

ORGANIC NANOPARTICLE-ASSISTED STRESS MODULATION IN STEVIA REBAUDIANA: BIOCHEMICAL IMPACTS OF M6 AND M6+IAA IN *IN VITRO* CULTURES

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

¹ Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria

² Department of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

* Presenting Author: elisab@abv.bg

SUMMARY

Stevia Rebaudiana is sensitive to oxidative stress during *in vitro* cultivation. This study explores the use of organic nanofibers (M6), formed from L-valine and nicotinic acid, as carriers for the auxin IAA in the *in vitro* propagation of *Stevia rebaudiana*.

M6 nanofiber are emerging nanomatirials with the potential to modulate stress responses. IAA is a plant hormone implicated in stress regulation. *In vitro* plant growth often suffers due to suboptimal conditions like poor gas exchange, high humidity, and low light. To mitigate these stressors, culture media are commonly supplemented with growth-enhancing substances. Results indicate that M6 alone significantly improved shoot growth, soluble sugar levels, phenolic content, and steviol glycosides. Conversely, the M6+IAA combination notably enhanced root initiation, caffeoylquinic acid accumulation, and antioxidant enzyme activity. These findings support the potential of nanofiber-based hormone delivery systems to boost plant development and therapeutic metabolite production.



The study is based on the hypothesis that organic nanofibers used as carriers for plant growth hormones will positively influence the physiology, biochemistry, and morphology of *in vitro* *Stevia Rebaudiana* plantlets, thereby enhancing the production of pharmacologically valuable antioxidant compounds. In this research, we employed organic peptidomimetics as a delivery system for the auxin indole-3-acetic acid (IAA) to investigate the impact of nanofibers on the propagation and antioxidant enzyme activity of *in vitro* *Stevia rebaudiana* Bert. For this purpose, the IAA carrier used was an organic compound composed of two valine and nicotinic acid fragments, linked and dimerized via a diaminohexane spacer, with the ability to form nanofibrillar networks.

Objective:

To evaluate how different concentrations of M6 and M6-IAA nanofibers affect oxidative stress biomarkers in *Stevia Rebaudiana*

RESULTS

The application of M6-Indolyl Acetic Acid (M6-IAA) induced significant changes in oxidative stress markers in a concentration-dependent manner. (Fig. 1). Hydrogen peroxide and MDA levels were highest in control plants, indicating elevated oxidative stress. M6-IAA at 1 mg/L notably reduced H₂O₂ levels, while 10 mg/L caused a partial increase. The lowest H₂O₂ content was observed at 50 mg/L. MDA levels declined consistently with treatment, suggesting membrane protection. Proline content increased with M6-IAA, peaking at 10 mg/L, whereas SH-group levels decreased at all concentrations. These results indicate that M6-IAA modulates stress responses, with optimal effects varying by marker and concentration. 10 mg/l M6 is optimal; reduces oxidative stress(Fig 2). Enhances antioxidant activity. Maintains physiological balance without signs of toxicity

