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Short Communication

USE AUXINS AND ANTIOXIDANTS TO OPTIMISE NUTRIENT MEDIUM OF TOMATO TISSUE CULTURE

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Calli characteristics of tomato grown in vitro *on different nutrient media supplemented with auxins and antioxidants in various combinations were studied. Explants were cut from the hypocotyl, apex, and cotyledons and cultivated on Murashige and Skoog medium supplemented with some auxins and antioxidants. It was found that the regeneration potential was higher in combinations with joint application of antioxidant and a phytohormone of the auxin group — indolyl-acetic acid. The obtained results demonstrate differential influence of specific biologically active substances on the manifestation of calli characteristics depending on the type of explant.*

Keywords: in vitro*, biologically active substances, calli, rhizogenesis.*

The method of tissue culture is widely used as a method of micropropagation of plant organisms *in vitro*, and therefore the optimisation of nutrient medium is an important issue for the intensification of biotechnology methods. The need of isolated tissues for nutrients is significantly different from the needs of the plant when grown *in vivo*. The main difference is that isolated organs require not only mineral salts from the environment, but also organic biologically active substances (BAS). In order to optimise nutrient media, BAS of different origin and functions (phytohormones, animal hormones, vitamins, amino acids, antioxidants, etc.) are used as growth regulators (Phillips and Garda, 2019; Sidik *et al*., 2024). For effective propagation *in vitro* it is important to take into account the concentration of BAS, as well cultivation conditions, including temperature, humidity, and light intensity (Pasternak and Steinmacher, 2024).

Previously, we studied the interaction of the antioxidant ionol, kinetin, which is a phytohormone of the cytokinin group, and acetone in different *in vitro* media (Gorbatenko *et al.,* 2014; 2020; Kuklenko *et al*., 1995; Prosandeeva *et al*., 2002). The goal of this study was to determine the influence of addition of auxin IAA (Indole-3-acetic acid) and NAA (1-Naphthaleneacetic acid), and the synthetic antioxidant phenoxane (class of spatially complex phenols) to the nutrient media on some calli characteristics of tomato plants cultivated *in vitro*.

Explants of tomato (*Lycopersicum esculentum*, variety 'Novichok') were used in the experiments. Seedlings were grown from aseptic tomato seeds, obtained by treatment with 20% potassium permanganate solution for 2 minutes and then by 4% calcium hypochlorite solution for 15 minutes, followed by three washes with sterile distilled water. Explants were cut from the hypocotyl, apex and cotyledons of 14 day seedlings and then planted in flasks with cultivation medium.

Two experiments were conducted. In the **first experiment** the following cultivation media were used: group I (control) — the basic Murashige and Skoog medium (MS) (Murashige and Skoog, 1962); group II — MS + natural antioxidant glutathione-SH ($2.26 \cdot 10^{-5}$ M); group III — MS + phytohormone of the auxin group 2,4-dichlorophenoxy-

acetic acid (1.0·10-5 M); and group IV — a combination of antioxidant and phytohormone: MS + glutathione-SH +2,4 -D.

In the **second experiment** the following nutrient media were used: group I (control) — MS; group II — MS + phytohormone auxin IOC ($1.0 \cdot 10^{-5}$ M); group III — MS + antioxidant phenoxane ($0.5 \cdot 10^{-4}$ M); and group IV — MS + IOC + phenoxane.

Explants were cultivated at 16-hour photoperiod and temperature 25 °C for 35 days (the first experiment) and 21 days (the second experiment). Statistical data processing was performed using Statistica software.

Figure 1 shows the reaction of explants to the presence in the medium of certain biologically active substances (the first experiment). The maximum absolute values of the shoot height of tomato seedlings in 35 days (5.4 cm) were found in the case of addition of the natural antioxidant glutathione-SH to MS medium. The obtained height was 25% higher than that of the control group. The lowest height of tomato seedlings was in the case of combined addition of auxine 2,4-dichlorophenoxyacetic acid and antioxidant. The absolute value was 56% lower than when the antioxidant was used alone. High values of height of seedlings were also noted in control variant (basic Murashige and Skoog medium). The use of phytohormones and antioxidants when used alone in the nutrient medium did not promote rhizogenesis.

