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THE INFLUENCE OF EXOGENOUS GROWTH REGULATORS ON THE CANNABINOID CONTENT AND THE MAIN SELECTION TRAITS OF HEMP (*CANNABIS SATIVA* L. SSP. *SATIVA*)

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Abstract: Hemp (*Cannabis sativa* L.) is a species sensitive to the influence of exogenous growth regulators, both in the treatment of vegetative plant tissues and *in vitro* culture. 1-naphthylacetic acid, indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, kinetin, 6-benzylaminopurine (BAP), gibberellic acid (GA_3), ascorbic acid and nicotinic acid of exogenous origin in the studied concentrations and doses caused a change in the content of cannabinoids in plants of the variety USO 31. Ascorbic acid, auxins and GA_3 significantly reduced the content of cannabinoids, whereas nicotinic acid and cytokinins increased it. Under the influence of nicotinic acid and BAP, a higher content of cannabinoid compounds was stably manifested during each of the three years of processing and it is inherited by at least one generation of descendants. An additional method to increase the level of non-psychotropic cannabinoids may be the treatment of vegetative plant tissues with cytokinin BAP (the concentration of 40 mg/l, the consumption rate of 30 ml/m^2, the phase of growth and development BBCH 51), which, in contrast to high concentrations of nicotinic acid, significantly increased the content of cannabidiol, and, to a lesser extent, tetrahydrocannabinol. The selection traits of the hemp – stem total length, mass and fiber content, seed productivity and sex determination significantly increased under treatment. A wide range of possibilities for phytohormones of exogenous origin in regulating cannabinoid accumulation, morphogenesis of hemp plants and their productivity was confirmed. Different hemp genotypes may have different responses to plant growth regulators and concentrations, which should be established in each case.

Key words: hemp, phytohormones, vitamins, cannabinoids, productivity, sex determination.

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Introduction

Phytohormones are physiologically active substances that are synthesized in certain tissues and at different stages of plant development, occupying a central place in the regulation of growth, especially the differentiation of plants in general, as well as in the culture of isolated cells and tissues. Based on physiological functions, phytohormones are divided into five groups: 1) auxins; 2) gibberellins; 3) cytokinins; 4) gaseous compounds (such as ethylene); 5) compounds that induce growth retardation and aging (e.g., abscisic acid). According to the latest data, growth regulators also include jasmonic acid, brassinosteroids and salicylic acid (Neumann et al., 2020). Some phytohormones have been discovered relatively recently, but auxins, for example, have been known for more than a hundred years, playing a role in endocytosis regulation, cell polarity, cell cycle, embryogenesis, histogenesis and organogenesis, but their other broad physiological functions and mechanisms still remain unclear (Sauer et al., 2013; Sudan et al., 2014). Of considerable scientific interest is the study of the effectiveness of phytohormones of exogenous origin as regulators of growth and development on hemp plants (*Cannabis sativa* L.) – a worldwide multi-purpose culture. The researchers' attention is focused on the possibility of increasing the yield of stems, fibers and seeds of hemp in the open ground and on the development of new cultivation systems specific to different genotypes of medical use in the conditions of closed ground (Burgel et al., 2020; Mendel et al., 2020).

Within the known studies, hemp had a significant response to the exogenous use of 1-naphthylacetic acid (NAA) at the concentration of 5 mg/l, 10 mg/l and 20 mg/l and 6-benzylaminopurine (BAP) at the concentration of 10 mg/l, 25 mg/l and 50 mg/l, influencing apical dominance, branching of shoots, bast fiber formation and flavonoid content. Hemp is characterized by a standard response to cytokinin treatment comparable to that observed in peas and beans (Mendel et al., 2020). A synthetic analogue of NAA, auxin, increased the yield of bast fibers, and the reaction to it differed from the standard response. This finding requires further study to establish the possible synergistic effect of this phytohormone with other growth regulators, namely cytokinins and gibberellins, which can be achieved, for example, by studying phytohormones in the vascular structures of the stem by immunological methods (Mendel et al., 2020).

In other studies, the height of hemp plants decreased significantly due to the use of NAA (28%), BAP (18%) and a mixture of NAA and BAP (15%). The length of internodes also decreased by 58% (when using NAA) and by 30% (when using a mixture of NAA and BAP), and the number of internodes decreased by 15% (NAA), 10% (BAP) and by 14% (when using a mixture of NAA and BAP), compared to control without treatment (Burgel et al., 2020). NAA changed the habit of inflorescence, which became more compact, although the seed yield and content of cannabinoid compounds remained similar to the control variant.

