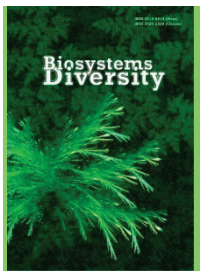

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Morphogenesis, pigment content, phytohormones and yield of tomatoes under the action of gibberellin and tebuconazole

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One of the main tasks of contemporary plant physiology is regulation of growth and development of cultivated plants in order to optimize the productive process. The scientific community focuses its attention on the use of natural activators and growth inhibitors. We investigated the effect of foliar treatment with 0.005% solution of gibberellic acid and 0.025% solution of the anti-gibberellic preparation tebuconazole on morphogenesis, leaf mesostructure, content of photosynthetic pigments, balance of endogenous phytobrybrides and lymphocyte B and productivity of tomatoes. The vegetation experiment was carried out in the conditions of soil-sand culture in vessels with a 10-liter volume. The treatment was carried out in the budding phase. Morphometric parameters were measured every 10 days. The mesostructure of the middle tier leaves was studied in the fruit formation phase, and the chlorophyll content was determined in the raw material by spectrophotometric method. Analytical determination of endogenous phytohormones – indolyl-3-acetic (IAA), gibberellic acid and abscisic (ABA) acids and cytokinins – zeatin (Z), zeatin-O-glucoside (ZG), zeatinribozide (Znila) and isopentenyladenosine (iPA) was performed by high performance liquid chromatography – mass spectrometry (HPLC-MS). With gibberellic acid treatment plant height increased significantly, while with tebuconazole it decreased. Gibberellic acid increased the number of leaves per plant, and tebuconazole did not change it. The preparations increased the number of leaf blades per leaf, the total number of leaf blades per plant, the weight of the raw material of leaves, the area of leaf blades and the area of the leaves at the end of the study period. The dry matter weight of stems and roots under the action of gibberellic acid increased, and during the treatment of tebuconazole decreased. Gibberellic acid increased the dry matter of the whole plant, and tebuconazole did not change it. Under the action of tebuconazole the content of chlorophyll in the leaves increased, while under the action of gibberellic acid it decreased. Both regulators increased the volume of columnar parenchyma cells. Gibberellic acid increased the size of spongy parenchyma cells, while tebuconazole did not change them. It is revealed that the action of exogenous gibberellic acid in stems and leaves increased the content of endogenous IAA and gibberellic acid, and tebuconazole decreased their content. The ABA content in stems and leaves increased with tebuconazole treatments and decreased with exogenous gibberellic acid. The total cytokinin content in the leaves was higher than in the stems in both the control and the experiment samples. Growth regulators induced an increase in the cytokinin pool in leaves and a decrease in stems. Gibberellic acid increased the content of all five forms of cytokinins in the leaves, and tebuconazole increased only two isoforms. In the stems under the action of both growth regulators the content of Z decreased and iP increased. The content of ZR and iPA in stems increased after the application of the retardant and decreased under the action of growth stimulant. The ZG content exceeded the control after gibberellic acid treatment and was in trace concentrations under the action of tebuconazole. Growth regulators optimized the productivity of tomato plants: under the action of gibberellic acid there was a considerable increase in the number of fruits per plant, and after the use of tebuconazole the average weight of one fruit significantly increased. The obtained results demonstrated that anatomical-morphological and structural-functional rearrangements in tomato plants under the action of exogenous gibberellic acid and tebuconazole occurred against the background of changes in the balance and distribution of endogenous hormones. Increased photosynthetic activity, stimulation of growth processes of some plant organs and inhibition of others increased the biological crop capacity.

Keywords: *Lycopersicon esculentum*; growth stimulants and inhibitors; morphometry; leaf apparatus; plant hormones; crop capacity.

Introduction

The hormonal system is one of the most important factors that regulates plant growth and morphogenesis (Zhou et al., 2020). In studying the mechanisms of action of phytohormones, treatment of organs with exogenous native phytohormones or their synthetic analogues or modifiers is widely used, followed by analysis of fast and slow feedback, changes in metabolism and hormonal status of the whole plant (Rademacher, 2016; Zhou et al., 2020). Nonetheless, the lack of experiments with exogenous hormones only is obvious, because intact plants have a complex interaction between individual endogenous hormones, and plant treatment with a hormone, analogue or modifier, leads to changes in synthesis and metabolism (Wen et al., 2018; Kuryata et al., 2019; Cavalcante et al., 2020) and in the ratio of other components of the hormonal complex (Soumya et al., 2017; Mao et al., 2018; Qiu et al., 2019), which, in their turn, can lead to changes in morphological and physiological programs (Jabir et al., 2017; Khodanitska et al., 2019; Song et al., 2019). In particular, exogenous use of GA_3 significantly affects the entire hormonal complex of plants. Treatment of sugar cane seedlings with exogenous GA_3 increased IAA and decreased ABA, but did not affect cytokinin content (Qiu et al., 2019). GA_3 at concentrations of 100, 200 and 300 mg/L increased the content of endogenous GA_3 and IAA in the first and sixth leaves of camellia (Wen et al., 2018). After foliar treatment with exogenous HA_3, the balance of phytohormones in tomatoes changed. The content of active GA_3, SC, IAA and ABA increased under the action of the growth stimulant (Khalloufi et al., 2017). After treatment of *Brassica campestris* L. plants with

exogenous GA_3 acid under conditions of cold stress in the tops of the shoots, an increase in the content of endogenous GA_3, IAA and cytokinins was observed (Song et al., 2019). We have previously found that pretreatment of plants in the budding phase with exogenous GA_3 caused an increase in the content of endogenous GA_3, IAA and ABA in zuccini leaves and stems (Rogach et al., 2020). It is shown that after treatment with exogenous GA_3, the content of endogenous IAA and ABA decreased while GA_3 increased in the stems of sweet pepper plants. Native phytohormone enhanced the accumulation of endogenous GA_3 and IAA and inhibited ABA in sweet pepper leaves (Rogach et al., 2021).

