EFFECT OF MICROPROPAGATION ON *PHYSALIS PERUVIANA* L. ENZYME AND NON-ENZYME ANTIOXIDANT ACTIVITY

Maria Geneva*, Eli Zayova, Elisaveta Kirova, Mariana Sichanova

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

*Corresponding author: Maria Geneva, e-mail: boykova2@yahoo.com

Introduction This study examines how plant growth regulators influence the antioxidant activity of micropropagated *Physalis peruviana* L., a medicinal species valued for its nutrient-rich fruits and diverse bioactive compounds. Because *in vitro* propagation exposes plantlets to stressful conditions such as high humidity, low light, and limited gas exchange, the accumulation of reactive oxygen species can lead to oxidative stress. Plants counteract this through enzymatic and non-enzymatic antioxidant defence systems, making it important to understand how culture conditions and growth regulators affect these responses.

Materials and methods The explants were cultured on MS medium supplemented with 7.0 g L^{-1} agar, 3.0% sucrose and different cytokinins - 6-benzylaminopurine (BAP), zeatin, and thidiazuron (TDZ) - at concentrations of 0.5 and 1.0 mg L^{-1} to evaluate shoot multiplication. After five weeks of cultivation, the percentage of formed shoots was determined, as well as the height and the average number of shoots per explant. Rooting was induced on half-strength MS medium supplemented with 7.0 g L^{-1} agar, 2.0% sucrose (control variant), and auxins (IBA and IAA) at 0.1 and 0.5 mg L^{-1}. The regenerated plantlets were successfully acclimatised *ex vitro*, achieving a 100% survival rate after two months, indicating the effectiveness of the propagation protocol.

Figure 1. Micro propagation of *P. peruviana*: Shoot formation on MS medium supplemented with: a) 1.0 mg L^{-1} BAP; b) 1.0 mg L^{-1} Zeatin; c) 1.0 mg L^{-1} TDZ. *In vitro* rooting of *P. peruviana* on half-strength MS medium with: d) 0 mg L^{-1} auxins – control; e) 0.1 mg L^{-1} IBA; f) 0.1 mg L^{-1} IAA; g) *Ex vitro* acclimatized plants grown on soil: sand: perlite (2: 1: 1 v/v/v) for two months.

Table 1. Effect of citokinines on the micropropagation of *P. peruviana* shoots after five weeks of cultivation

PGR (mg L^{-1})	Micropropagation rate (%)	Average number of shoots (explant^{-1})	Average shoots height (cm)
0.5 BAP	75	2.9 ± 0.18^{b}	2.3 ± 0.20^{e}
1.0 BAP	90	5.3 ± 0.36^{d}	1.2 ± 0.11^{a}
0.5 Zeatin	65	1.8 ± 0.16^{a}	1.7 ± 0.15^{c}
1.0 Zeatin	70	2.3 ± 0.19^{ab}	1.4 ± 0.12^{b}
0.5 TDZ	80	4.0 ± 0.27^{c}	2.0 ± 0.18^{d}
1.0 TDZ	95	6.6 ± 0.41^{e}	1.5 ± 0.13^{bc}