Increasing the biomass of inflorescences in closed soil led to an increased gathering of cannabinoids per unit area (Burgel et al., 2020). In general, one important detail should be noted: the nature of the response to phytohormones is specific to each genotype (variety) (Burgel et al., 2020).

In hemp, apical dominance prevents the development of lateral shoots (branching of the inflorescence). It can be suppressed in two ways: the removal of apical meristems (Ačko et al., 2019) and the use of phytohormones that affect the height and lateral branching of plants, leading to increased seed yield, as proved in other crops, such as *Solanum tuberosum* L. (Kumlay, 2014), *Moringa oleifera* Lam. (Brockman et al., 2020). It is indole-3-acetic acid (IAA), which is synthesized at the top of the shoot in young leaves and transported basipetally to the roots, that plays a key role in maintaining apical dominance (Brockman et al., 2020), which is important to consider when developing a model for the optimal use of exogenous growth regulators for hemp.

An important issue in the cultivation of crops is to increase the resistance of plants to drought, which is becoming a complex environmental problem due to anthropogenic activities. The mechanism of response to drought includes morphophysiological, biochemical, cellular and molecular processes in the plants, including changes in the root system, leaf structure, osmotic regulation of water content, stomatal activity, etc. (Ilyas et al., 2021). Abscisic acid is considered to be the main hormone that enhances the drought resistance of plants. Jasmonic acid and salicylic acid, ethylene, auxins, gibberellins and cytokinins, as a result of interaction with each other (the synergism of action), play a vital role in regulating various phenomena in plants to adapt to drought (Ilyas et al., 2021; Ullah et al., 2018). The intensification of research into the mechanisms of hemp resistance to drought with the use of phytohormones of exogenous origin may also become one of the priority areas in crop production.

As for other crops, phytohormones are extremely important in the cultivation of hemp *in vitro* (Galán-Ávila et al., 2020; Chaohua et al., 2016; Lata et al., 2016), which is used for the genetic transformation of plants, microclonal propagation, obtaining new starting breeding material (by chemical mutagenesis or polyploidy), storage of germplasm, and so on. Obtaining cannabinoids (specific chemical compounds of cannabis used in the pharmaceutical industry and medical practice) from calluses and cell suspension cultures was impossible. The lateral roots may form a small amount of these metabolites, but their synthesis is completed over time, and as a result, cannabinoids are negligible for industrial applications (Wróbel et al., 2018). It will be advisable to develop (improve) a highly efficient protocol for direct *in vitro* regeneration of hemp plants from different explants. The main problems are the induction of callusogenesis and strong apical dominance, which are overcome through the use of auxin antagonist α-(2-oxo-2-phenylethyl)-1*H*-indole-3-acetic acid and synthetic cytokinin derivative 6-benzylamino-9-

(tetrahydroxypyranyl) purin (Smýkalová et al., 2019). It was determined that the most effective ratios of "auxin: cytokinin" in the nutrient medium for the induction of callusogenesis are 2 : 1, 2 : 2, 2 : 3 and 3 : 2 µm (Thacker et al., 2018). Regarding root regenerating plants, NAA (Smýkalová et al., 2019) or indole-3-butyric acid (Chaohua et al., 2016) are usually added to the medium.

The aim of our study was to assess the effect of exogenous growth regulators on the content of cannabinoids and the main selection traits of hemp.

Material and Methods

The plant material was a variety of industrial monoecious hemp (*Cannabis sativa* L.) of Central European type USO 31. Its plants contain non-psychotropic cannabinoid combinations and tetrahydrocannabinol (THC) within the pale of the Ukrainian law, not exceeding 0.08%. Three-year field research was carried out in northeastern Ukraine, on the southern border of the mixed forest zone, lying in the lowest part of Ukrainian Polissia. The height above the sea was 166 m, and location area coordinates are: 51°39' N and 33°59' E. The soils used for crop rotation were dark and light gray forest, slightly podzolized loams formed on moraine clay. The fertilizer application rate was $N_{120}P_{90}K_{90}$. The weather conditions during the research period (2016–2019) were various and characterized by deviations from the average annual air temperature, precipitation and relative humidity, which made it possible to comprehensively assess the content of cannabinoid compounds and the level of selection traits in different weather conditions. In the period of 2016–2019, the air temperature during the growing season was 1.5–2.1 °C higher than the long-term average. The hottest weather was in 2018. The average monthly air temperature in July was 6.5°C, and in August 6.2°C higher than the long-term average. Within four years, the amount of precipitation per month was 3–19 mm lower than the long-term average. The uneven distribution of precipitation was observed both during the month and during the growing season of hemp. In September 2016, August 2018 and August 2019, only 3.2, 2.2, 0.8 and 9.9 mm of precipitation were detected, respectively. No sharp fluctuations in relative humidity were observed, except in April and August 2016–2019, when the relative humidity was 2–5% higher than the long-term average. In general, abiotic environmental factors for the normal growth and development of hemp were particularly favorable in 2016 and 2017.