Gibberellin-like compounds and industrial growth regulators made on their basis are widely used in agricultural production to intensify histo- and morphogenesis, accelerate cell proliferation and differentiation. It results in a more branched root system, strengthenened leaf apparatus, which can provide active synthesis that is sent to generative and storage organs. Consequently, an increase in plant height due to elongation of the internode was recorded in sugar cane (Qiu et al., 2019). Treatment of camellias with GA_3 at doses of 100, 200 and 300 mg/L increased the content of chlorophyll in the leaves, its fluorescence parameters and the rate of photosynthesis (Wen et al., 2018). Foliar treatment with exogenous GA_3 significantly reduced the negative effects of salinity, enhanced growth and increased yields of *Solanum lycopersicum* L. plants (Khalloufi et al., 2017). We previously found that exogenous GA_3 increased the linear size of eggplant and pepper plants, the number of leaves on the plant, the raw weight of the leaves and the dry weight of the whole plant increased under the action of the preparation. Under the action of the growth stimulant, the chlorenchyma thickened, the cell volume of the columnar parenchyma increased, but the chlorophyll content in the leaves decreased. Such changes in the leaf apparatus under the action of GA_3 led to an increase in the quantitative indicators of the productivity elements of eggplant and peppers (Rogach et al., 2020, 2021).

The approach "from the opposite" can be compelling and informative enough. It consists in the effect on plants of inhibitors of certain phytohormones, followed by the study of the relationship between the components of the whole hormonal complex. One of the most common groups of inhibitors are antihiberine preparations – retardants (Kim et al., 2018; Ahmad et al., 2019) and ethylene producers (Shevchuk et al., 2019).

The inhibitory effect of retardants, depending on the chemical structure, is known to be determined by blocking the synthesis, or reducing the activity of already synthesized gibberellins (Rademacher, 2016). However, in the contemporary scientific literature one can find some research papers that analyze changes in the hormonal complex of plants during their treatment with individual retardants. In particular, paclobutrazole at a concentration of 50 mg/L reduced the content of GA_3 in radish leaves (Jabir et al., 2017) and in flax plants (Kim et al., 2018) to trace concentrations. The same preparation at a dose of 1 g/m^2 crown inhibited the synthesis of GA_1, GA_3 and GA_4 in mango plants (Cavalcante et al., 2020). Medium and high concentrations of paclobutrazol decreased the content of GA_3 and IAA and increased the content of ABA in magnolia leaves. The decrease in GA_3 content was clearly correlated with the increase in ABA content (Shi et al., 2021). One of the most active retardants of the triazole group is uniconazole. High and medium doses increased the content of ABA and decreased the content of GA_3 and IAA in magnolia plant leaves. The increase in ABA content was clearly correlated with a decrease in the amount of GA_3 (Shi et al., 2021).

Rapeseed plants were treated with uniconazole at doses of 0.5, 1.0, 1.5 and 2.0 mg/L, which led to a decrease in GA_3 and IAA and an increase in cytokinins and ABA in roots and shoots (Zuo et al., 2020). Spraying plants of *Brassica campestris* L. with uniconazole inhibited growth, slowed down the process of budding and flowering, changed the content of endogenous gibberellins (Song et al., 2019). Gibberellin inhibitors uniconazole and ethephon caused an increase in ABA, zeatin and zeatinriboside and decreased GA_3 levels in maize plants. Unicanozole turned out to be more effective than ethylene-producing ethephon in influencing the hormonal status of plants (Ahmad et al., 2019). The retardants metconazole and paclobutrazole inhibited the synthesis of gibberellins in mango fruits (Cavalcante et al., 2020). We have previously found that EW-250 decreased the content of endogenous GA_3, IAA and increased ABA in eggplant leaves and stems during budding treatment (Rogach et al., 2020). The application of this preparation in sweet pepper culture caused a decrease in the levels of GA_3, IAA and ABA in the stems. Under the action of the retardant, the level of ABA in the leaves did not change, while GA_3 and IAA decreased it (Rogach et al., 2021).

