

Effect of silver nanofibers on non-enzymatic antioxidant defense in *Stevia rebaudiana* subjected to drought

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BACKGROUND: *Stevia rebaudiana* B. is a herbaceous perennial plant of the warm and humid tropical climate of Asteraceae family and is known as stevia, sweet leaf, honey leaf, and candy leaf. Stevia leaves contain many biologically active substances, which have beneficial effects on human health. *Stevia rebaudiana* is popularly used as an unusual plant with natural sweetener potential, without the harmful effects of sugar and artificial sweeteners. The antioxidant activity of *in vitro* cultivated *S. rebaudiana* is influenced by growth regulators, amino acids and silver nanoparticles added to the nutrient media.



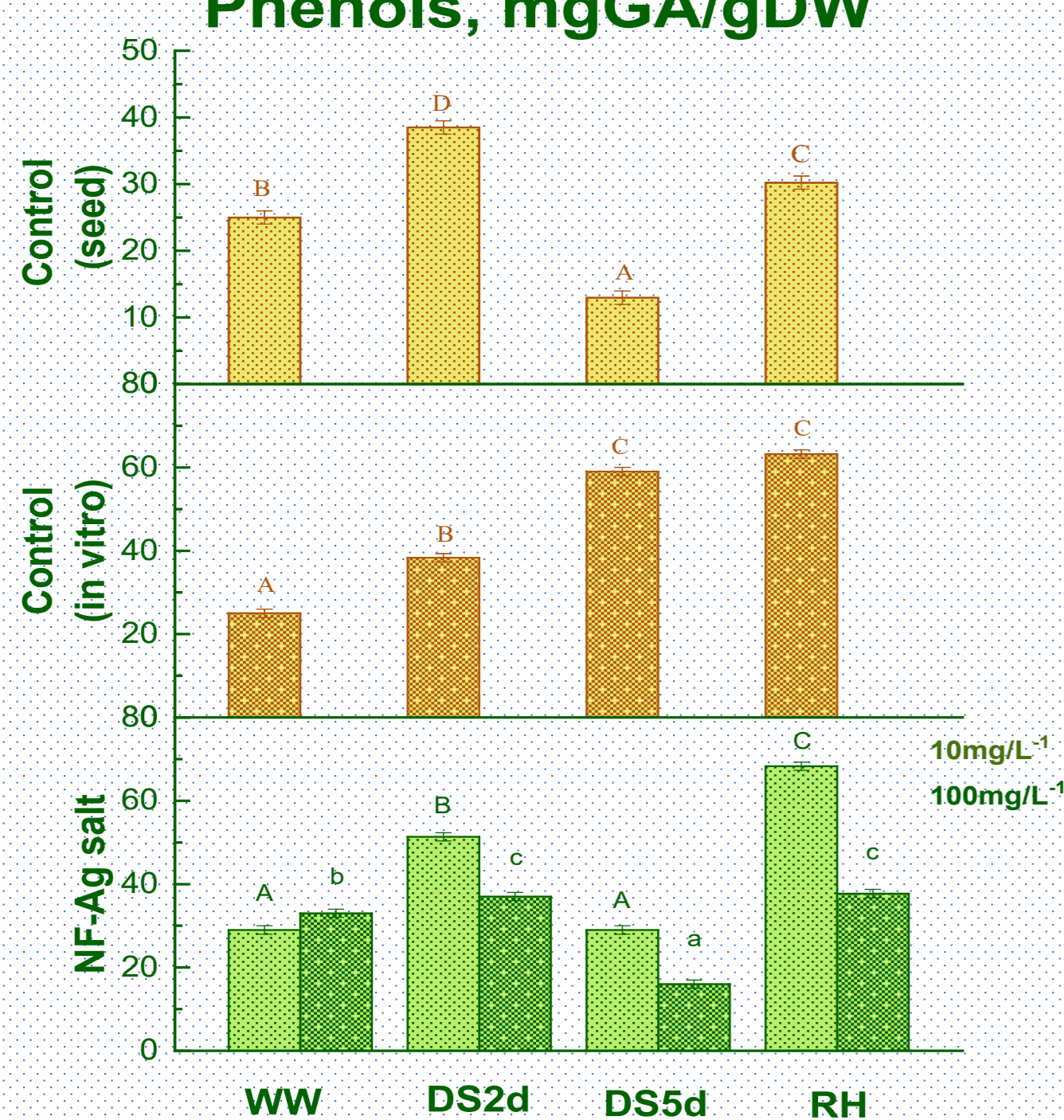
Stevia rebaudiana two months after *ex vitro* adaptation

OBJECTIVE: This study aimed to evaluate the influence of the concentration (10 and 100 mg L⁻¹) of Ag nanofibers on the non-enzymatic antioxidant metabolites (phenols, flavonoids, water-, lipid-soluble antioxidants and total antioxidants measured by the FRAP method) in *S. rebaudiana*. Two months after adaptation, two-month-old plants were examined for the effects of drought.

