Analysis of Drought Tolerance in *Stevia rebaudiana*, Propagated by Seed and *in vitro* with Silver Salt of Peptidomimetic Nanofiber

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Water stress is a significant threat to the growth and productivity of Stevia rebaudiana, a plant valued for its natural sweetening compounds. This study investigates the drought tolerance of Stevia rebaudiana across three cultivation methods: seed germination, in vitro propagation, and in vitro growth with silver salt nanofiber treatment. The evaluation focused on key stress markers, including malondialdehyde (MDA), proline, hydrogen peroxide (H_2O_2), and sulfhydryl (SH) groups.

Plant Material and Growth Conditions:

Stevia (Stevia rebaudiana) plants were propagated both from seeds (control seeds) and in vitro on Murashige and Skoog (MS) medium (control-in vitro). Additionally, in vitro propagation was conducted on MS medium supplemented with NF1-Ag salt at concentrations of 10 mg L^{-1} and 100 mg L^{-1} for a period of one month. After this initial growth period, plantlets with well-developed root systems were transferred to small vessels (8 cm in diameter) containing a soil mixture of soil, sand, and perlite in a 1:1:1 ratio. The plantlets were maintained in a growth chamber under controlled conditions, and to ensure high humidity, they were initially covered with a transparent polyethylene membrane. The polyethylene cover was removed after 2 weeks.

Acclimatization and Drought Stress Treatment

After one month of adaptation in the growth chamber, the plants were transferred to a greenhouse for further development. After one week of acclimatization in the greenhouse, the plants were transplanted into vessels containing 4 kg of soil and sand mixture in a (3:1 v:v) ratio. The plants were then allowed to grow uniformly for 2 months, after which those exhibiting uniform growth were selected for drought stress treatment. The selected plants were subjected to drought stress for periods of 2 and 5 days, followed by a rehydration period of 3 days.

Fresh leaf material (approximately 300 mg) was homogenized with 0.1% (w/v) trichloroacetic acid for determination of proline, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content. Free proline was derivatized with acid ninhydrin and absorbance was read at 520 nm according to Bates et al. (1973).Malondialdehyde content was determined as thiobarbituric acid-reagent product according to Kramer et al. (1991) by using the extinction coefficient 155 mM^{-1} cm^{-1}. Hydrogen peroxide content was estimated spectrophotometrically according to Alexieva et al. (2001). The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2. The total phenolics content was measured according to Swain and Goldstein (1964).

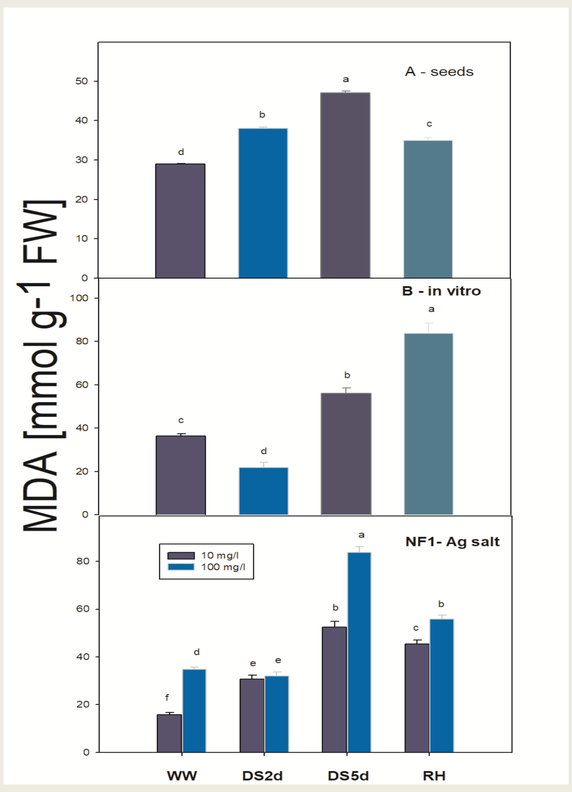

Fig1. Malondialdehyde content –ww-controls plants; ds2- two-day dried plants; ds5d- five-day dried plants; RH-recovery plants