Inhibition of apical and intercalary meristems due to inhibition of gibberellin synthesis under the influence of retardants led to a decrease in the linear size of plants and consequently reduced the demand for assimilates for growth of one of the largest plant acceptors – stems. At the same time, the compensatory increase in the activity of lateral and marginal meristems led to increased branching of the shortened stem and redistribution of excess plastic substances for growth and formation of lateral plant organs – leaves and fruit. In particular, paclobutrazol and prohexadione in closed soil at concentrations of 40, 80 and 160 ppm inhibited linear growth and enhanced stem branching in flaxseed, accelerated seed maturation and increased fruit yield. Under the action of paclobutrazol, the oil content in the seeds decreased, and under the influence of prohexadione it increased (Kim et al., 2018). Paclobutrazol increased the chlorophyll content of lily leaves due to chlorophyll and enhanced the transportation and utilization of photoassimilates in maize (Ahmad et al., 2019). The same preparation at a dose of 50 g/L increased the rate of photosynthetic processes in radish plants (Jabir et al., 2017). Uniconazole in all applied concentrations reduced the height of magnolia plants by shortening the internodes and did not affect the stem diameter and reduced the number of nodes on the plant (Shi et al., 2021). The same preparation at doses of 0.5, 1.0, 1.5 and 2.0 mg/L caused a decrease in the stem linear size and its thickening in rapeseed plants in direct proportion to the dose. The retardant increased leaf area and dry matter mass of shoot and root in low and medium concentrations (Zuo et al., 2020). Spraying plants of *Brassica campestris* L. with uniconazole inhibited growth, slowed down the process of budding and flowering (Song et al., 2019).

Gibberellin inhibitors – uniconazole and ethephon – inhibited the growth of maize plants, promoted better dry matter accumulation and faster grain filling. The use of triazole-derived retinondant unicanozole turned out to be more effective than ethylene-producing ethephon (Ahmad et al., 2019). The retardants metconazole and paclobutrazole enhanced carbohydrate accumulation in mangoes (Cavalcante et al., 2020). We previously found that EW-25 reduced the linear size of eggplant and pepper plants, under the action of the preparation the number of leaves on the plant, the weight of the raw substance of the leaves and the dry matter of the whole plant increased. Under the action of the retardant, the chlorenchyma thickened, the cell volume of the columnar parenchyma and the chlorophyll content in the leaves increased. Such changes in the leaf apparatus under the action of EW-25 led to an increase in the quantitative indicators of the productivity elements of eggplant and pepper (Rogach et al., 2020; Rogach et al., 2021).

Therefore, the analysis of the literature testified to the fact that the exogenous use of GA_3 and its retardant inhibitors, especially from the group of triazoles, often leads to the same result – optimizing the production process of crops and increasing their yields, despite the opposite changes in the hormonal field, that are conditioned. Anyway, the literature possesses virtually no comparative systematic studies of the regulation of growth rate, morphogenesis, formation of photosynthetic apparatus and hormonal status of plants of nightshade vegetables under the action of GA_3 and triazole-derived retardants. Furthermore, the components of the regulation system of donor-acceptor relations in plants under the action of these preparations remain unknown.

Taking into consideration the aforementioned, the aim of the research was to study the effect of exogenous growth regulators – GA_3 and EW-250 – on morphogenesis, photosynthetic pigment content, endogenous phytohormone balance and productivity of *Lycopersicon esculentum* Mill. tomato plants. Moreover, it required determining the role of morphological, mesostructural and hormonal components in the regulation of donor-acceptor relations.

Materials and methods

The vegetation experiment was carried out in the conditions of soil-sand culture in opaque plastic vessels with a 10-litre capacity. Grey forest podzolic coarse-grained medium-loam soil was used in a 3:1 mixture with

sand. The plants were grown under controlled conditions at a temperature of + 20/17 °C (day/night), light intensity was 190 μmol/(m^2 • s), photoperiod was 16/8 years (day/night), relative humidity was 65 ± 5%, substrate humidity was maintained at 60% of total moisture content. They were watered daily with Knop's solution at the rate of 250 mL per vessel.

Bobcat tomato plants were sprayed once to make leaves completely wet with 0.005% solution of gibberellic acid (GA_3) (Power Grown, USA) and 0.025% solution of tebuconazole (EW-250) (Bayer, Germany) in the budding phase. Control plants were sprayed with distilled water. The repetition of the vegetation experiment is tenfold.

Analysis of morphological parameters was performed every 10 days from the day of treatment. Individual organs were weighed on laboratory scales to determine their mass. Leaf area was determined by cutting (Official methods of analysis of Association of Analytical Chemists International (18th ed.) Association of Analytical Chemists, Gaithersburg, Maryland). The average area of leaf blades was determined by multiplying the length of the leaf blade by its width and by a conversion factor of 0.75.

Leaf mesostructure was analyzed during carpogenesis (30th day after treatment). For anatomical analysis, the leaves of the middle tier were selected, which had completely finished growing. The plant material was stored in a mixture of equal parts of ethyl alcohol, glycerin, water with the addition of 1% formalin. The size of individual chlorenchyma cells was determined on preparations obtained by partial maceration of leaf tissues. Macerating agent – 5% solution of acetic acid in hydrochloric acid (2 mol/L). The dimensions of the anatomical elements were determined on a microscope Mikmed-1 (Lomo-Microsystems, RF) using an ocular micrometer MOV-1-15 × (Lomo-Microsystems, RF). It was done thirty-five times.

The chlorophyll content was determined in the raw material by spectrophotometric method on a spectrophotometer SF-16 (RF). The experiment was repeated five times (Official methods of analysis of Association of Analytical Chemists International (18th ed.). Association of Analytical Chemists, Gaithersburg, Maryland).