The treatment of plants in the assessment nursery (the area – 1 m^2, the number of repetitions – four) and artificially isolated areas was carried out by spraying according to the options presented in Table 1.

In order to identify the likelihood of epigenetic effects under the influence of the prolonged and repeated action of growth regulators, mature seeds were collected from treated plants in the isolated nursery, which were then resown and the plants were treated with the appropriate stimulator for three years. After such a triple exposure, on the fourth year, the descendants were analyzed for cannabinoid content.

Table 1. Types of hemp cropper treatments with exogenous growth regulators.

Substance	Solution strength (mg/l)	Solution application rate (ml/m^2)	BBCH-scale treatment phase
Ascorbic acid (C)	400	30	BBCH 51 + BBCH 59
Nicotinic acid (PP)	200	30	BBCH 51 + BBCH 59
Succinic acid (SA)	200	30	BBCH 51 + BBCH 59
1-naphthaleneacetic acid (NAA)	20	30	BBCH 51
Indole-3-acetic acid (IAA)	200	30	BBCH 51
2,4-dichlorophenoxyacetic acid (2,4-D)	20	30	BBCH 51
Kinetin (KIN)	10	30	BBCH 51
6-benzylaminopurine (BAP)	40	30	BBCH 51
Gibberellic acid (GA_3)	100	30	BBCH 51

Note: Phenological phases of growth and development were determined by reference to the BBCH-scale (the abbreviation BBCH derives from the names of the originally participating stakeholders: "Federal Biological Institute, Federal Plant Variety Office and Chemical Industry") adapted for hemp (Mishchenko et al., 2017).

In order to identify cannabinoid compounds during the threshing of hemp plants grown in an assessment nursery with a feeding area of 30 cm × 5 cm (phase BBCH 89), a combined sample of plant material was taken from each area of 1 m^2, dried and stored at a laboratory temperature. Before the analysis, the samples were dried to constant weight at a temperature of 105 °C in an oven, ground to a fine powder and thoroughly mixed. The samples weighing 0.5 g were taken in two repetitions, and 5 ml of methanol was added (the ratio "plant type: extractant" – 1: 10). The extraction time was 24 h. After that, the extract was filtered using a paper filter. In the obtained methanol extracts of the samples, the quantitative content of cannabinoid compounds was determined by gas chromatography on an HP 6890 Series GC System chromatograph (Producer – Hewlett-Packard, USA). Chromatography conditions were: – capillary column – Agilent Technologies Inc. 19091J-413 (HP-5), length – 30 m, diameter – 0.320 mm, phase – 0.25 µm, SN: USN493366H, constant flow – 1.5 ml/min, carrier gas – helium; – injector – auto-injector 7683, Split 20 : 1, evaporator temperature – T = 250 °C; oven – $T_{initial}$ = 100°C, held for 2 minutes, heating – 15 °C/min, T_{final} = 280 °C, held for 11 minutes; – detector – flame ionization; – sample – 1.0 µl. Compounds were identified by retention time. The concentration of cannabinoids was determined using an internal standard (stearic acid methyl ester at a concentration of 0.392% of the sample).

Conditions for the microclonal propagation of hemp *in vitro* were as follows: hormonal medium Murashige and Skoog, supplemented with ascorbic acid 5 mg/l, 15 mg/l and 30 mg/l, photoperiod – 16 h, relative humidity – 60–80%, air temperature – 22–24 °C (records were performed on the 35th day of cultivation).