Results of the second experiment concerning the effect of studied BAS on growth of tomato callus are presented in Table 1. Effect of the phytohormone auxin (IAA) and the

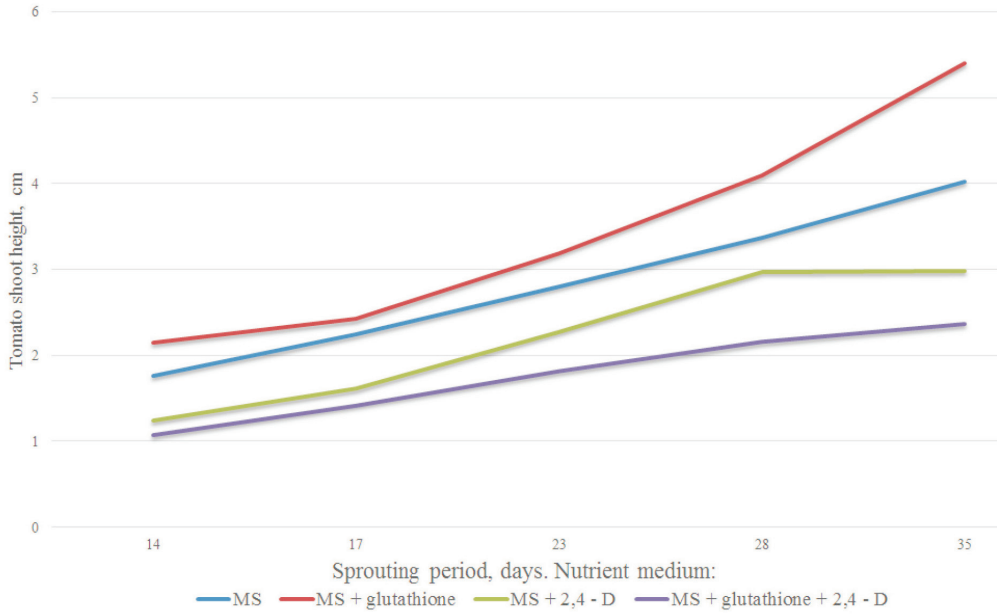

Fig. 1. Shoot height of tomato seedlings *in vitro* depending on the nutrient medium.

Table 1. Tomato calli characteristics on media with auxins and antioxidants

Characteristics	Nutrient medium			
	MC	MC + IOK	MC+ phenoxane	MC + IOK + phenoxane
Hypocotyl				
Callus:				
Weight, mg	47.15 ± 4.42	95.56 ± 11.50***	50.75 ± 5.37	105.09 ± 8.27**'
Size, cm	2.22 ± 0.24	3.16 ± 0.20**	2.55 ± 0.22	3.17 ± 0.22**
Frequency of root formation, %	44	88	50	100
Cotyledons				
Callus:				
Weight, mg	32.66 ± 4.03	129.73 ± 16.59***	94.60 ± 14.91**	94.73 ± 13.96***
Size, cm	0.28 ± 0.02	0.69 ± 0.04	0.75 ± 0.04	0.67 ± 0.03
Frequency of root formation, %	–	79	80	100
Apexes				
Weight, mg	²5.50 ± 2.27	143.20 ± 12.96***	15.60 ± 0.86	45.00 ± 4.24***
Shoot height, cm	0.52 ± 0.023	1.10 ± 0.16**	0.68 ± 0.05**	1.28 ± 0.09***
Frequency of root formation, %	–	70	–	100

antioxidant phenoxane was slightly different from those in the first experiment. Manifestation of calli characteristics depended on the origin of explants and the composition of the medium. It was found that joint use of IAA and the synthetic antioxidant phenoxane, compared with application of only phenoxane, provided a synergistic effect, what illustrated by the statistically significant increasing (2.07 times) of callus weight of hypocotyl explant and also significant higher (2.23 times) than control. In turn, the combined effect of the both studied substances exceeded by only 9.1% the effect when indolyl-3-acetic acid (IAA) was used alone. It is important that with the combined action of IAA and phenoxane, the frequency of root formation reached 100% versus 44.4% in the control. Statistically significant difference of size of calli originated from explants from hypocotyls in variants with phytohormone and antioxidant was not found.

In calli originated from cotyledons and apexes the highest weight was observed in the combination MS + IAA. It was also found that the frequency of roots formation was 100% in calli induced from cotyledons and apexes in the variant with combined use of IAA and phenoxane. The statistical significance of the obtained results was noted on both types of explants.

In general, the obtained results indicate the complexity of the process of interaction of different BAS in media, which must be taken into account when selecting the composition of the nutrient media components to further improve of effectiveness *in vitro* cultivation. It is proved that the regeneration potential was higher with joint application of the antioxidant and phytohormone of the auxin group — indolylacetic acid. The obtained results demonstrate differential influence of specific BAS on the manifestation of calli characteristics depending on the type of explant.

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It is advisable to use antioxidants as growth regulators (Shoring *et al.*, 1999; Shamraev *et al.,* 2002) and as substances that reduce the level of oxidative stress, which is an important factor that optimises the regenerative potential and increases the efficiency of biologically active compounds.

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