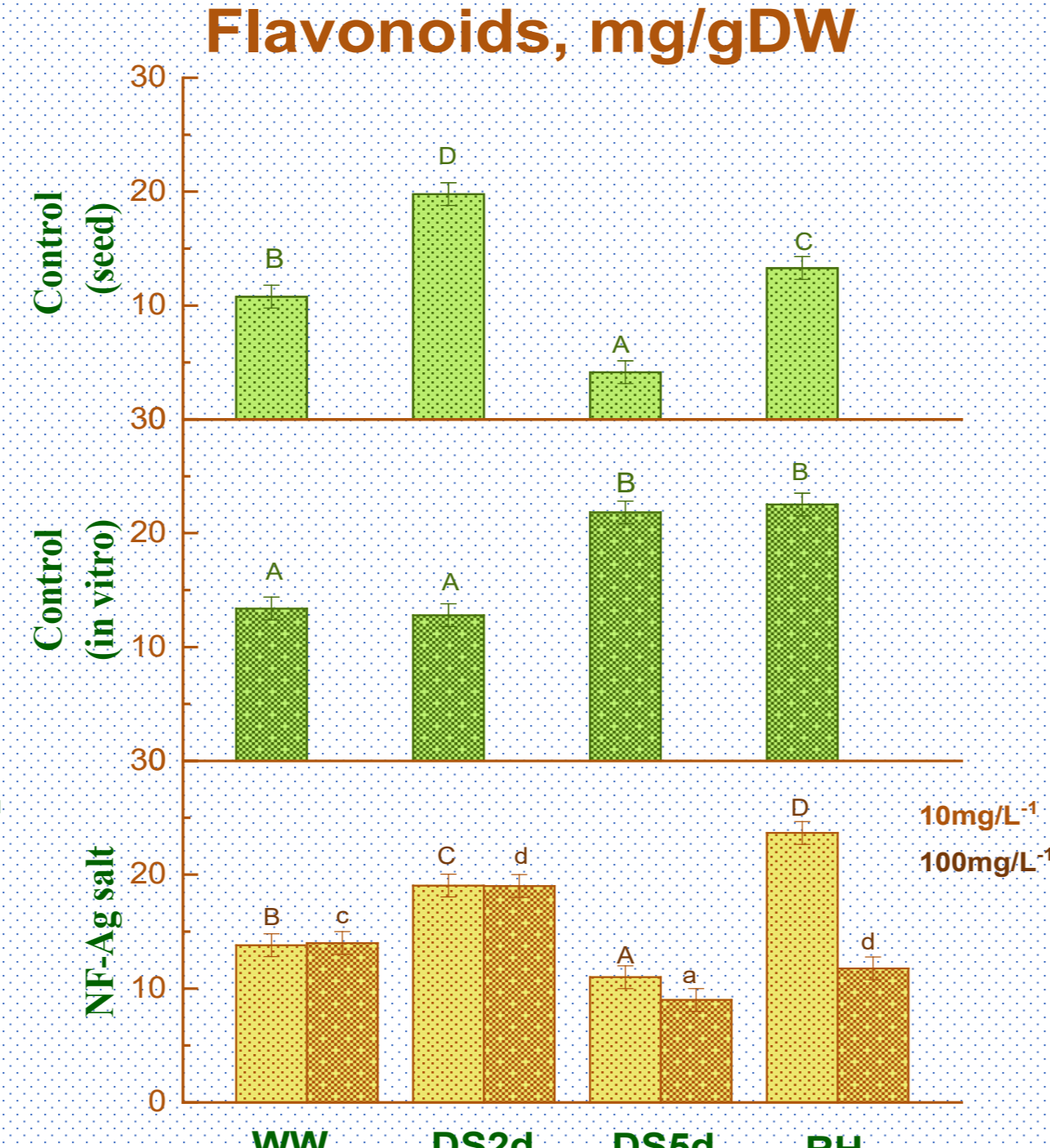
MATERIALS AND METHODS

The nanofibers Ag salt was synthesized by aspartic acid derivatives self-organized with a monomeric molecular structure containing one residue of L-Asp with one hydrophilic head which bonds one Ag ion (NF-Ag salt). Shoot explants (1.5–2 cm) were cultured on MS media including vitamins supplemented with 3.0% sucrose, 7.0 g L⁻¹ agar, and 100 mg L⁻¹ NF-Ag salt. All conditions for *in vitro* propagation, adaptation and cultivation of plants *ex vitro* were met. To quantify the main complexes of biologically active substances in *Stevia rebaudiana*, several phytochemical spectrophotometric methods were used to measure the non-enzymatic antioxidant activity.