In order to determine phytohormones a portion of the material (2 g) was triturated in liquid nitrogen and homogenized in 10 mL of extraction solution (methanol, water, formic acid in a ratio of 15:4:1) and extracted for 24 hours. The extracts were centrifuged for 30 minutes at 15,000 rpm at a temperature of + 4 °C in a K-24 centrifuge (Janetski, Germany). The supernatants were drained, and 5 mL of extraction solution was added to the precipitate and kept for another 30 minutes, then centrifuged again. The combined supernatants were evaporated to 5 mL using a Typ 350P vacuum evaporator (Poland). Further purification of phytohormones was performed by the method (Kosakivska et al., 2020) on two solid-phase columns SPE C18, Sep-Pak Plus, Waters and SPE Oasis MCX, 6 cc/150 mg, Waters. Column C18 was used to remove lipophilic substances, proteins and pigments. The SPE Oasis MCX column was sorbed with IAA, ABA, GA_3 and cytokinins. Elution of IAA, ABA, GA_3 was performed with 100% methanol, cytokinins – with alkaline eluent: 60 mL of 100% methanol and 2.5 mL of 26% ammonia were adjusted to 100 mL with ultrapure water. The obtained eluents were evaporated to dryness on a vacuum rotary evaporator at a temperature not exceeding + 40 °C. The dry residue of each fraction was reduced to 200 μL with 45% methanol before analysis.

Analytical determination of phytohormones was performed by high performance liquid chromatography on Agilent 1200 LC liquid chromatograph with diode-matrix detector G 1315 V (USA) together with a single-quadrupole mass spectrometer Agilent G6120A. An Agilent ZORBAX Eclipse Plus C18 column with a lipophilic-modified sorbent with a particle size of 5 μm (reverse phase chromatography) was used for chromatographic separation. After chromatographic separation of the sample components in a volume of 20 μL with a solvent system of methanol, ultrapure water, acetic acid in a volume ratio of 45:54.9:0.1, IAA and ABA were detected in the UV absorption region at analytical wavelengths of 280 and 254 nm. After separating the samples with a solvent system, acetonitrile, ultrapure water, acetic acid (30:69.9:0.1) detected GA_3 on the signal of the mass detector. Samples with cytokinins were separated by a solvent system of methanol, water, acetic acid (35:64.5:0.5), and detection was performed at 269 nm. The rate of the mobile phase of solvents during the detection of IAA and ABA was 0.7 mL/min, GA_3 and cytokinins – 0.5 mL/min. Unlabeled IAA, ABA, GA_3, trans-zeatin-O-glucoside (t-ZG), trans-zeatin (t-Z), trans-zeatinriboside (t-ZR), isopentenyladenine (iP) and isopentenyladenine were used as standards in the construction of the calibration tables (IPA, Sigma-Aldrich, USA).

The content of the analytes in the samples was monitored using a mass spectrometer in a combined mode (electrospray and chemical ionization at atmospheric pressure) with negative polarity of ionization of analyte molecules in the analysis of IAA, GA_3, ABA and positive in the analysis of cytokinins (CTC). For quantitative analysis of GA_3, the MSD SIM mass detector signal was used (setting 50% of the scan time of the ionized molecule/charge indicator 345). If the content of phytohormone was less than 2.01 ng/g of crude substance, then in the table this value is indicated as traces.

The experiments were conducted in three biological and three analytical repetitions. Analysis and calculation of phytohormone content was performed using Agilent OpenLAB CDS ChemStation Edition software (rev. C.01.09).

The results were statistically processed using the computer program Statistica 6.0 (StatSoft Inc., USA). One-way analysis of variance was used (differences between the mean values were calculated according to the ANOVA criterion with the Bonferroni correction and considered plausible at $P < 0.05$) (Van Emden, 2008).

Results

Foliar treatment of tomatoes in the budding phase with 0.005% aqueous solution of GA_3 and 0.025% aqueous solution of EW-250 influenced the rate of growth processes. During the growing season, the height of the shoots was dominated by plants treated with GA3 solution, while with application of the retardant in the first half of the growing season, plant growth did not differ significantly from the control sample, and in the second – it slowed down. In the phase of fruit formation, the linear size of plants treated with GA_3 exceeded the control sample by 23.6%, while under the action of EW-250 they were 8.6% lower than the control sample (Fig. 1).

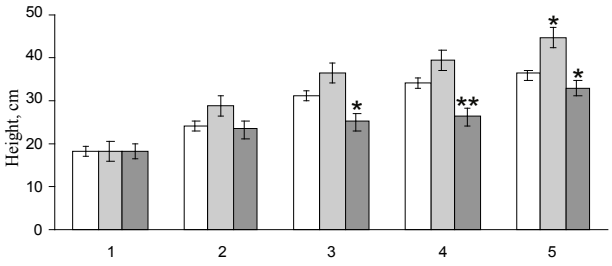

Fig. 1. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on the height of *Lycopersicon esculentum* Mill. gb. Bobcat: treatment in budding phase; n = 10; x ± SE; white – control, light grey – 0.005% gibberellic acid, dark grey – 0.025% tebuconazole; differences between the mean values were calculated using the Bonferroni-corrected ANOVA criterion; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ compared to control at this stage of vegetation; *1* – date of processing; *2* – 10th; *3* – 20th; *4* – 30th; *5* – 40th day after processing

Since the leaf is the main donor of plastic substances in the plant, the analysis of the influence of growth regulators on the leaf apparatus was carried out. It turned out that after treatment with GA_3 solutions the number of leaves on the plant increased by 16.1%, and under the action of EW-250 it did not change compared to the control sample (Fig. 2). GA_3 and EW-250 increased the number of leaf blades per leaf by 11.8 and 22.4%, respectively (Fig. 3). The consequence of such changes in the leaf apparatus was an increase in the total number of leaf plates on the plant, both during GA_3 treatment (29.8%) and after the application of EW-250 (19.8%, Fig. 4).