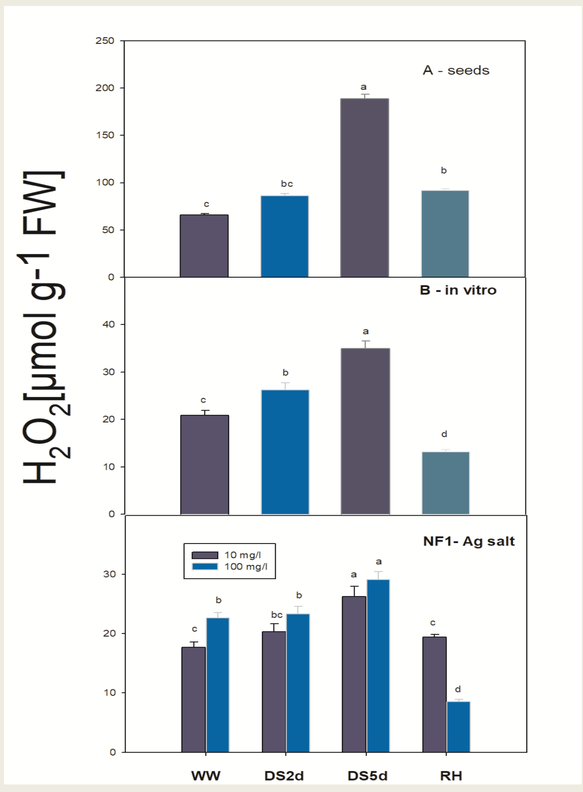

Fig2. Hydrogen peroxide content –ww-controls plants; ds2- two-day dried plants; ds5d- five-day dried plants; RH-recovery plants

Our results demonstrated that *Stevia* plants propagated in vitro exhibited higher drought tolerance compared to those grown from seeds, as evidenced by lower MDA and H2O2 levels, indicating reduced lipid peroxidation and oxidative stress (Fig.1; Fig.2) MDA content increased significantly under drought stress (especially under DS5d conditions) and decreased after rehydration (RH). Plants treated with 100 mg/L silver salt showed lower MDA content compared to those treated with 10 mg/L, suggesting that higher concentrations of silver salt treatment may provide better protection against lipid peroxidation.NF1-Ag salt treatment generally decreased MDA levels compared to untreated plants, suggesting better protection against drought-induced oxidative damage. Compressive analysis The figures compare the oxidative stress responses (via H_2O_2 and MDA levels) in plants propagated by seeds, in vitro, and those treated with silver salt nanofibers. Under drought conditions (DS5d): Plants propagated by seed showed higher oxidative stress (both in H_2O_2 and MDA levels), indicating higher sensitivity to drought. In vitro propagated plants show moderate levels of stress. Silver salt-treated plants (NF1-Ag salt) showed the lowest stress markers, suggesting increased drought tolerance due to the treatment.