Phenols, mgGA/gDW

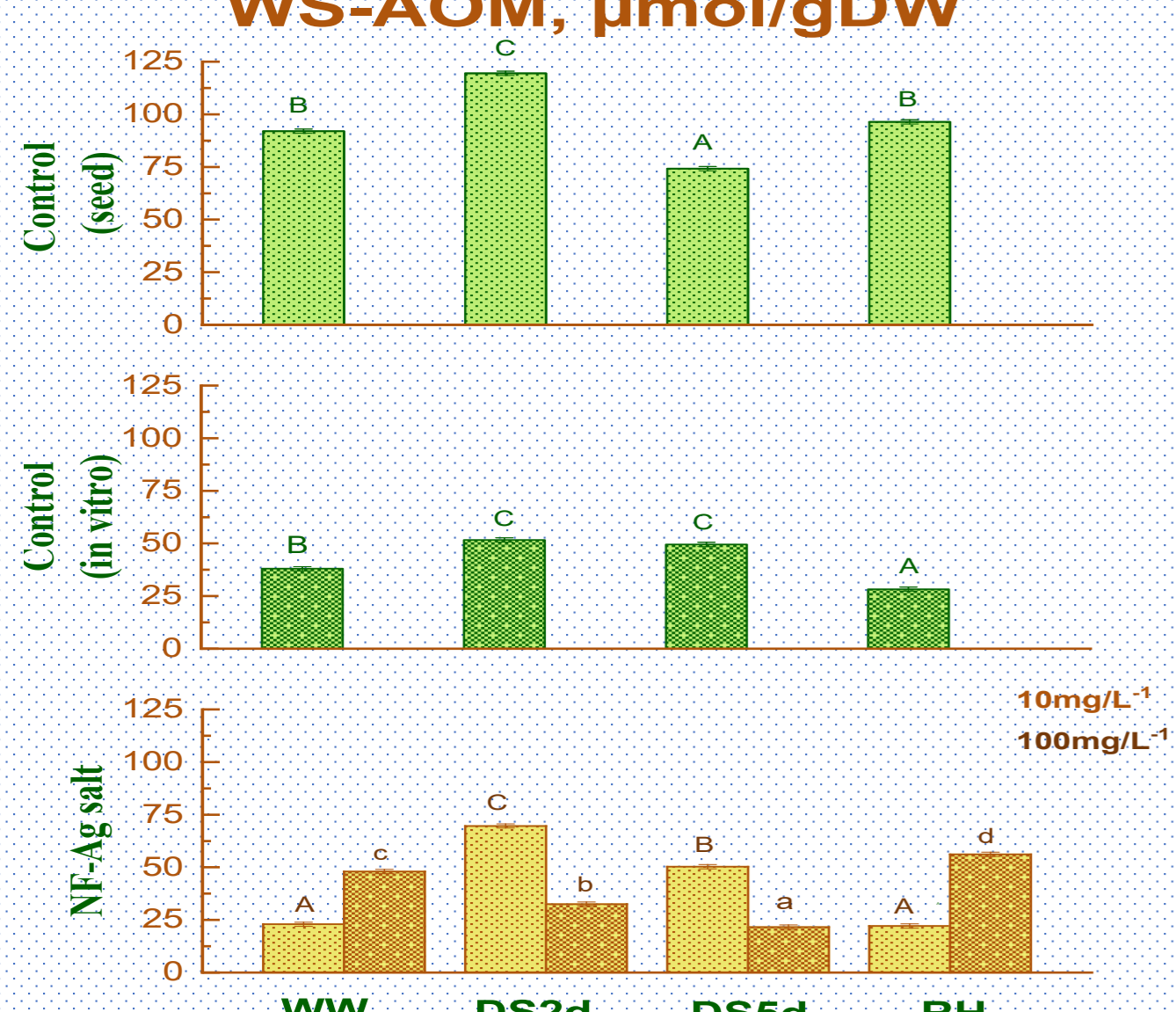


Flavonoids, mg/gDW

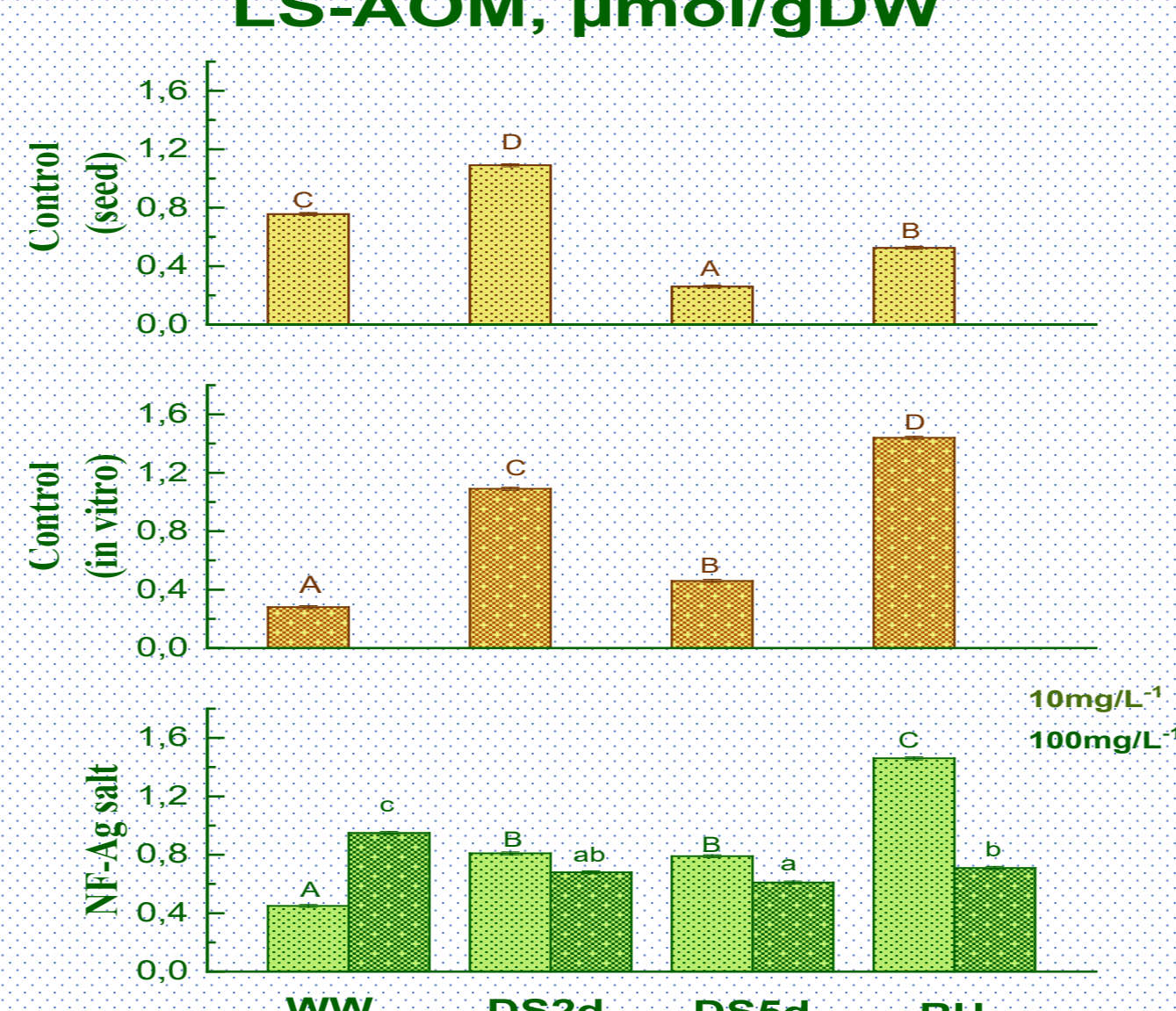


Content of low molecular metabolites with antioxidant capacity as phenols, flavonoids, water-soluble-, lipid-soluble antioxidants and total antioxidants measured by the FRAP method in *Stevia rebaudiana* plants propagated by seeds (Control-seeds), *in vitro* propagated on MS medium (control-*in vitro*) and *in vitro* propagated on MS medium supplemented with various concentrations (10 and 100 mg L⁻¹) of NF-Ag salt, with subsequent soil adaptation, grown for two months in soil and drought-stressed for 2 and 5 days, respectively.