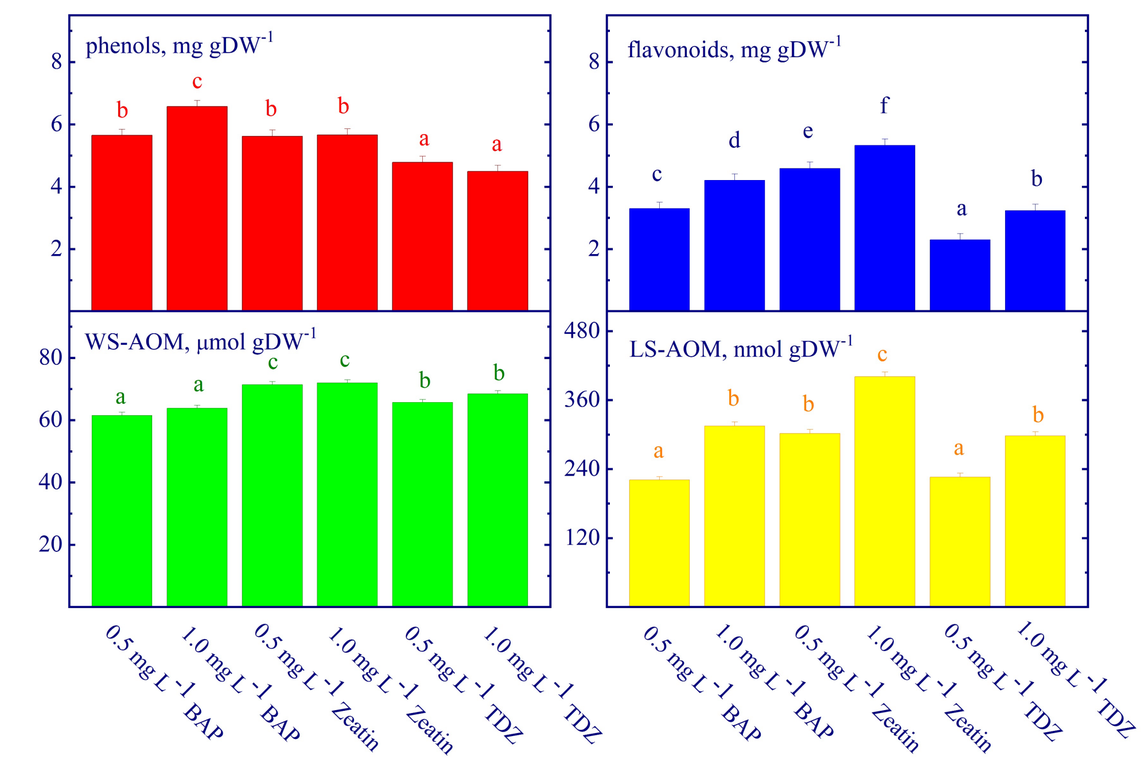

Figure 2. Content of metabolites with antioxidant properties in the *in vitro* propagated *P. peruviana*. Values are means ± SE, n=3; different letters indicate significant differences assessed by Fisher LSD test (P≤0.05) after performing ANOVA multi-factor analysis.

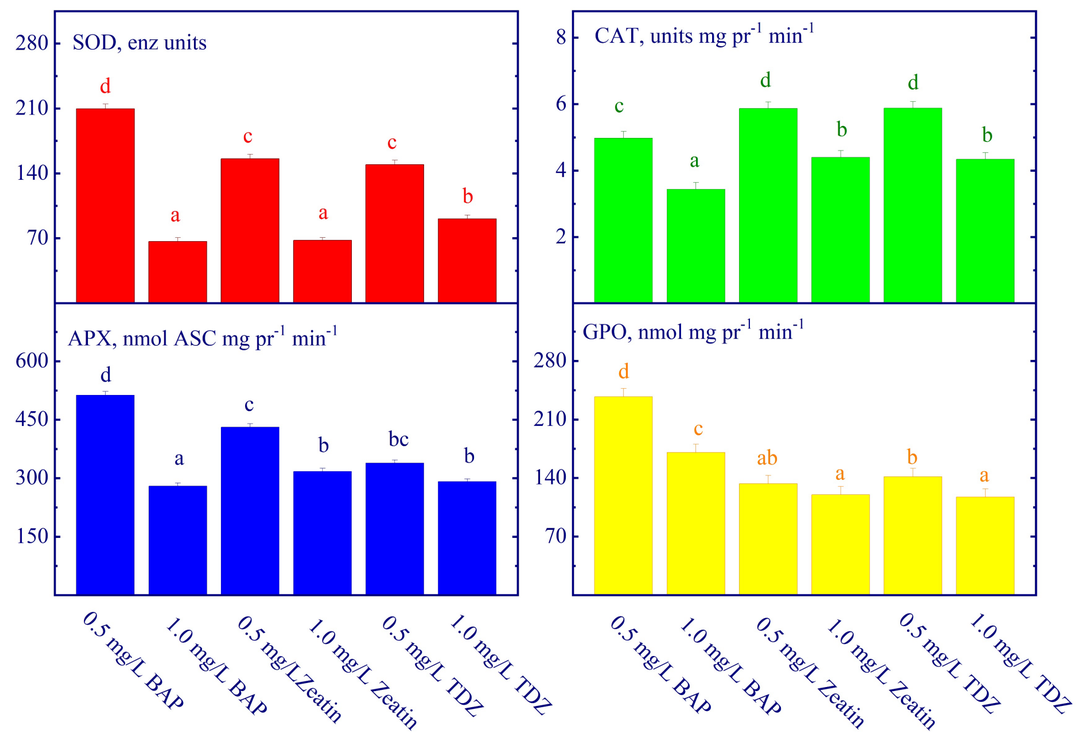

Figure 3. The activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPO), and ascorbate peroxidase (APX) in the *in vitro* propagated *P. peruviana*. Values are means ± SE, n=3; different letters indicate significant differences assessed by Fisher LSD test (P≤0.05) after performing ANOVA multi-factor analysis

The results showed that antioxidant responses were strongly influenced by cytokinin concentration. Lower cytokinin levels (0.5 mg/L) led to greater accumulation of metabolites with antioxidant potential, suggesting enhanced non-enzymatic antioxidant capacity. In contrast, higher cytokinin concentrations increased antioxidant enzyme activity, indicating activation of enzymatic defence mechanisms. These findings demonstrate that the type and concentration of plant growth regulators play a critical role in modulating oxidative stress responses during micropropagation. Overall, the research provides insight into optimising in vitro culture conditions to improve plant quality and antioxidant potential, supporting the use of micropropagation for large-scale production of Physalis peruviana with functional and medicinal value.