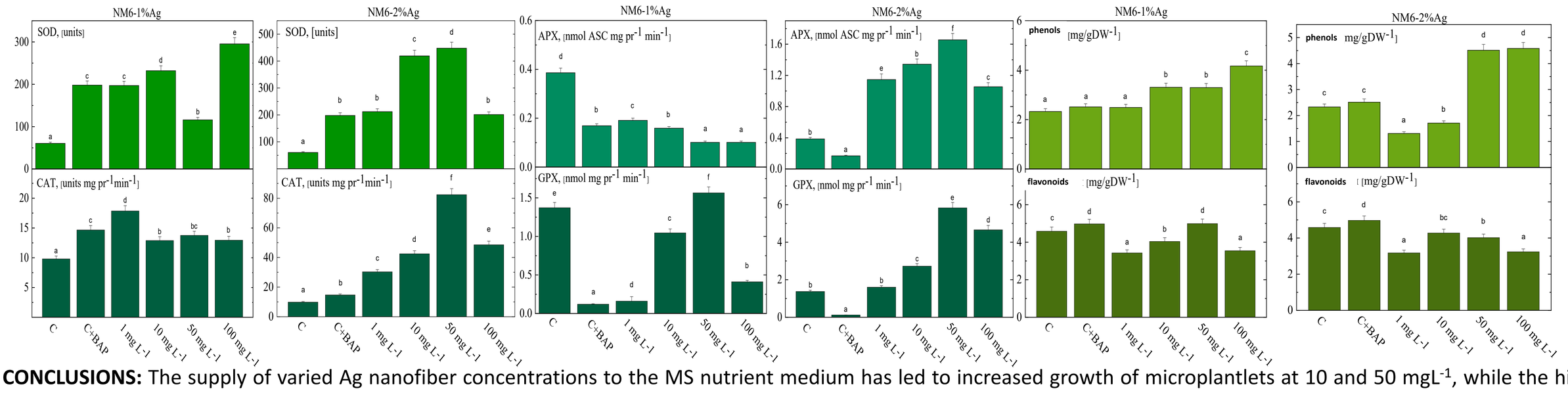
RESULTS:

Biometry of *Stevia rebaudiana* Bert microseedlings, treated with NM6 – 1%Ag and NM6-2%Ag in concentrations 1, 10, 50, 100 mg L^{-1}

variants	FW microplantlets g	Height microplantlets cm	Number microplantlets explants^{-1}	Root formation %
control plants	0.115	5.97	1.01	0.04
1 mg L^{-1} BAP	0.353	7.58	1.70	0.00
1 mg L^{-1} NM6-1%Ag	0.305	6.83	1.39	15.81
10 mg L^{-1} NM6-1%Ag	0.334	7.30	1.34	18.30
50 mg L^{-1} NM6-1%Ag	0.371	8.39	1.45	42.92
100 mg L^{-1}NM6-1%Ag	0.285	6.58	1.40	37.12
LSD	0.026	0.62	0.11	2.50

variants	FW microplantlets g	Height microplantlets cm	Number microplantlets explants^{-1}	Root formation %
control plants	0.115	5.97	1.01	0.04
1 mg L^{-1} BAP	0.353	7.58	1.70	0.00
1 mg L^{-1} NM6-2%Ag	0.377	8.28	2.44	13.68
10 mg L^{-1} NM6-2%Ag	0.412	8.63	3.35	17.13
50 mg L^{-1} NM6-2%Ag	0.464	10.79	3.25	28.43
100 mg L^{-1} NM6-2%Ag	0.193	5.35	1.25	26.00
LSD	0.033	0.69	0.20	1.80

Antioxidant enzymes activity, total phenolic and flavonoid contentof *Stevia rebaudiana* Bert microseedlings, treated with NM6 – 1%Ag and NM6-2%Ag in concentrations 1, 10, 50, 100 mg L^{-1}



CONCLUSIONS: The supply of varied Ag nanofiber concentrations to the MS nutrient medium has led to increased growth of microplantlets at 10 and 50 mgL^{-1}, while the highest concentration of 100 mg L^{-1} inhibited growth. A significant increase in the percentage of root formation was also observed, which is almost zero in controls and plants grown in an MS with BAP adding. NM6-2% Ag application led to a higher activity of the enzymes with antioxidant activity (SOD, CAT, APX and GPX), than NM6-1% Ag adding. The enzyme activity levels rose with an increase in NM6-2% Ag concentration from 1 to 50 mg L^{-1}, while the addition of 100 mg L^{-1} led to a decrease in their activity.

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