Evaluation of the enzyme antioxidant activity of ex vitro adapted stevia pretreated with Ag salt peptidomimetic, under the drought

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Background

For centuries, plants have been used as a valuable food source, as flavors, colorings and components with use in cosmetic and pharmaceutical industries. However, this has a detrimental effect on the natural population of the medicinal plant, putting many of them in danger of going extinct. The plants' natural habitats are quickly exhausted due to climate change and developing agriculture. Biotechnological methods for propagation are used to prevent the destruction of natural populations of plants. Plants in vitro systems allow the cultivation of individual cells, tissues, organs or entire plants. Stevia rebaudiana Bert. is a source of alternative calorie-free sweeteners, gaining popularity globally. The two main steviol diterpene glycoside, stevioside and rebaudioside A provide the sweet taste of the plant and are 150-450 times sweeter than sucrose for human taste receptors. The in vitro technique of S. rebaudiana propagation is graceful for producing high quantity and quality seedlings, for supporting the cultivation of plants of improved quality and high content of stevioside and rebaudioside A, higher production of biomass, wider adaptability and viability.

Aims

This study aims to evaluate *Stevia rebaudiana*'s physiological response to water deficit applied *ex vitro* after being cultivated *in vitro* on MS media supplemented with amino acid nanofibers carriers of silver at varied concentrations. The effect of 2 and 5 days of drought is monitored, followed by 3-day rehydration, on the enzyme antioxidant system of *ex vitro* adapted to the soil *S. rebaudiana* plantlets propagated in the MS nutrient medium, with 10 and 100 mg L^{-1} Ag salt of nanofibres, synthesized by asparagine acid derivative (NF1-Ag salt).

Material and Methods

The organic compound used for the silver salt synthesis was dekanoyl-L-Asp-N-hexylamide salts, which can self-organized into nanofibers with monomeric molecular structures. The Ag salt of organic nanofibers is obtained through interactions of the organic compound with an equivalent amount of silver nitrate ($AgNO_3$) in basic solutions (NF1-Ag salt), and containing one residue of L-Asp with one hydrophilic head which bonds one Ag ion.

The *Stevia rebaudiana* plants were propagated by seeds (control-seeds) and *in vitro* on an MS medium (*control-in vitro*) and on MS medium containing NF1-Ag salt at 10 and 100 mg L-1 concentrations for one month. After that, plantlets with a well-developed root system were transferred to small vessels (8 cm in diameter) containing a mixture of soil, sand and perlite (1:1:1) and were maintained in a growth chamber. The plants were covered with a transparent polyethylene membrane to provide high humidity. The polyethylene was open after 2 weeks. After 1 month of adaptation, the plants were transferred to a greenhouse for further growth development. After 1 week of acclimatization, the plants were transferred to vessels with 4 kg of soil: sand (3: 1, V: V). After 2 months plants with visibly uniform growth were selected and drought-stressed for a period of 2 and 5 days, after that were rehydrated for 3 days. To evaluate the antioxidant properties, plant samples were taken for analysis on days two and five of the drought as well as upon 3 days of rehydration.

