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"REGOPLANT" AND "STIMPO" INFLUENCE ON THE CONTENT OF FREE AMINO ACIDS, PROLINE AND ON THE LIPID PEROXIDATION REACTION INTENSITY IN HELIANTHUS ANNUUS L. GROWN ON TECHNOSOL

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The aim of study was to investigate the effect of new growth regulators "Stimpo" and "Regoplant", produced by State Enterprise Interdepartmental Science and Technology Center — ISTC "Agrobiotech", on the proline and free amino acids content, and lipid peroxidation reactions intensity in comperison to the action of gibberellic acid and "Treptolem" (a second-generation growth regulator) in 14 day sunflower sprouts. The plants were cultured on soil substrates made from the coal mine rock waste of the Central Concentrating Factory in the Chervonohrad mining region.

It was shown that the free amino acids and proline content increased, and the intensively of lipid peroxidation reactions (evaluated by the content of malonic dialdehyde) decreased. This indicated to the increase of plants resistance to unfavorable conditions of the coal mine rock under the action of the growth regulators studied.

One could assump that "Stimpo" and "Regoplant" are promising agents in phytochemical treatment of coal mine rock dumps.

Key words: *Helianthus annuus* L., "Stimpo", "Regoplant", "Treptolem", substrates of coal dumps, free amino acids, proline, lipid *peroxidation reactions*.

Plant growth regulators belong to the third generation of biotechnological breakthroughs. "Regoplant" (TU U 24.2-31168762-006) and "Stimpo" (TU U 24.2-31168762-005) have a wide spectrum of action and a bioprotective effect. Previously, "Stimpo" and "Regoplant" were not used as growth stimulators for the phytochemical treatment of rock dump technoland. Therefore, in this research their influence on the content of free amino acids and proline, and the intensity of lipid peroxidation reactions was investigated to evaluate resulting plant resistance to unfavorable edaphic conditions of rock dumps.

Effectively cleansing soils of pollution and technogenic changes caused by heavy metals is an important issue everywhere, including Chervonohrad mining region (ChMR). The recultivation of such soils is problematic, the process is multi-stage and has several disadvantages [1, 2]. Biologically, recultivation aims to improve soil productivity with plants [3]. Phytorecultivation is one of approaches used to improve soils of the Central enriching plant (CEP) using plants which accumulate heavy metals [1, 4]. These plants tolerate the effects of heavy metal in concentrations, toxic for most plant species [5]. Scientists have found how to identify such tolerant plants [6, 7]. Particularly, plants of the genera *Calamagrostis*, *Phragmites*, and *Brassica napus* L. were used to recultivate substrates of coal mine wastes at the CEP [8–10]. There are also studies of compounds which can potentially enhance plant tolerance during phytorecultivation [11–14].

Two growth regulator (GR) preparations, "Regoplant" (TU U 24.2-31168762-006) and "Stimpo" (TU U 24.2-31168762-005), were produced in ISTC "Agrobiotech" (Kyiv, Ukraine). The preparations have a wide range of action and bioprotection effect. The main components of "Stimpo" and "Regoplant" are metabolites of microscopic fungi and the bacterium *Streptomyces avermetilis*. These preparations were produced *in vitro* in fungal culture, isolated from root systems of ginseng plants. The preparations contain a complex of amino acids, fat acids, polysaccharides, phytohormones, microelements and metabolites of *Streptomyces avermitilis*, which include avermectins (complex anthelmintic macrolide antibiotics). Thus they enhance the physiological indices of plants grown on normal soils [12, 15–18]. Improvement of soils under heavy metal pollution is a long-term process and requires effective biotechnologies to reverse those disturbed habitats to their previous natural state. Hence, plant growth regulators are needed there to create optimal growth conditions and increase plant tolerance to unfavorable edaphic and microclimatic conditions.

Sunflower plants are relatively tolerant to heavy metals and can accumulate several of them [11, 19, 20]. However, their metabolism is not studied in conditions of growing the plants on substrates of rock dumps and treatment with the mentioned GR. Our work aimed to evaluate the effect of "Stimpo" (S) and "Regoplant" (R) compared to gibberellic acid (GA) and another GR of previous (second) generation, "Treptolem" (T), on free amino acid content (particularly, proline) and intensity of lipid peroxidation (LPO) reactions in sunflower plants grown on substrates of ChMR dumps.

Materials and Methods

Previously, we have determined the optimal concentrations of GR for sunflower plants, namely 0.5 ml/l of "Stimpo" and 0.1 ml/l of "Regoplant" . Optimal concentrations of GA (10 mg/l) and "Treptolem" (1 ml/l) were taken from other publications [11, 19, 21, 22]. Seeds were soaed in 1 hr in solutions of the mentioned concentrations and then washed in distilled water. Seed germination was performed in Petri dishes in darkened thermostat at 22 °C for 3 days, and then seeds were planted into black (not burnt-out) and red (burnt-out) substrates of rock dumps for 14 days. Sprouts soaked in distilled water and planted into garden soil were used as control. Substrates were collected at CEP dumps, in Silets village, Sokalsky district of Lviv region at the depth of 20 cm.