Under the action of growth regulators, the leaf raw matter mass increased. In particular, under the influence of GA_3, this figure increased by 0.91 times per plant, while after treatment with retardant – only 0.22 times (Table 1). GA_3 increased the stem and root raw weight, while EW-250 decreased it. During GA_3 treatment, the stem weight increased 1.22 times

and the root weight 0.75 times. Under the influence of EW-250, the mass of stems and roots tended to decrease compared to the control sample (Table 1). Growth regulators also affected the accumulation of dry matter of the whole plant. In the fruit formation phase, GA_3 induced an increase in dry weight of 0.93 times, while EW-250 did not change it (Table 1).

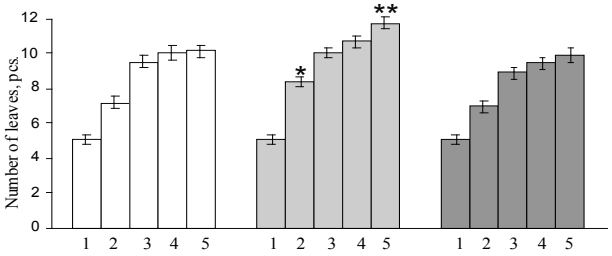

Fig. 2. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on the number of leaves on *Lycopersicon esculentum* Mill. gb. Bobcat: see Fig. 1

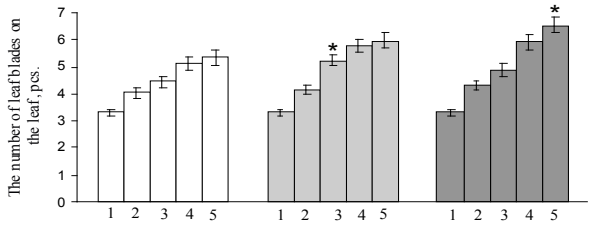

Fig. 3. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on the number of leaf blades on the leaf of *Lycopersicon esculentum* Mill. gb. Bobcat: see Fig. 1

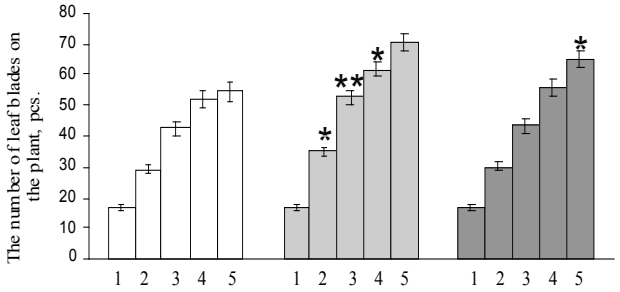

Fig. 4. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on the number of leaf blades on *Lycopersicon esculentum* Mill. gb. Bobcat: see Fig. 1

The area of leaves on the plant is one of the main indicators that affect crop capacity. It was found that during the whole research period the area of leaf blades after treatment with GA_3 increased by 3.3–7.7 cm^2. Phytohormone treatment led to an increase in the area of the leaf blade during carpogenesis by 44.8% (Fig. 5). When using a retardant, the average area of the leaf blade did not differ from the control sample (Fig. 4). In the fruit formation phase, the leaf surface area on the plant during GA_3 and EW-250 treatments exceeded the control sample by 79.9 and 7.1% (Fig. 5).

Taking into consideration that the content and ratio of photosynthetic pigments are an indicator of the efficiency of the assimilation apparatus, the effect of exogenous treatment with growth regulators on the amount of chlorophyll in tomato leaves was studied. It turned out that under the action of EW-250 the amount of chlorophyll in the leaves during the growing season increased significantly. In the fruit formation phase, this indicator exceeded the control sample by 44.1%. Under the action of GA_3, the chlorophyll content decreased by 11.3% (Fig. 7).

Table 1

The effect of foliar treatment with solutions of gibberellic acid and tebuconazole on the mass of vegetative organs of *Lycopersicon esculentum* Mill. plants, gb. Bobcat (n = 10, x ± SE)

Parameter	Control sample	Gibberellic acid	Tebuconazole
Mass of leaf raw matter, g	4.24 ± 0.16	8.08 ± 0.38***	5.15 ± 0.17**
Mass of stem raw matter, g	6.48 ± 0.27	14.41 ± 0.67***	5.98 ± 0.28
Mass of root raw matter, g	5.05 ± 0.23	8.81 ± 0.32***	4.73 ± 0.15
Mass of dry matter of the whole plant, g	4.52 ± 0.18	8.72 ± 0.36***	4.45 ± 2.08
Number of flowers per plant, pcs.	2.21 ± 0.08	3.88 ± 0.14***	4.05 ± 0.18***

Note: treatment of plants was in the budding phase, determination of indicators was in the fruit formation phase; * – P < 0.05; ** – P < 0.01; *** – P < 0.001, one line was compared by the difference between the mean values, calculated according to the ANOVA criterion with the Bonferroni correction.