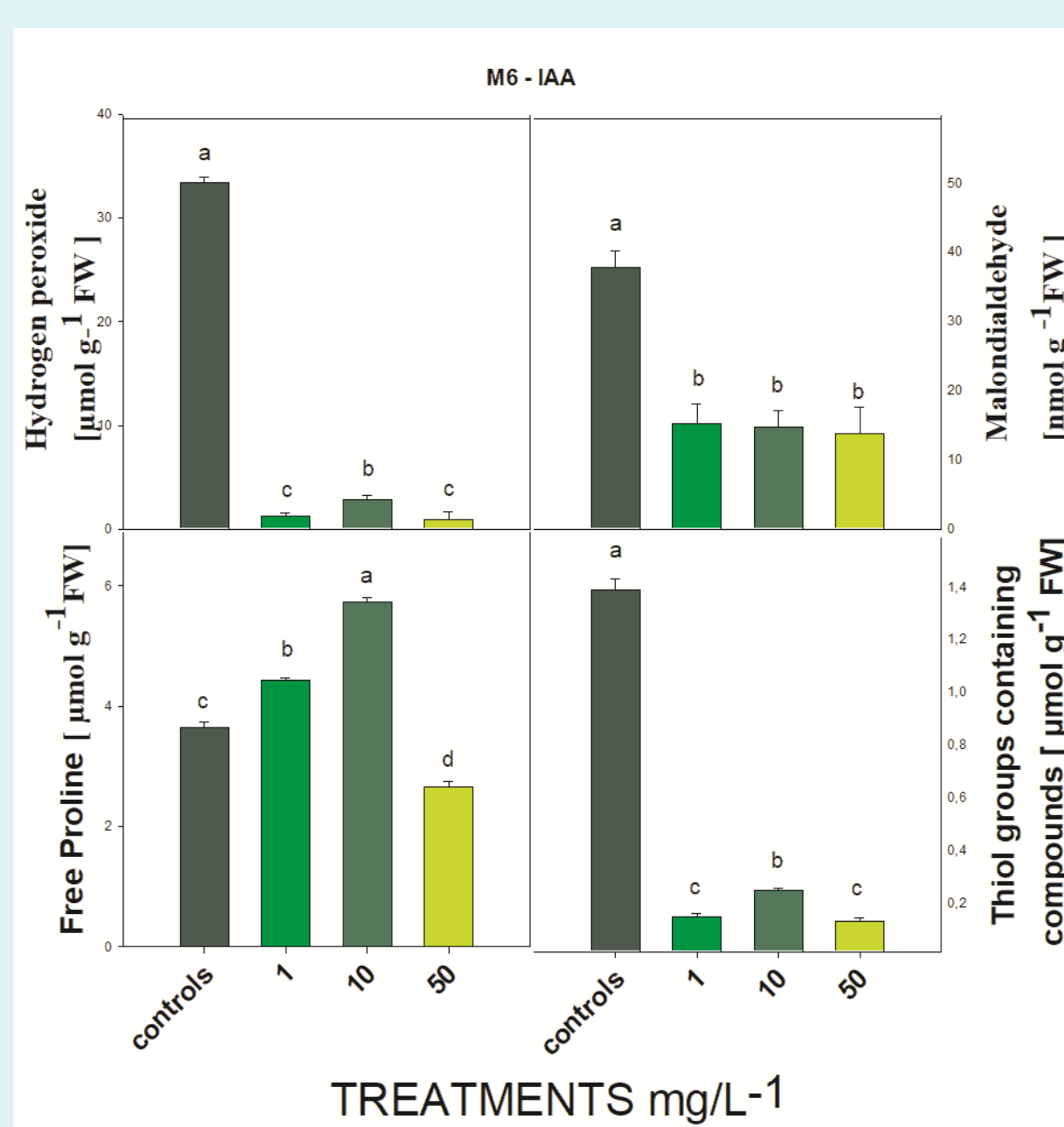


Figure 1: Dose-dependent effects of M6-indolyl acetate (M6-IAA) on biochemical stress markers in plants. The figure shows the levels of hydrogen peroxide (H₂O₂), malondialdehyde (MDA), proline, and sulfhydryl (SH) groups in plants treated with varying concentrations of M6-IAA (0, 1, 10, and 50 mg/L).