In the phase BBCH, 89 plants from each variant in the evaluation nursery were collected to determine selection traits: total stem length, technical stem length (distance from the root collar to the inflorescence), stem diameter (determined in the middle of the technical stem length), stem and fiber mass, fiber content, the mass of seeds from one plant, and the mass of one thousand seeds. Twenty plants were selected from all repetitions of each variant. Statistical data processing on the selection traits was performed by variation statistics methods ($\bar{x}$, $s_{\bar{x}}$, CV) using the Application Software Package "OSGE" (Ukrainian AAS, Institute of Plant Production©, TK "EliteSystems gr.", 1992, 93). The comparisons among the different treatments were made with the least significant difference (LSD) test and the Student's t-test. Statistical significance was determined at the level of $p < 0.05$.

Results and Discussion

Phytohormones, as well as vitamins and other physiologically active substances, play a very important role in plants. They have a role in the division of cells and their differentiation, forming of tissues, embryogenic processes, and ontogenesis. In addition, they influence inducible enzyme synthesis and the main vital processes of plants (breathing, water exchange, photosynthesis, excretion, etc.). Phytohormones are highly active physiologically, causing significant changes in development and growth at very low concentrations. Treating the plants with such phytohormones as NAA, IAA, 2,4-D, KIN, BAP, GA_3 and vitamins (ascorbic and nicotine acids) in the doses mentioned resulted in the change of cannabinoid content. In the variant with SA treatment, the content of cannabidiol (CBD), THC and cannabigerol (CBG), according to the three-year average, remained virtually unchanged (Table 2).

Table 2. The effect of exogenous growth regulators on the content of cannabinoid compounds (average for 2016–2018).

Variant	Content (%)		
	cannabidiol	tetrahydrocannabinol	cannabigerol
Untreated	0.1271	0.0030	0.0041
C	0.0276	0.0006	0.0021
PP	0.1419	0.0064	0.0082
SA	0.1264	0.0030	0.0040
NAA	0.0634	0.0027	0.0023
IAA	0.0461	0.0022	0.0034
2,4-D	0.2386	0.0069	0.0050
KIN	0.2173	0.0054	0.0049
BAP	0.2654	0.0044	0.0046
GA_3	0.0443	0.0010	0.0023
LSD_{05}	0.0455	0.0009	0.0009

Ascorbic acid, as an antioxidant, favored a decrease in the content of the identified compounds: CBD – with 0.1271%, and in control with near 0.0276% in the variant with processing; THC – with 0.0030%, and with near 0.0006% in the variant with processing; and CBG – with 0.0041%, and with near 0.0021% in the variant with processing, or by 78.3%, 80.0% and 48.8%, respectively, regarding the option without processing (Figure 1). Such data assert that ascorbic acid highly inhibits decarboxylation of the corresponding acidic forms of cannabinoids (CBDA, THCA and CBGA) into their neutral forms.

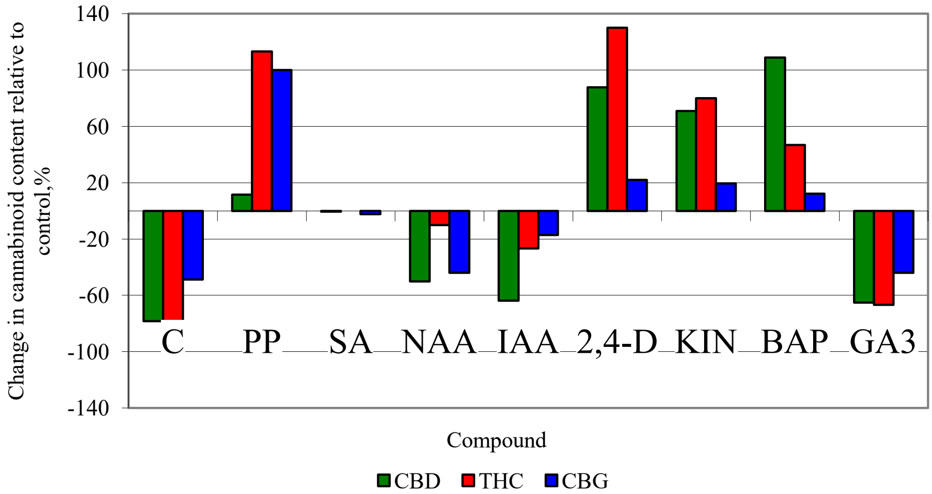

Figure 1. Changes in the content of cannabinoids in the plants of the USO 31 variety under the influence of growth regulators of exogenous origin, relative to control (+ or –), % (average for 2016–2018).