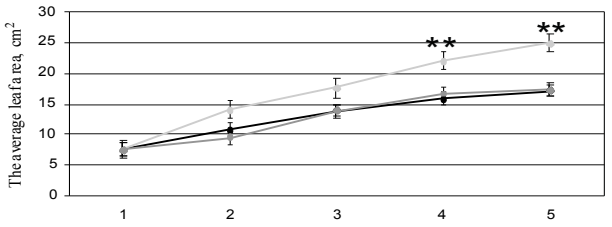

Fig. 5. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on the area of leaf blade of *Lycopersicon esculentum* Mill. gb. Bobcat: see Fig. 1

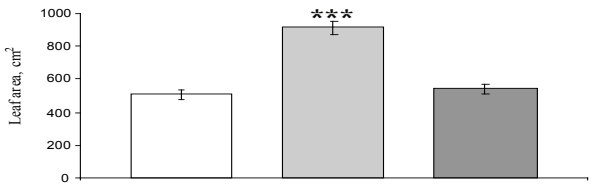

Fig. 6. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on the leaf area of *Lycopersicon esculentum* Mill. gb. Bobcat: treatment in budding phase; n = 10; x ± SE; white – control, light grey – 0.005% gibberellic acid, dark grey – 0.025% tebuconazole; differences between the mean values were calculated using the Bonferroni-corrected ANOVA criterion; * – P < 0.05; ** – P < 0.01; *** – P < 0.001 compared to the control at this stage of vegetation

Leaf mesostructural organization is an important indicator that determines the effectiveness of the photosynthetic apparatus of the plant and significantly affects its productivity. It is found that after treatment with EW-250 chlorenchyma thickened by 28.6 ± 1.41 μm, and after the use of GA_3 it thickened by 24.4 ± 1.21 μm. In general, the thickness of the leaf blade after the application of EW-250 increased by 15.1%, while with GA_3 treatment it decreased by 12.8%. Exogenous GA_3 and EW-250 increased the volume of columnar parenchymal cells by 29.8% and 92.2%, respectively. The size of spongy parenchyma cells increased only with growth stimulant treatment (Table 2).

The effects of foliar treatment of plants with GA_3 and EW-250 solutions on the distribution of endogenous IAA, GA_3 and ABA in tomato organs were studied. Under the action of exogenous GA3 in stems and leaves there was an increase in the content of endogenous IAA on 47.2% and 185.5%, while content of EW-250 was reduced by 20.0% and 59.2%, respectively (Fig. 6). Exogenous GA_3 increased the content of endogenous GA_3 in stems and leaves by 51.4% and 61.1%, correspondingly, while EW-250 decreased its content by 39.9% and 55.5%, respectively. The content of ABA increased by 67.2% for EW-250 treatments and decreased by 37.6% after the use of exogenous GA_3 in stems, and EW-250 increased its content by 26.7%, and GA_3 decreased by 50.6% in leaves.

Growth regulators affected the cytokinin content in the aboveground vegetative tomato organs. Five forms of cytokinins were identified in leaves and stems under control conditions: zeatin (Z), zeatinriboside (ZR), zeatin-O-glucoside (ZG), isopentenyladenine (iP), isopentenyladenosine (iPA) (Table 3).

Table 2

Influence of foliar treatment with solutions of gibberellic acid and tebuconazole on leaf mesostructural parameters of *Lycopersicon esculentum* Mill. gb. Bobcat (n = 35, x ± SE)

Parameter	Control sample	Gibberellic acid	Tebuconazole
Leaf blade thickness, μm	190.2 ± 6.2	165.8 ± 5.0**	218.8 ± 9.8*
Upper epidermis thickness, μm	20.03 ± 0.54	13.93 ± 0.55***	14.72 ± 0.29***
Chlorenchyma thickness, μm	158.3 ± 5.3	141.6 ± 4.1*	193.0 ± 9.3**
Lower epidermis thickness, μm	11.91 ± 0.39	10.25 ± 0.32**	11.05 ± 0.23
Columnar parenchyma cell volume, μm^3	5111 ± 255	6635 ± 310***	9822 ± 311***
Length of the spongy parenchyma cells, μm	18.31 ± 0.51	21.66 ± 0.40***	18.38 ± 0.37
Width of spongy parenchyma cells, μm	15.39 ± 0.46	18.80 ± 0.40***	16.37 ± 0.39

Note: see Table 1.

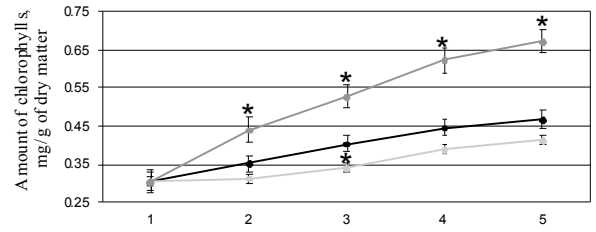

Fig. 7. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on content of chlorophylls (*a* + *b*) in leaves of *Lycopersicon esculentum* Mill. gb. Bobcat: see Fig. 1

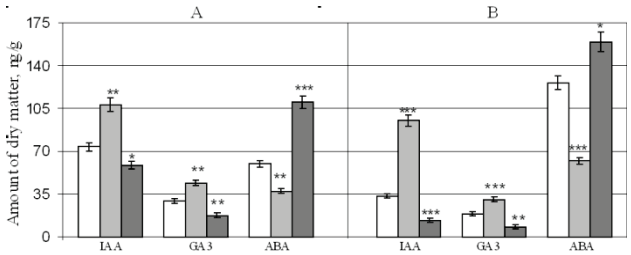

Fig. 8. Effect of foliar treatment with gibberellic acid and tebuconazole solutions on the content of endogenous phytohormones in stems and leaves of *Lycopersicon esculentum* Mill. gb. Bobcat: treatment in budding phase; n = 9 (3 (biological) × 3 (analytical)), x ± SE; white – control, light grey – 0.005% gibberellic acid, dark grey – 0.025% tebuconazole; *A* – the stem; *B* – the leaves; differences between the mean values were calculated using the Bunferoni-corrected ANOVA criterion; * – P < 0.05; ** – P < 0.01; *** – P < 0.001