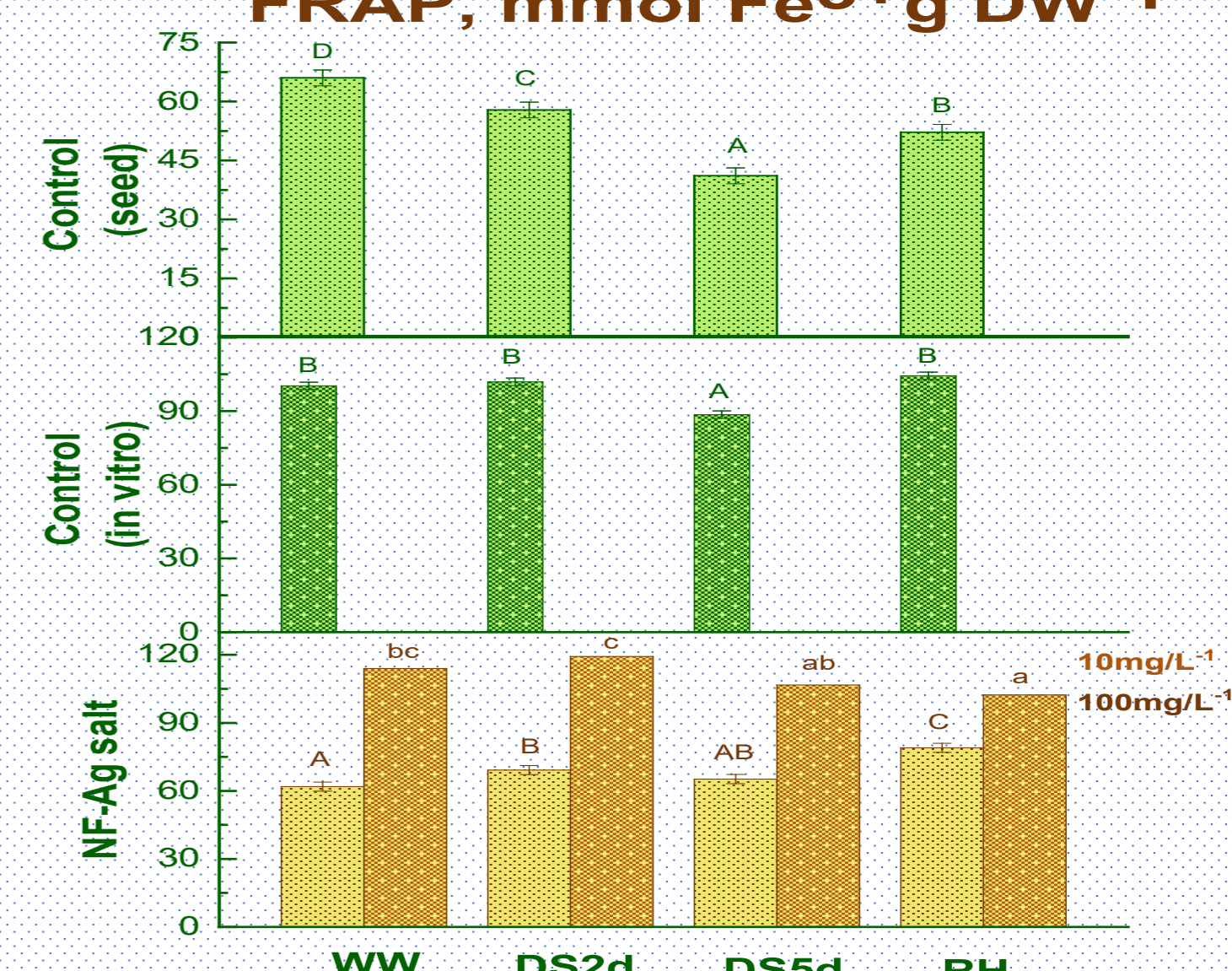
WS-AOM, μmol/gDW



LS-AOM, μmol/gDW



FRAP, mmol Fe³⁺+g DW⁻¹



Results: The obtained results demonstrated that, the application of 10 mg L⁻¹ and 100 mg L⁻¹ NF-Ag salt in MS nutrient medium during micropropagation and subsequent drought of the *ex vitro* adapted plants led to a higher content of most metabolites with antioxidant potential in short-term drought (2nd day) and their lower content in long-term drought (5th day). With few exceptions, rehydration leads to an increase in the content of metabolites with antioxidant capacity, compared to the fifth day of drought, at both tested concentrations. Further studies are needed to evaluate the influence of the studied nanofibers NF-Ag salts on plantlets' soil adaptation.

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Conclusions: In this study, the investigated nanofibers support the metabolism of *Stevia rebaudiana* under stress conditions and demonstrate great potential in plant-tissue cultures. It is a challenge for scientists to look for progress and help achieve physical and mental health more easily.