

Influence of Nanofiber enriched with Ag salt on the accumulation of metabolites in *Stevia Rebaudiana Bertoni*

Elisaveta Kirova^{1*}, Maria Petrova¹, Kamelia Miladinova-Georgieva¹, Maria Geneva¹, Lyubomir Vezekov, Daniela Tsekova², Mariana Sichanova¹

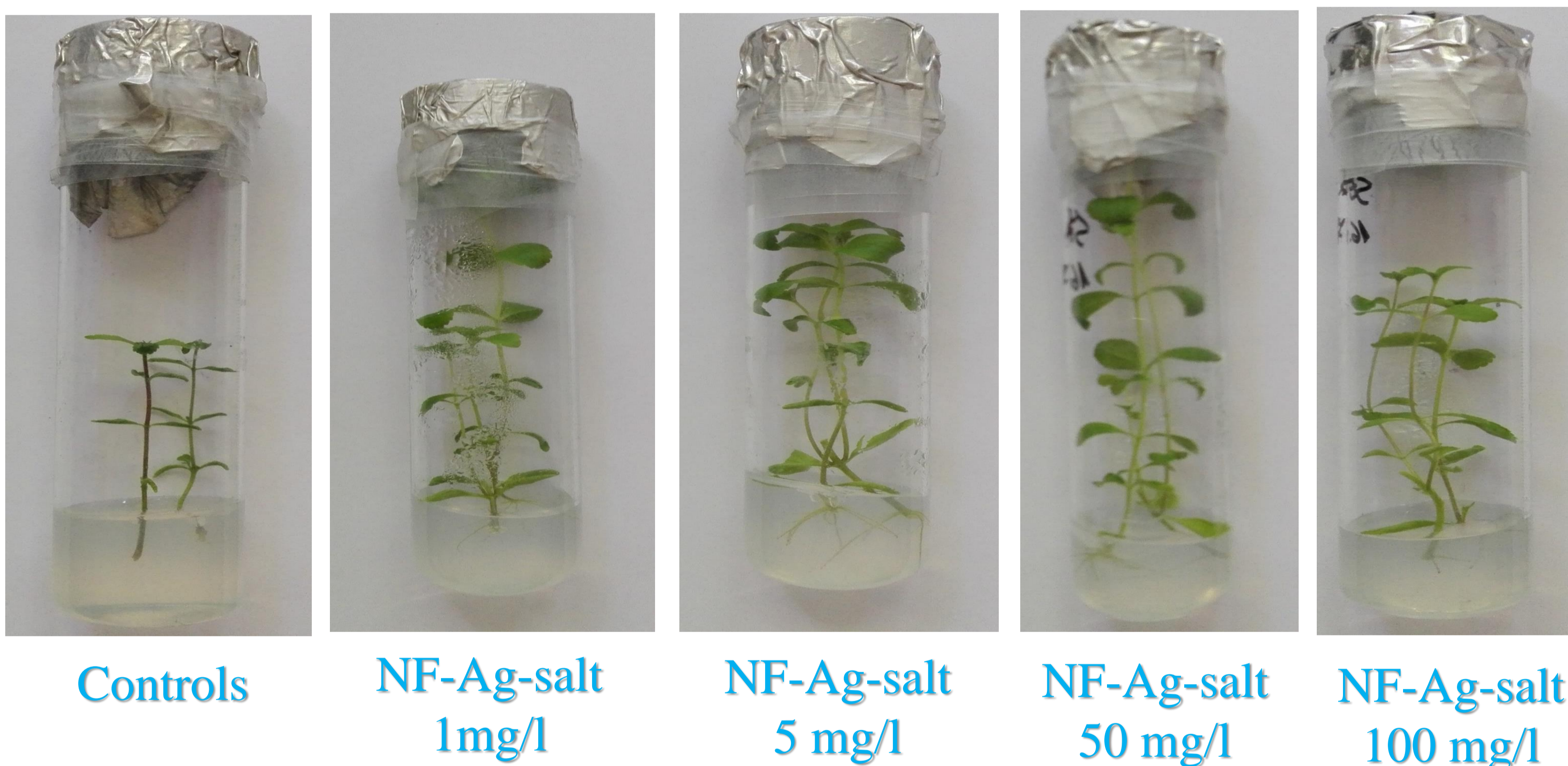
¹Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bldg. 21, 1113 Sofia, Bulgaria