The effectiveness of hemp saturation with ascorbic acid, as an important metabolite and antioxidant, in combination with other components of the antioxidant system, for protecting plants from damage by oxidation products, is proven *in vitro*. In order to prevent the accumulation of phenolic compounds and their oxidation products, ascorbic acid was added to the nutrient medium at the concentrations of 5 mg/l, 15 mg/l and 30 mg/l. Increasing the concentration of ascorbic acid increases the length of the shoots and the number of internodes formed on them (according to average data). It also increases the efficiency of microclonal propagation, and the frequency of a positive, rapid response to cannabinoid compounds, supplemented with 30 mg/l of ascorbic acid. Therefore, in cultivating hemp *in vitro*, it is advisable to use this antioxidant to augment the efficiency of microclonal propagation (Table 2).

Table 2. The effect of ascorbic acid on the traits of hemp *in vitro*.

The concentration of C in Murashige and Skoog medium (mg/l)	Microclones				Plants with a negative reaction to cannabinoids (%)	
	length (cm)		number of internodes			
	$\bar{x} \pm s\bar{x}$	CV (%)	$\bar{x} \pm s\bar{x}$	CV (%)	1st passage	2nd passage
0	11.37 ± 0.710	27.9	7.0 ± 0.39	24.6	40	50
5	11.39 ± 0.847	33.2	7.3 ± 0.57	35.0	75	85
15	12.45 ± 0.668	24.0	7.4 ± 0.29	17.8	80	95
30	12.81 ± 0.421	14.7	7.9 ± 0.39	22.1	85	100

To a lesser extent, but at a reliable level of significance, the content of cannabinoid compounds was reduced by auxins (NAA and IAA) and gibberellins (GA_3). In particular, plants treated with the solution of NAA, prior to the phase of biological maturity, accumulated 0.0634% of CBD, 0.0027% of THC and 0.0023% of CBG. Plants of the variant with IOC synthesized 0.0461% of CBD, 0.0022% of THC and 0.0034% of CBG. The variant with GA_3 synthesized 0.0443%, 0.0010% and 0.0023% of the CBD, THC, and CBG. Such patterns can be explained by the fact that auxins are actively involved in the physiological mechanisms of specific hemp compound synthesis (they are inhibitors of their formation). Under the influence of gibberellins, there is a decrease in the general level of cannabinoids due to the increase in the proportion of male flowers in the inflorescence, which contain much fewer cannabinoids than the perianth of female flowers, as well as due to the increase in the total biomass of the inflorescence.

Nicotinic acid and cytokinins (2,4-D, KIN and BAP) contributed to the increase in cannabinoids. Moreover, due to the treatment with nicotinic acid, the content of THC (0.0064%) and CBG (0.0082%) increased approximately twice. As a result of exposure to 2,4-D, the content of THC approximately doubled (0.0069%). The content of CBD doubled (0.2654%) under the action of BAP, which is the largest increment value within the studied options. In addition, it was noted that, under the influence of nicotinic acid and BAP, a higher content of cannabinoid compounds is stably manifested during each of the three years of processing and is inherited by at least one generation of descendants (Table 3). Such changes cannot be explained in terms of the chromosomal theory of heredity, and they can be regarded as epigenetic only if the genotype × environment interaction is zero.

Changes of sex in hemp under the influence of photoperiod and the removal of plant tops of unisexual hybrids, created as a result of the crossing of a dioecious form of hemp with a monoecious one, have already been described (Hall et al., 2012; Mishchenko, 2018). The sex composition of F_1 hybrids grown in the field was mainly represented by female plants and a small number of monoecious plants. The sex structure shifted towards the female sex, which is fully embedded in the theory of the genotypic sex determination of hemp. Under the influence of the reduced duration of daylight, the sexual structure of hybrids shifted towards the male sex. The content of the female plants decreased (by 36.6–92.3%), the content of monoecious sexual types increased, and male plants were observed. When combining two factors of influence – the photoperiod and the removal of growth points – this pattern was even clearer. The change in the ratio of sexual types of same-sex hybrids under the influence of photoperiod is difficult to explain in terms of the theory of the genotypic sex determination of hemp – it is epigenetic. (Mishchenko, 2018). The displayed changes in the sex of hemp flowers under the influence of external conditions are epigenetically determined, despite the fact that it is normal for this species to be identified by a syngamous method of sex determination when chromosomal sex determination is realized with a fusion of male and female gametes. Environmental conditions (or signals) allowed for regulating both the sex of hemp flowers and the reproduction system seeds, maintaining the status of sex chromosomes in the genome (Maletsky, 2008; Maletsky et al., 2013).