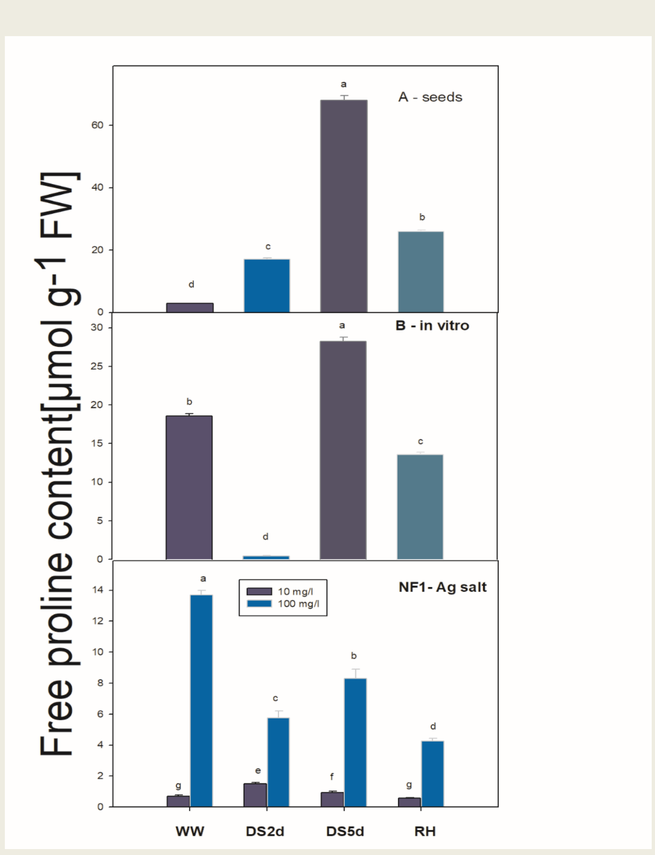

Fig3. Proline content–ww-controls plants; ds2- two-day dried plants; ds5d- five-day dried plants; RH-recovery plants

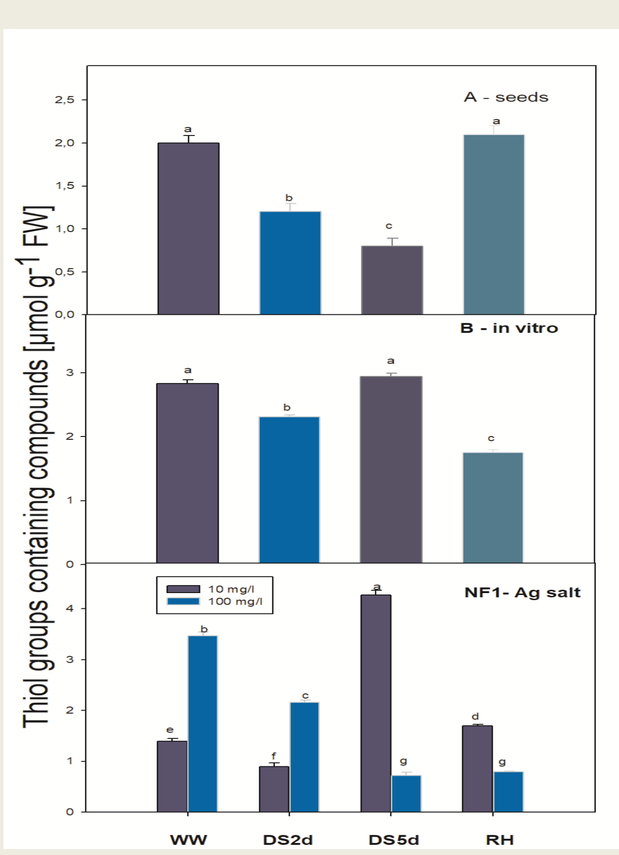

Fig4. Thiol groups containing compounds - ww-controls plants; ds2- two-day dried plants; ds5d- five-day dried plants; RH-recovery plants

Proline accumulation was significantly higher in vitro propagated plants, suggesting an enhanced osmoprotectant response (Fig.3; Fig.4). Additionally, SH group content, indicative of protein stability and cellular defense mechanisms, was substantially elevated in plants treated with silver salt nanofibers (100 mg/l Ag salt). These findings suggest that in vitro propagation, particularly with silver salt nanofiber treatment (100 mg/l Ag salt), enhances the drought resilience of *Stevia rebaudiana*.

In the comparative analysis, *in vitro* propagated *Stevia rebaudiana* treated with silver salt of peptidomimetic nanofiber likely shows superior drought tolerance compared to seed-propagated plants, reflected by lower oxidative damage (lower MDA, H_2O_2 levels) and higher protective compounds (proline, SH-groups). This suggested that nanotechnology could offer a valuable tool for enhancing stress tolerance in plants.

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