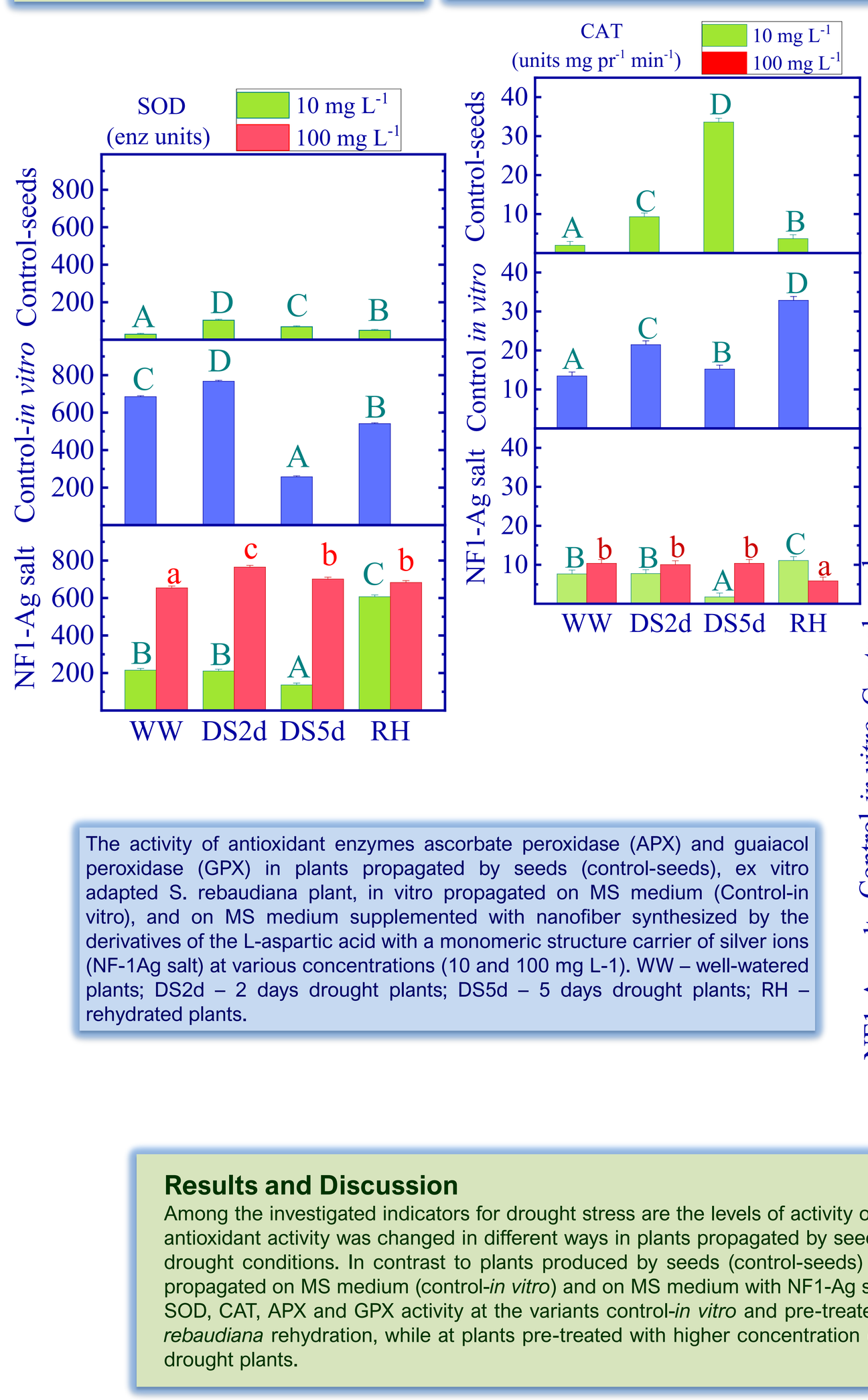
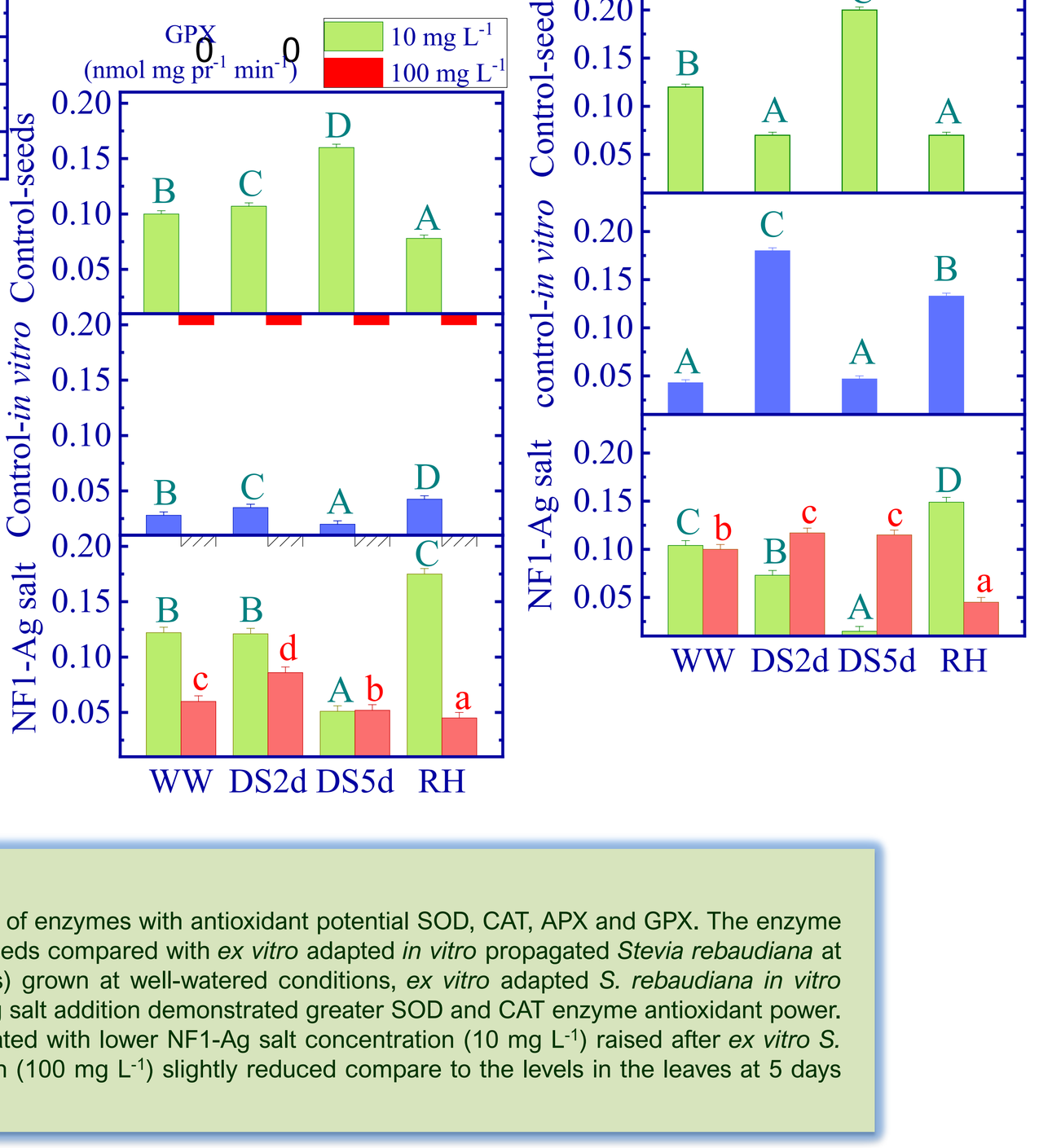