Table 3. Changes in the content of cannabinoid compounds in the descendants of plants treated with exogenous growth regulators for three years (2019).

Variant	Content (%)		
	cannabidiol	tetrahydrocannabinol	cannabigerol
Untreated	0.1323	0	0
C	0.1344	0	0
PP	0.1596	0.0070	0.0123
SA	0.1365	0	0
NAA	0.1310	0	0
IAA	0.1335	0	0
2,4-D	0.1402	0	0
KIN	0.1347	0	0
BAP	0.2540	0.0035	0.0115
GA_3	0.1299	0	0
LSD_{05}	0.0384		

Note: 0 – the compound was not identified within the sensitivity of the gas chromatograph and the research methods used.

From the standpoint of epigenetic effects, a promising direction to increase the level of non-psychotropic cannabinoids is the treatment of vegetative plants with cytokinin BAP, which, in contrast to high concentrations of nicotinic acid, significantly increases the content of CBD and less psychotropic THC. This technique is recommended for the use in crop production as an adjunct in the cultivation of industrial varieties of medical hemp. However, once again, it should be noted that different genotypes (varieties) may have different reactions to phytohormones and their concentrations, which must be established in each case.

The treatment of hemp plants with phytohormones, vitamins and succinic acid also affected the change of (economic) selection traits. According to the results of the control of the unprocessed plants, the total length of the plants (14.6–32.6 cm) treated with the studied compounds of the above concentration and dose significantly exceeded that of the former. GA_3 contributed to the increase of technical length at a reliable level (210.5 cm compared to 180.9 cm), which has a positive effect on increasing the quantitative and qualitative characteristics of the fiber. The variants treated with ascorbic acid had a significantly shorter technical length (163.6 cm), i.e., this compound contributed to the increase of the inflorescence size, which is positive for the increase of seed productivity. No significant difference was found for stem diameter after treatments in regard to control. There was only a tendency to its increase, especially under the influence of vitamins, SA, NAA, cytokinins (KIN and BAP) and GA_3 (Table 4).

Table 4. The variability of selection traits relating to hemp plants treated with exogenous plant growth regulators (average for 2017–2018).

Statistical indicator	Processing option									
	without processing	C	PP	SA	NAA	IAA	2,4-D	KIN	BAP	GA_3
Total length (cm)										
$\overline{x} \pm s\overline{x}$	225.4±4.90	230.0±5.62	255.7±5.44	258.0±5.92	253.0±5.66	240.0±4.46	247.4±3.73	245.9±4.99	248.0±4.66	250.1±6.56
CV (%)	6.9	7.8	6.7	7.3	7.0	5.8	4.8	6.4	6.0	8.3
Technical length (cm)										
$\overline{x} \pm s\overline{x}$	180.9±6.06	163.6±5.88	171.1±6.98	174.7±5.15	192.7±5.50	179.2±4.58	189.6±5.91	181.1±5.12	193.7±5.72	210.5±6.74
CV (%)	11.0	11.4	12.9	9.3	9.1	8.0	8.4	8.9	9.1	10.2
Stem diameter (mm)										
$\overline{x} \pm s\overline{x}$	8.74±0.483	9.26±0.330	9.10±0.406	9.88±0.529	9.24±0.506	8.68±0.322	8.44±0.254	9.22±0.434	9.84±0.584	9.30±0.512
CV (%)	17.8	11.2	14.1	16.9	17.3	11.8	9.4	14.8	18.6	17.4
Stem mass (g)										
$\overline{x} \pm s\overline{x}$	15.5±4.11	19.2±1.58	20.0±1.96	19.9±2.26	20.5±2.22	16.2±1.17	16.3±0.77	18.3±1.73	20.7±2.44	18.2±2.18
CV (%)	28.3	26.0	31.1	35.9	33.7	22.4	15.0	29.9	37.1	37.8
Fiber mass (g)										
$\overline{x} \pm s\overline{x}$	4.50±0.480	6.10±0.503	5.59±0.561	5.21±0.449	6.54±0.576	5.54±0.432	4.98±0.296	5.89±0.536	6.52±0.750	5.72±0.583
CV (%)	33.5	25.8	31.7	27.2	28.6	25.2	18.8	28.8	36.6	32.2
Fiber content (%)										
$\overline{x} \pm s\overline{x}$	32.6±0.67	31.4±0.81	28.3±1.15	27.0±1.12	32.6±0.66	33.1±0.50	30.7±1.19	34.4±0.82	31.4±0.79	32.6±1.02
CV (%)	6.6	8.4	12.8	13.2	6.4	5.0	11.8	8.1	8.2	9.1
Seed mass (g)										
$\overline{x} \pm s\overline{x}$	4.00±0.300	3.86±0.375	5.94±0.260	5.80±0.240	6.10±0.304	4.17±0.457	3.91±0.455	3.07±0.400	4.74±0.464	3.00±0.168
CV (%)	10.6	11.2	14.3	17.1	16.9	11.8	9.4	9.9	9.9	15.5
Mass of 1000 seeds (g)										
$\overline{x} \pm s\overline{x}$	16.2±0.20	18.0±0.18	18.2±0.18	17.7±0.16	18.5±0.29	18.4±0.27	18.1±0.17	17.3±0.59	17.8±0.18	16.4±0.38
CV (%)	6.5	8.5	10.0	10.0	9.1	9.2	5.0	11.5	6.2	10.1