²Department of Organic Chemistry, University of Chemical Technology and Metallurgy, 8 "St. Kl. Ohridski" Blvd, Sofia 1756, Bulgaria

* corresponding author: *elisab@abv.bg

SUMMARY; *Stevia (Stevia rebaudiana Bertoni)* is herbaceous perennial medicinal plant belonging to Asteraceae family and originating from Paraguay and Brasil. In order to determine the optimal conditions for *in vitro* propagation of the plant to standard MS nutrient medium various concentrations (1, 10, 50 100 mg L⁻¹) of nanofibers have been added which had been previously enriched with silver salt. Control plants were grown on pure MS without growth regulators and nanofibers added. The aim of using nanofibers as silver carriers in direct organogenesis was to establish the proper concentration for maximal accumulation of biologically active substances, sugars and secondary metabolites with antioxidant potential.

MATERIALS AND METHODS: To determine the optimal conditions for *in vitro* propagation of the plant in standard MS medium, various concentrations (1, 10, 50 100 mg L⁻¹) of nanofibers were added, which were previously enriched with silver salt. Control plants were grown on pure MS without added growth regulators and nanofibers. NF-enriched with silver salt in concentrations of 1, 5, 10, 50, 100 mg / l MS are included in the MS nutrient medium. Control plants were grown on MS medium without plant growth regulators and other additives. The medium contains a constant quality of agar-agar (0.7%) and sucrose (3%). The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 20 minutes at a pressure of 1.1 kg cm⁻². *In vitro* cultures are maintained in growth room conditions at 22 ± 2°C, relative humidity 70% and 16 h photoperiod at 40 μmol m⁻²s⁻¹ illumination provided by white fluorescent lamps.



The purpose of using NF-enriched with Ag salt in different concentrations is to determine which of them plants accumulate the greatest amount of biologically active substances such as sugars and substances with antioxidant activity and the inclusion of nanotechnology for their rapid production.

RESULTS: Fresh biomass, shoot height, number of stem branches and rooting percentage were assessed for morphological characterization of the plants. A comparative characteristic of biometric parameters of *in vitro* propagated *Stevia* plants was accomplished based on obtained results. It was demonstrated found that highest shoot fresh biomass and stem branching was determined in variants grown on medium containing 50 mg/l Ag-salt enriched nanofibers. Increasing the concentration of nanofibers enriched with silver salts from 1 to 100 mg L⁻¹ disclosed/showed that biomass accumulation was mainly due higher branching as the mean stem length per plant was reduced Table 1.

Under various stress conditions plants usually accumulate osmolytes like proline, sugars and carotenoids. The addition of NF-Ag salt to MS medium led to significant decrease in the amount of stress markers like proline, MDA and H₂O₂ and to increase in SH-group containing compounds (Fig.1).

In conclusion, it can be said that the concentration of Ag-salts is particularly important for the accumulation of metabolites with antioxidant activity and their inclusion in the future in nanotechnology.