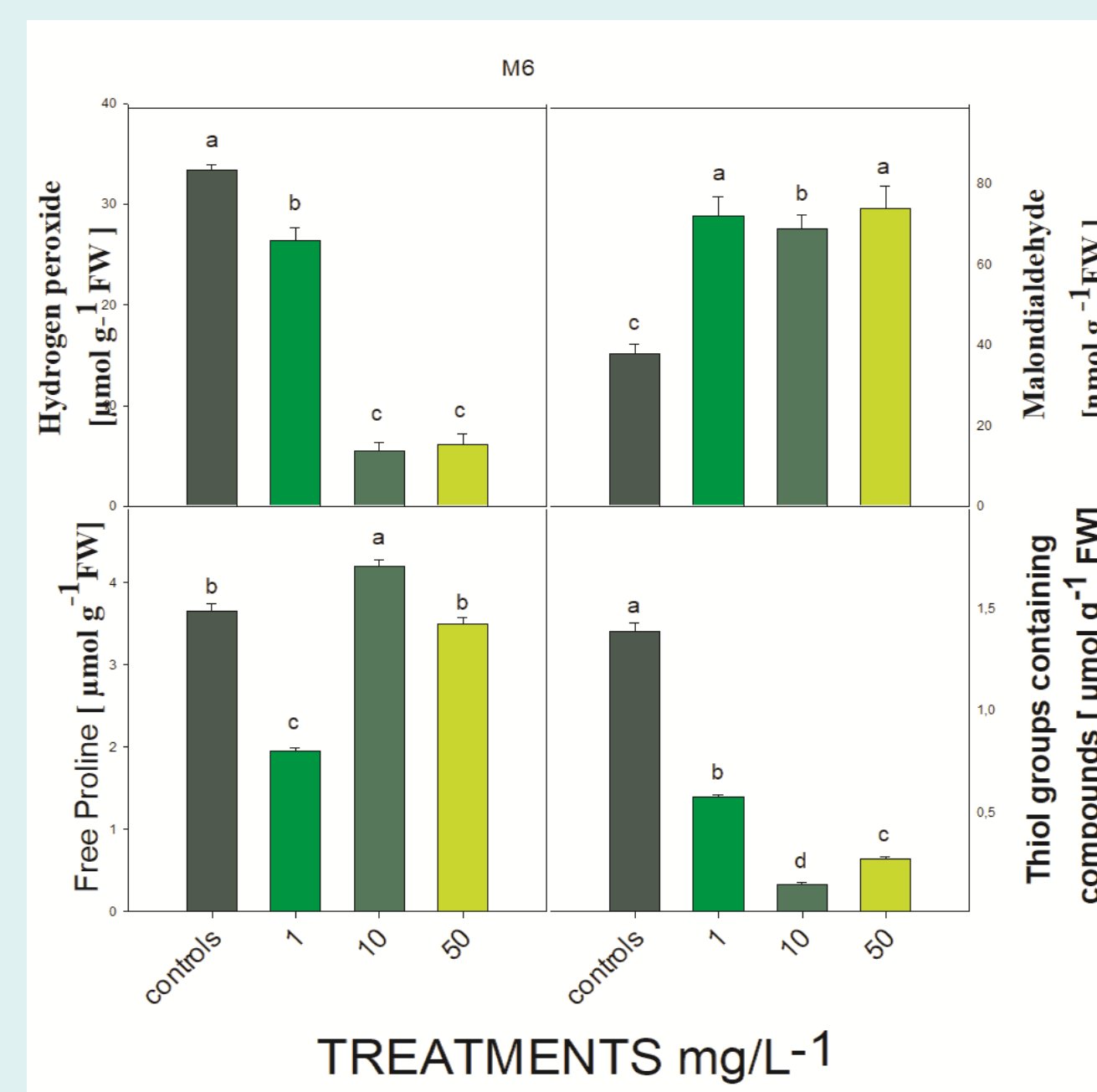


Figure 2: Effect of different concentrations of M6 (1, 10, and 50 mg/L) on hydrogen peroxide (H₂O₂), malondialdehyde (MDA), proline, and sulfhydryl (SH) content in plants. Data are presented as mean \pm SE. Control plants showed the highest H₂O₂ and SH levels, while M6-IAA treatments reduced oxidative stress markers in a concentration-dependent manner.

Conclusion: Nanofiber-based strategies hold promise for importing plant tissue culture resilience. tailored concentration is critical - higher doses may become counterproductive