Biochemical indices were analyzed at the 14^{th} day. Free amino acid content was determined spectrophotometrically according to [23] on KFK-3 at 580 nm. Recalculated for mg/100 g of studied compound. Proline content was estimated by changes in optical density of reaction mixture at 520 nm according to [24], calculating for mg/100 g of raw mass. Lipid peroxidation reaction intensity was determined spectrophotometrically by malondialdehyde content at 530 and 600 nm following [25]. Data was statistically processed in programs MS Excel and Statistics.

Results and Discussion

Helianthus annuus L. plants are suitable for dump recultivation, due to their tolerance to heavy metals (HM) [8, 11], that is metallic elements with density more than 6 g/cm^3, atomic weight no less that 50 carbon units. Soils easily accumulate heavy metals and conversely are slowly and laboriously purged. The elements can induce diseases in plants, animals and humans.

The resistance of plants can increase due to changes in the synthesis of amino acids, which, under various stresses, perform regulatory and protector functions. Free amino acids are involved in the formation of various forms of nitrogen, and in maintaining the cellular osmotic potential. They also can respond to the environmental stress factors [26, 27]. They act as buffers, binding anions and cations, reducing their concentration in the cell [8]. This is definitely a positive phenomenon for plants that grow in environments with high contents of toxic substances [28]. Hence, we started with studying the effect of GR on the content of free amino acids in sunflower plants grown on the coal dump substrates (Fig. 1).

The content of free amino acids increased in sunflower seeds treated with GR and grown on the garden soil. This is a possible indication of increased protein synthesis. Plants treated with "Stimpo" and Regaplanet, had higher content of free amino acids than those treated with "Treptolem" and GA, grown in the garden soil or on the substrates of ChMR.

Free amino acid content was higher in plants grown on black substrate compared to those grown on red substrate. A possible reason for that is more acidic pH of the black substrate, where plants accumulate heavy metals faster and neutralize them with amino acids.

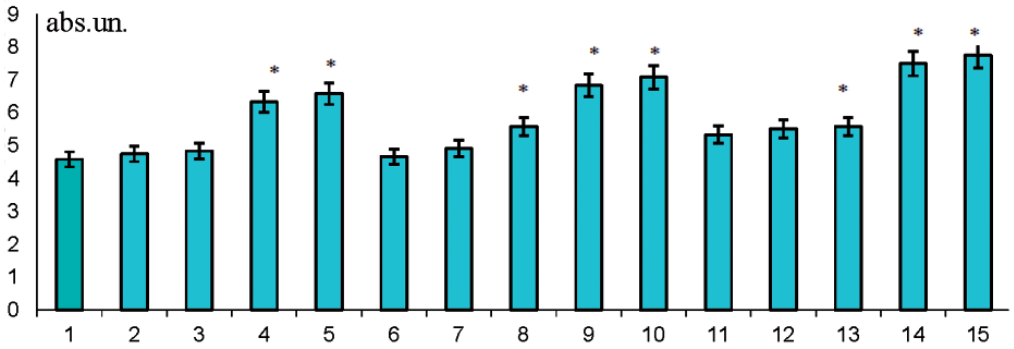

Fig. 1. **Influence of growth regulators on the content of free amino acids in *Helianthus annuus* L. grown on soil substrates (mg/100 g of raw mass)**

Hear and after: *1. Control — Garden soil; 2. Garden soil + GA; 3. Garden soil + Treptolem (T); 4. Garden soil + Stimpo (S); 5. Garden soil + Regoplant (R); 6. Red substrate; 7. Red substrate + GA; 8. Red substrate + T; 9. Red substrate + S; 10. Red substrate + R; 11. Black substrate; 12. Black substrate + GA; 13. Black substrate + T; 14. Black substrate + S; 15. Black substrate + R.

* — $P < 0.05$ compared with control.

The effect of "Regoplant" was stronger compared to "Stimpo". The difference between the increases in the content of free amino acids on the garden soil was 26%, 6% at the red substrate and 7% at the black substrate. Indices of plants grown on the garden soil and not treated with GR were used as control. Obviously, that increase in the content of free amino acids may be explained by adaptation to adverse edaphic conditions of substrates [9, 10, 29].

Stressed plant organisms, particularly influenced by HM, continuously adapt to preserve and restore the dynamic constancy of their internal environment [30]. One of the mechanisms by which plants adapt to adverse conditions is the accumulation of osmoactive substances, among which proline plays an important role. This compound is involved in protective reactions, in particular in stabilizing the cytoplasm [27]. Therefore, further work was to determine the content of proline (Fig. 2).