Figure 1. The activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in plants propagated by seeds (control-seeds), *ex vitro* adapted *S. rebaudiana* plant, *in vitro* propagated on MS medium (Control-*in vitro*), and on MS medium supplemented with nanofiber synthesized by the derivatives of the L-aspartic acid with a monomeric structure carrier of silver ions (NF-1Ag salt) at various concentrations (10 and 100 mg L-1). WW – well-watered plants; DS2d – 2 days drought plants; DS5d – 5 days drought plants; RH – rehydrated plants.



The activity of antioxidant enzymes ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in plants propagated by seeds (control-seeds), ex vitro adapted S. rebaudiana plant, in vitro propagated on MS medium (Control-in vitro), and on MS medium supplemented with nanofiber synthesized by the derivatives of the L-aspartic acid with a monomeric structure carrier of silver ions (NF-1Ag salt) at various concentrations (10 and 100 mg L-1). WW – well-watered plants; DS2d – 2 days drought plants; DS5d – 5 days drought plants; RH – rehydrated plants.

Results and Discussion

Among the investigated indicators for drought stress are the levels of activity of enzymes with antioxidant potential SOD, CAT, APX and GPX. The enzyme antioxidant activity was changed in different ways in plants propagated by seeds compared with *ex vitro* adapted *in vitro* propagated *Stevia rebaudiana* at drought conditions. In contrast to plants produced by seeds (control-seeds) grown at well-watered conditions, *ex vitro* adapted *S. rebaudiana in vitro* propagated on MS medium (control-*in vitro*) and on MS medium with NF1-Ag salt addition demonstrated greater SOD and CAT enzyme antioxidant power. SOD, CAT, APX and GPX activity at the variants control-*in vitro* and pre-treated with lower NF1-Ag salt concentration (10 mg L^{-1}) raised after *ex vitro S. rebaudiana* rehydration, while at plants pre-treated with higher concentration (100 mg L^{-1}) slightly reduced compare to the levels in the leaves at 5 days drought plants.

Conclusions

The increased drought resistance of Stevia rebaudiana plants *in vitro* propagated and soil adapted can be attributed to their distinct enzyme antioxidant responses as compared to plants cultivated from seeds.

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