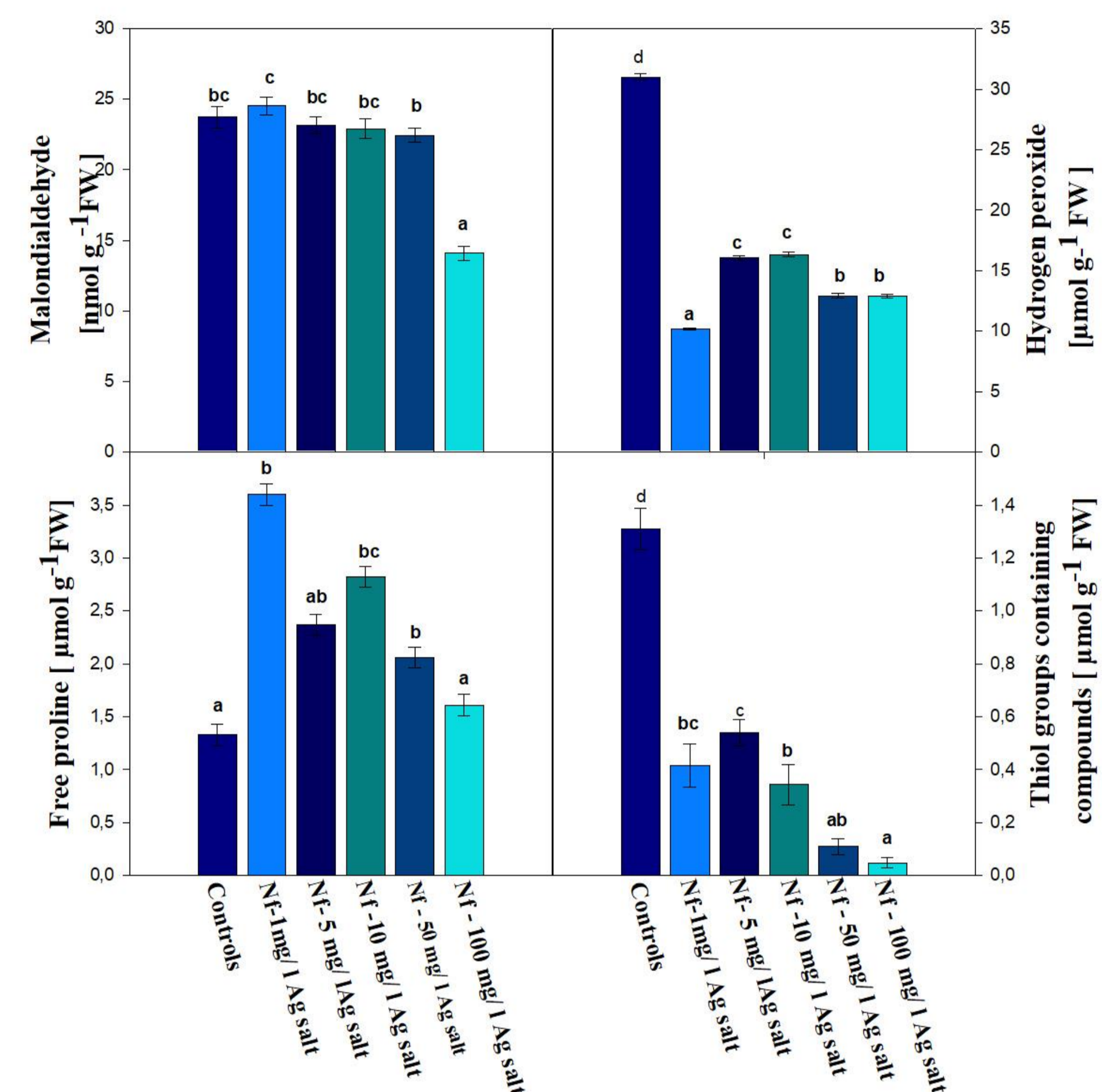


Figure 1. Influence of NF enriched with Ag salt in concentrations of 1, 5, 10 50, 100 mg / l on the antioxidant capacity of *Stevia*.