In plants treated with GR the proline content was higher compared to control. This may indicate increased photosynthesis and nitrogen accumulation on garden soil in experimental plants [31] and plants reaction to stress (heavy metals and the moisture deficit) on ChMR substrates [14, 24, 32]. In plants grown on garden soil, the effect of "Regoplant" was higher compared to "Stimpo": proline content increased by 491% in experiment with "Regoplant" compared to 352% under treatment with "Stimpo". The difference between indices was 139%. In plants grown on artificial substrates and treated with GR, the content of proline increased exceedingly: by 1011% and 1023% on the red substrate, and by 1112% and 1119% on the black substrate, for "Stimpo" and "Regoplant" respectively. That corresponded to the 12% difference in the effectiveness of "Regoplant" over "Stimpo" on red substrate, and the 7% difference on black substrate. The proline content in plants grown on garden soil without GR treatment was used as control in calculations.

The earliest stress reactions occur at the membrane level [33], therefore the next step in our work was to determine the activity of the lipid peroxidation, one of the main indicators of membrane integrity (Fig. 3).

Lipid peroxidation decreased in treated with GR sunflower plants grown on the garden soil or on the substrates of ChMR. Malonic dialdehyde content was lower after treatment with newer GR compared to GA and "Treptolem". Moreover, on the dump substrates, "Regoplant" caused the highest reduction of the MDA content, while the smallest reduction was caused by gibberelic acid.

MDA content was more reduced under treatment with "Regoplant" compared to "Stimpo". Interestingly, reduction percentage was almost the same in treated plants grown on garden soil, whereas there was an 8% difference of MDA content decrease in plants cultured on the red substrate, and an 11% difference on the black substrate respectively. Indicators of plants that grew on the garden soil without GR treatment were used as control.

In our opinion, the effects of Ukrainian growth regulators of different generations on plants are unequal because of the difference in their composition. Their basic component are growth substances of natural origin. The additives are different, for example in "Treptolem" those

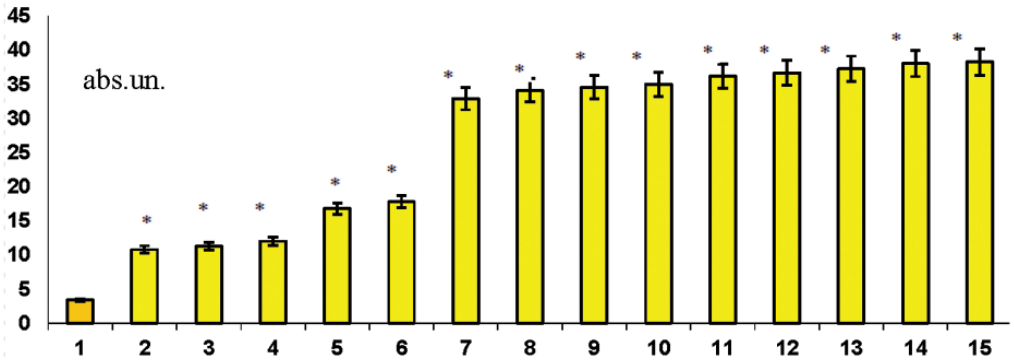

Fig. 2. **Proline content in *Helianthus annuus* L. grown on substrates of coal mines and treated with growth regulators (mg/100 g of raw mass)**

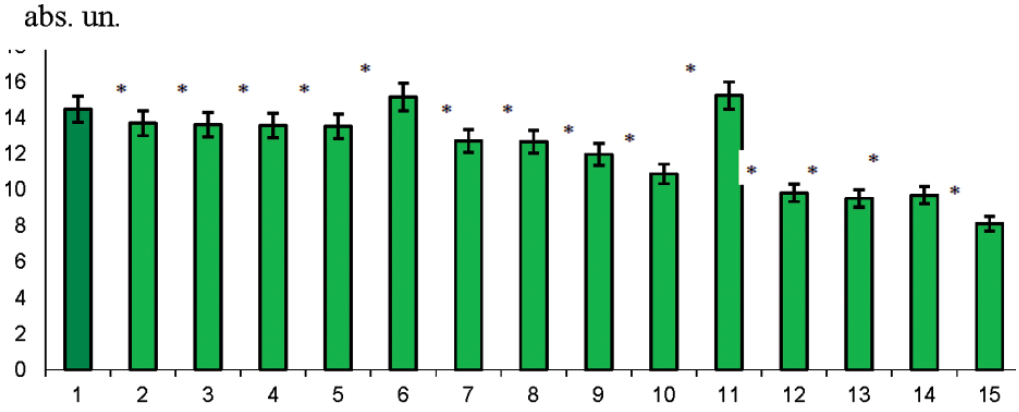

Fig. 3. **Influence of growth regulators on the content of malonic dialdehyde in *Helianthus annuus* L. grown on substrates of ChMR dump (mM/g of raw mass)**

are 2,6-dimethylpyridine-1 oxide and amber acid, without trace elements. There are no boron and molybdenum in "Stimpo" but they are present in "Regoplant" , which may explain the latter's greater effect on the plants, compared to "Stimpo" and "Treptolem" .