Note: The color highlights the options that are reliably different from the control of the Student's *t*-test.

The mass of the stem also increased insignificantly under the influence of processing. Nevertheless, at the same time, the fiber mass of the plant markedly increased, in particular by more than 35% in the variants treated with ascorbic acid, NAA and BAP (6.1 g, 6.5 g and 6.5 g, respectively, compared to 4.5 g in the variant without processing). Authentically lower fiber content was formed by plants treated with ascorbic acid and nicotinic acid, namely – 28.3% and 27.0% (in the control version, this figure was 32.6%).

The control variant seed mass (4.00 g) was higher than that obtained from plants treated with nicotinic acid and succinic acid and NAA (5.94 g, 5.80 g and 6.10 g, respectively). Hemp plants treated with GA_3 of the specified concentration formed seeds with lower mass (3.00 g). It was found that many variants of the experiment were also characterized by an increased mass of a thousand seeds. The highest rate was in the variant with the NAA treatment (18.5 g compared to the control – 16.2 g).

In general, one important detail should be noted: the nature of the response to phytohormones is specific to each hemp genotype (variety) (Burgel et al., 2020).

It is known that phytohormones change the sex of hemp, but these studies are mostly conducted on dioecious hemp or varieties of monoecious hemp with unstable monoecious signs (Myhal, 2004). The influence of gibberellins and partially auxins is most widely studied, while cytokinins were ignored. Therefore, in part, this issue still remains open and relevant. Sex control is very important for selection because the combination of a large proportion of female flowers in the inflorescence and a sufficient proportion of male flowers for the normal course of the pollination process is a necessary condition for the formation of the high seed productivity of hemp.

In the modern variety of monoecious hemp, the sexual structure is represented mainly by monoecious feminized female plants (84.0% of the total), with a share of less than 30% of male flowers in the inflorescence. When the 2,4-D trea tment was carried out, the action of phytohormone occasioned a shift of signs towards the female sex, in particular, 95.4% of monoecious feminized female plants were taken into account. The treatment with BAP, NAA, IAA, KIN and ascorbic acid caused a decrease in the number of monoecious feminized female plants with a small proportion of male flowers in the inflorescence – 85.0%, 75.0%, 73.5%, 72.8% and 59.4%, respectively, and an increase in the number of other sexual types. As a result of the GC_3 treatment, there was a shift of signs towards the male sex. The sexual structure was represented mainly by monoecious plants with a predominance of male flowers in the inflorescence. Nicotinic acid and SA contributed to the shift of the sexual structure towards the female sex (up to 100.0% in the first variant) (Figure 2).