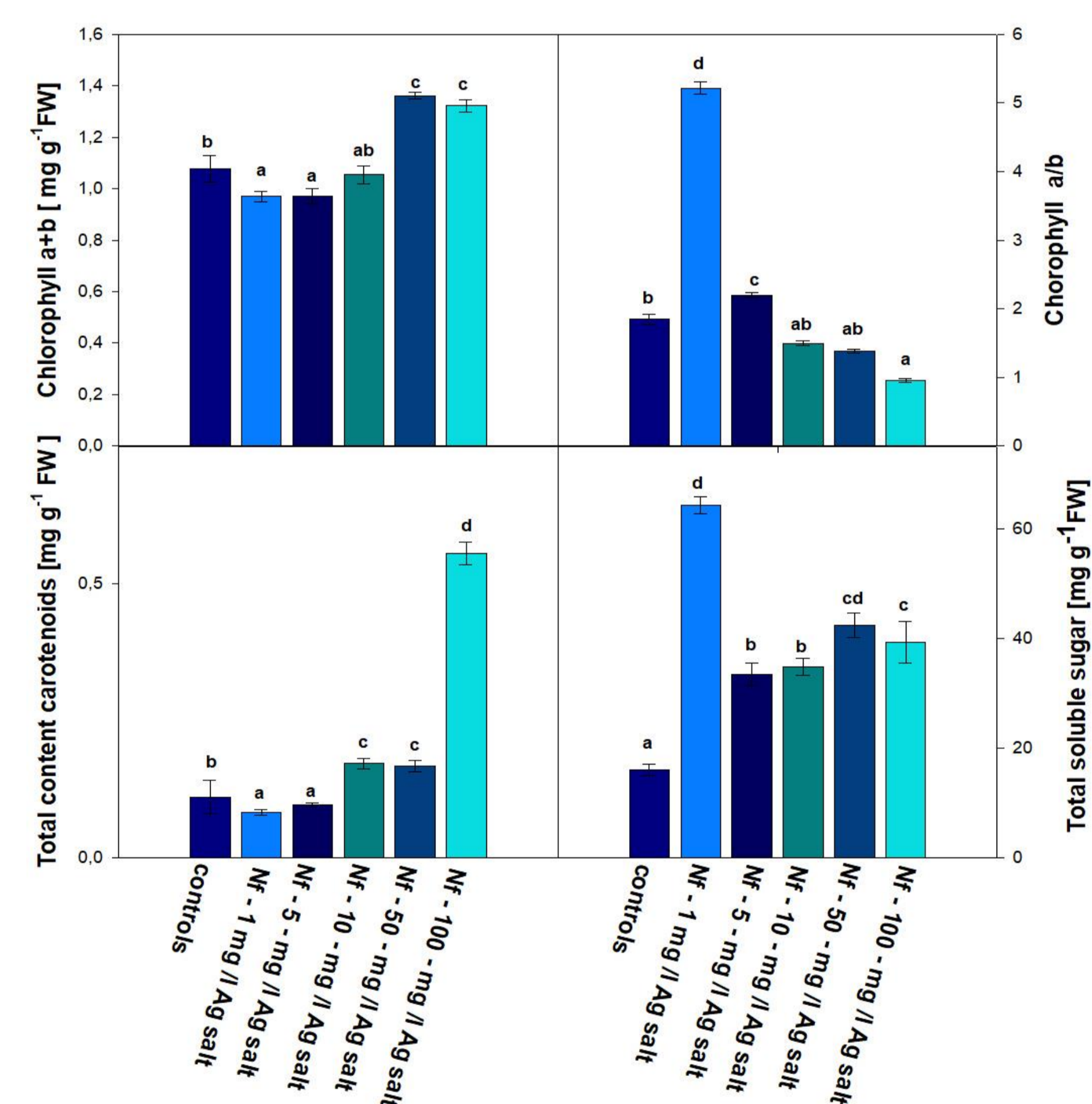


Figure 2. Effect of NF enriched with Ag salt in concentrations of 1, 5, 10 50, 100 mg / l on pigment content and soluble sugars.

Table 1. Effect of NF enriched with Ag salt in concentrations of 1, 5, 10 50, 100 mg / l on biometrics of plants.

Variants	FW shoot g	Average shoot length cm	Number of branches/plant	Rooting %
Controls + BAP	0,353±0,04	7,58±0,04	1,7±0,05	0,042
NF-Ag salt 1 mg/l	0,111±0,04	10,14±0,05	1,45±0,04	80,00
NF-Ag salt 5 mg/l	0,333±0,03	8,08±0,04	1,4±0,03	52,50
NF-Ag salt 10 mg/l	0,374±0,02	6,39±0,03	1,99±0,05	51,28
NF-Ag salt 50 mg/l	0,389±0,04	5,48±0,04	3,07±0,04	51,70
NF-Ag salt 100 mg/l	0,237±0,02	2,01±0,01	1,44±0,04	52,60