The obtained results indicate a decreased oxidative stress, as evidenced by an increase in the content of free amino acids and proline, and decreased content of malonic dialdehyde, which confirms lessened damage of membrane structures in the plants treated with GR and grown on substrates of the coal dumps waste mine.

Thus, the free amino acids and proline content increased, and lipid peroxidation decreased in sunflower plants treated with optimal concentrations of "Stimpo" and "Regoplant" and grown on substrates of coal mine rock dumps. Increased free amino acid and proline content, which are compounds with bioprotection functions, indicate lower oxidative stress in plants grown in unfavorable edaphic conditions of rock dump substrates, and preservation of cellular membrane integrity, which follows from decreased malonic dialdehyde content.

Thus, the optimal concentrations of "Stimpo" and "Regoplant" identified in the study can be recommended for use as stimulators of growth and metabolism in sunflower plants used for phytochemical treatment of coal mines in the Chervonograd mining region.

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ВПЛИВ «REGOPLANT» І «STIMPO» НА ВМІСТ ВІЛЬНИХ АМІНОКИСЛОТ, ПРОЛІНУ ТА ІНТЕНСИВНІСТЬ РЕАКЦІЙ ПЕРОКСИДНОГО ОКИСНЕННЯ ЛІПІДІВ У *Helianthus annuus* L. ЗА РОСТУ НА ТЕХНОЗЕМАХ

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Метою роботи було дослідити вплив нових регуляторів росту «Stimp»» та «Regoplant» (виробник Державне підприємство Міжвідомчий науково-технологічний центр — ДП МНТЦ «Агробіотех») порівняно з гібереліновою кислотою і регулятором росту другого покоління «Treptolem» на вміст вільних амінокислот, проліну та реакції пероксидного окиснення ліпідів у 14-добових паростків *Helianthus annuus* L. за росту на ґрунтових субстратах породного відвалу вугільних шахт Центральної збагачувальної фабрики у Червоноградському гірничопромисловому районі.

Встановлено збільшення вмісту вільних амінокислот, проліну та зниження інтенсивності реакцій пероксидного окиснення ліпідів (за вмістом малонового діальдегіду), що свідчить про збільшення стійкості рослин до несприятливих умов породного відвалу за дії регуляторів росту.

Одержані результати вказують на перспективність подальшого застосування «Stimpo» та «Regoplant» за фіторекультивації породних відвалів.

Ключові слова: *Helianthus annuus* L., «Stimpo», «Regoplant», «Treptolem», субстрати породних відвалів вугільних шахт, вільні амінокислоти, пролін, реакції пероксидного окиснення ліпідів.

ВЛИЯНИЕ «REGOPLANT» И «STIMPO» НА СОДЕРЖАНИЕ СВОБОДНЫХ АМИНОКИСЛОТ, ПРОЛИНА И ИНТЕНСИВНОСТЬ РЕАКЦИЙ ПЕРОКСИДНОГО ОКИСЛЕНИЯ ЛИПИДОВ У *Helianthus annuus* L. ПРИ РОСТЕ НА ТЕХНОЗЕМАХ

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Целью работы было исследовать действие новых регуляторов роста «Stimpo» и «Regoplant» (производитель Государственное предприятие Межведомственный научно-технологический центр — ГП МНТЦ «Агробиотех») по сравнению с гиббереллиновой кислотой и регулятором роста второго поколения «Treptolem» на содержание свободных аминокислот, пролина и активность пероксидного окисления липидов у 14-суточных проростков *Helianthus annuus* L. при росте на грунтовых субстратах породного отвала угольных шахт Центральной обогатительной фабрики в Червоноградском горнопромышленном районе.

Установлено увеличение содержания свободных аминокислот, пролина и снижение активности пероксидного окисления липидов (по содержанию малонового диальдегида), что свидетельствует об увеличении устойчивости растений к неблагоприятным условиям породного отвала при действии регуляторов роста.

Полученные результаты указывают на перспективность дальнейшего применения «Stimpo» и «Regoplant» при фиторекультивации породных отвалов.

Ключевые слова: *Helianthus annuus* L., «Stimpo», «Regoplant», «Treptolem», субстраты породных отвалов угольных шахт, свободные аминокислоты, пролин, реакции пероксидного окисления липидов.