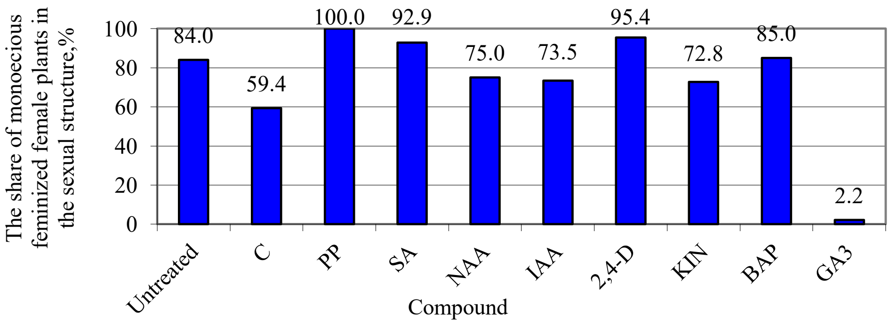

Figure 2. The influence of exogenous growth regulators on the formation of the sexual structure of hemp population (average for 2017–2018).

Plant growth regulators are successfully used in crop production to regulate plant morphogenesis, productivity and to control the biochemical composition of products, such as wheat (Iqbal and Ashraf, 2013), potatoes (Rogach and Rogach, 2015; Rogach et al., 2016), eggplant (Rogach et al., 2020), sweet pepper (Rogach et al., 2021), sugarcane (Qiu et al., 2019), coriander (Saleem et al., 2021), etc. Accordingly, there are wide opportunities for the use of phytohormones of exogenous origin in the regulation of cannabinoid accumulation and in the formation of valuable economic characteristics and productivity of hemp.

Conclusion

Hemp (*Cannabis sativa* L.) is a species sensitive to the effects of phytohormones, vitamins and other physiologically active substances. In plants of the variety USO 31, ascorbic acid as an antioxidant, auxins and GA_3 significantly reduced the content of cannabinoids, whereas nicotinic acid and cytokinins increased it. As a result of triple exposure to nicotinic acid and BAP, changes in the descendants persisted. An additional method to increase the level of non-psychotropic cannabinoids may be the treatment of vegetative plants with cytokinin BAP, which, in contrast to high concentrations of nicotinic acid, significantly increases the content of CBD and, to a lesser extent, THC. The selection traits such as stem total length, mass and fiber content, seed productivity and sex underwent the biggest changes under stimulator treatments. Different genotypes of hemp may have different reactions to phytohormones and their concentrations, which should be established in each case.

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UTICAJ EGZOGENIH REGULATORA RASTA NA SADRŽAJ KANABINOIDA I GLAVNE SELEKCIONE OSOBINE KONOPLJE (*CANNABIS SATIVA* L. SSP. *SATIVA*)

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R e z i m e

Konoplja (*Cannabis sativa* L.) je vrsta osetljiva na uticaj egzogenih regulatora rasta, kako kod tretiranja vegetativnih biljnih tkiva, tako i kod kultura *in vitro.* 1-naftilsićetna kiselina, indol-3-sirćetna kiselina, 2,4-dihlorofenoksisirćetna kiselina, kinetin, 6-benzilaminopurin (BAP), giberelinska kiselina (GA_3), askorbinska kiselina i nikotinska kiselina egzogenog porekla u ispitivanim koncentracijama i dozama izazvali su promenu sadržaja kanabinoida kod biljka sorte USO 31. Askorbinska kiselina, auksini i GA_3 značajno su smanjili sadržaj kanabinoida, dok su ga nikotinska kiselina i citokinini povećali. Pod uticajem nikotinske kiseline i BAP-a, veći sadržaj kanabinoidnih jedinjenja se stabilno ispoljavao tokom svake od tri godine prerade i nasleđuje ga najmanje jedna generacija potomaka. Dodatna metoda za povećanje nivoa nepsihotropnih kanabinoida može biti tretiranje vegetativnih biljnih tkiva citokininom BAP (koncentracije 40 mg/l, sa stopom unosa 30 ml/m^2, u fazi rasta i razvoja-BBCH 51), koji je, za razliku od visokih koncentracija nikotinske kiseline, značajno povećao sadržaj kanabidiola, a u manjoj meri i tetrahidrokanabinola. Selekcione osobine konoplje – ukupna dužina stabljike, masa i sadržaj vlakana, produktivnost semena i determinacija pola značajno su se povećali tokom tretmana. Potvrđen je širok spektar mogućnosti za upotrebu fitohormona egzogenog porekla u regulisanju akumulacije kanabinoida, morfogeneze biljaka konoplje i njihove produktivnosti. Različiti genotipovi konoplje mogu imati različite odgovore na regulatore rasta biljaka u različitim koncentracijama, što treba utvrditi u svakom slučaju.

Ključne reči: konoplja, fitohormoni, vitamini, kanabinoidi, produktivnost, determinacija pola.

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