In both stems and leaves, cytokinins were predominantly Z and ZR, as well as iPA in leaves in the GA_3 and EW-250 versions and iP in leaves in the EW-250 version. The total content of cytokinins in the leaves was higher than in the stems, both in the control and in the experimental variants. EW-250 induced a decrease in the cytokinin pool in stems by 11.6% and an increase in its leaves by 88.0%, respectively. When using exogenous GA_3, the cytokinin pool increased in leaves by 59.7% and decreased in stems by 33.1%. GA_3 increased the content of all 5 forms of cytokinins in the leaves, and EW-250 only two (iPA and iP). In the stems under the action of both growth regulators the content of C decreased and that of iP increased. The content of ZR and iPA in the stems increased after the application of the retardant and decreased under the action of the growth stimulant. ZG content exceeded control upon GA_3 treatment and the hormone was virtually absent after EW-250 administration.

The results of our research revealed a positive effect of growth regulators on the tomato productivity. GA_3 and EW-250 growth regulators increased the number of flowers per plant by 1.67 ± 0.06 and 1.84 ± 0.09 per plant, respectively. Moreover, after foliar treatment with GA_3 and EW-250 solutions, the number of fruits on tomato plants increased by 44.3% and 29.6%, correspondingly (Table 4). There was a significant increase in fruit diameter under the action of the retardant and the absence of such changes after the use of growth stimulants. The average weight of one fruit under the action of GA_3 remained virtually unchanged and increased after treatment with EW-250 by 41.1%. The change in the quantitative indicators of productivity elements under the action of growth regulators has led to an improvement in the biological productivity of the crop. The most significant fruit yield from the plant increased after the use of EW-250 and amounted to 205.9 ± 10.24 g per plant. When treated with GA_3 solution, this parameter increased to 119.1 ± 2.91 g per plant (Table 4).

Table 3

Effect of gibberellic acid and tebuconazole on the content of cytokinin isoforms in stems and leaves of *Lycopersicon esculentum* Mill plants. gb. Bobcat (ng/g of raw matter, n = 9 (3 (biological) × 3 (analytical)), x ± SE)

Organ	Parameter	Conrtol sample	Gibberellic acid	Tebuconazole
Stem	Zeatin	62.6 ± 3.0	17.5 ± 0.8***	47.9 ± 2.2*
	Zeatinriboside	42.5 ± 2.1	36.6 ± 1.7	45.5 ± 2.1
	Zeatin-O-glucoside	10.8 ± 0.5	15.5 ± 0.7**	traces
	Isopentenyladenine	3.6 ± 0.1	10.8 ± 0.4***	10.4 ± 0.4***
	Isopentenyladenosine	4.3 ± 0.2	2.3 ± 0.1***	5.5 ± 0.3*
	The amount of cytokinins	123.7 ± 6.2	82.8 ± 4.1**	109.3 ± 5.4
Leaf	Zeatin	46.9 ± 2.3	61.4 ± 3.0*	23.4 ± 1.2***
	Zeatinriboside	134.6 ± 6.7	161.4 ± 8.1	28.9 ± 1.4***
	Zeatin-O-glucoside	traces	43.3 ± 2.1***	traces
	Isopentenyladenine	13.4 ± 0.7	45.3 ± 2.2***	114.0 ± 0.7***
	Isopentenyladenosine	15.3 ± 1.4	232.9 ± 11.0***	223.3 ± 10.1***
	The amount of cytokinins	194.9 ± 9.7	311.3 ± 15.6**	366.4 ± 16.3**

Note: see Table 1.

Discussion

One of the important tasks of contemporary plant physiology and biochemistry is to study mechanisms of hormonal regulation of crop growth and development. Endogenous phytohormones take part in the management of physiological and biochemical processes at different levels of organization of the plant organism, the regulation of morphogenesis and plant production process. Changes in the rate of growth processes under the action of exogenous growth regulators and their synthetic analogues and modifiers, in particular gibberellin-like compounds and their inhibitors, are due to changes in the balance of endogenous phytohormones (Rademacher, 2016; Khalloufi et al., 2017; Song et al., 2019; Tkalich et al., 2021; Tsyliuryk et al., 2021). Therefore, the effect of gibberellins and anti-gibberellin preparations on the growth, development and productivity of cultivated plants requires further study (Jabir et al., 2017). Activation of growth processes and changes in the balance of endogenous hor-

mones under the influence of exogenous gibberellins were observed in the organs of tomato plants (Khalloufi et al., 2017) and zucchini (Song et al., 2019).

Table 4

Effect of gibberellic acid and tebuconazole on plant productivity elements of *Lycopersicon esculentum* Mill. gb. Bobcat (n = 10, x ± SE)

Parameter	Control sample	Gibberellic acid	Tebuconazole
Fruit diameter, cm	5.02 ± 0.18	5.25 ± 0.22	6.03 ± 0.28*
Average fruit weight, g	57.45 ± 2.67	58.86 ± 2.88	81.05 ± 4.74**
Number of fruits per plant, pcs.	4.33 ± 0.11	6.25 ± 0.32***	5.61 ± 0.19***
Weight of fruit from one plant, g	248.8 ± 9.9	367.9 ± 12.8***	454.7 ± 20.2***

Note: treatment of plants was in the budding phase, determination of indicators was in the fruit ripening phase; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$, one row was compared by the difference between the mean values calculated by the ANOVA criterion with the Bonferroni correction.

It is found that the application of exogenous GA_3 and EW-250 led to significant changes in the growth rate of tomato plants, as indicated by our previous studies (Rogach et al., 2020, 2021). The increase in the linear shoot size of GA_3-treated plants and the raw material masses of stem and leaf roots correlated with the increase in the content of endogenous growth stimulating hormones GA_3 and IAA in stems and leaves. On the contrary, EA-250 reduced GA_3 and IAA in tomato stalks and leaves, while ABA increased with inhibition of stem growth and increase in crude weight of all vegetative organs during GA_3 treatments and leaf weight of EW-250. It is found that there was a relatively low content of GA_3 in the control and experimental variants against the background of a sufficiently high level of ABA in tomato stems and leaves. The results obtained are similar to our previous ones (Rogach et al., 2020, 2021) and those of other authors (Ahmad et al., 2017), in particular when using similar regulators on tomato plants (Khalloufi et al., 2017) and flax (Kim et al., 2018).

The IAA content in tomato stems and leaves after retardant application was lower than in the control sample. One of the main centers of auxin biosynthesis is located at the top of the shoot (Khalloufi et al., 2017), which, in our opinion, led to a significant content of endogenous IAA in tomato stem tissues in the control sample and a GA_3 variant.

Exogenous GA_3 is shown to enhance growth and formation processes (Rogach et al., 2020, 2021). Under the action of the retardant, the growth of the stem in height was inhibited, but branching intensified and more leaves were laid (Kuryata et al, 2019). As a result of slowing down the linear growth of the stem, plastic substances were directed to the formation of new leaves.

The synthesis of chlorophyll in plants is known to be under the control of phytohormones of the cytokinin group (Rogach et al., 2020, 2021). It is revealed that under the action of EW-250, in contrast to GA_3, the content of chlorophyll increased (Rohach, 2017). The obtained results are consistent with the nature of the accumulation of cytokinins in the leaves of experimental plants. Under GA_3 and EW-250, the amount of cytokinins did not exceed the control by 59.7% and 88.0%, respectively.

Anatomical-morphological and physiological-biochemical changes under the action of growth regulators were realized through the restructuring of donor-acceptor relations and redistribution of flows of plastic substances between vegetative and generative organs (Kuryata et al., 2019; Poprotska et al., 2019). Exogenous GA_3 contributed to the formation of a more powerful leaf apparatus, increasing the number of leaves, their weight and area. After treatment with EW-250, these figures remained virtually unchanged compared to control samples. Increased photosynthetic activity enhanced the donor function of the leaf as well as the number of generative organs intensified the acceptance of newly formed plastic substances, which ultimately increased crop productivity.

Therefore, the application of exogenous multi-vector growth regulators – GA_3 and EW-250 in soil-sandy culture changed the growth rate of eggplants, affected the number of leaves on the plant, weight of raw material, leaf surface area and mesostructure of leaves, chlorophyll content, balance of endogenous phytohormones in aboveground vegetative organs, which led to the activation of the photosynthetic apparatus, resulting in increased biological crop capacity.

Conclusions

The research results demonstrate that exogenous growth regulators of plants with different directions of GA_3 effect (native stimulant hormone) and EW-250 (triazole-derived retardant), modulating the dynamics and distribution of endogenous plant phytohormones *Lycopersicon esculentum* Mill. Bobcat hybrid variety, induced changes in morphogenesis, structure and function of the leaf apparatus and optimized crop capacity.

It is found that foliar treatment with GA_3 increased plant height, while EW-250 inhibited stem elongation. Growth regulators induced formation of new leaves, accumulation of their biomass, increasing the area of a single leaf blade and the area of leaves on the plant. GA_3 also increased the biomass of stems and roots, and the dry matter mass of the whole plant was greater than in the control sample of both growth regulators. EW-250 increased the amount of chlorophyll ($a + b$) in the leaves. EW-250 thickened the leaf blades due to the growth of chlorenchyma cells while GA_3 significantly reduced their thickness. At the same time, an increase in the volume of cells of the columnar parenchyma was observed under the action of both preparations, yet the use of EW-250 turned out to be more effective.

Moreover, exogenous GA3 increased the content of endogenous IAA and GA_3 in stems and leaves. Conversely, EW-250 reduced GA_3 and IAA levels in tomato stems and leaves, and increased ABA levels. Treatment of plants with the studied growth regulators caused a decrease in the pool of cytokinins (CK) in the stems and led to its increase in the leaves. After spraying with GA_3 solution, the level of iP and ZR increased. Under the action of the retardant, the increase in the CK pool occurred exclusively due to iP and iRA.

Hence, anatomical-morphological and structural-functional rearrangements in tomato plants under the action of GA_3 and EW-250 are caused by changes in the balance and distribution of endogenous hormones. Increased photosynthetic activity, stimulation of growth processes of some plant organs and inhibition of others increased the biological crop capacity. The obtained results give a new practical approach to increasing tomato yield. Nevertheless, some questions about the molecular and physiological mechanisms of influence of the studied growth regulators require further investigation.

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