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Effect Of Growth Regulators On The Content Of Basic Cannabinoids In The Plants Of Monoecious Cannabis Sativa.

VA Serkov[1]*, MV Danilov[1], VV Kosheliaev[2], and AA Volodkin[2].

[1]FSBSI Penza Research Institute of Agriculture, 442731, 1b, Michurina St., working settlement Lunino, Luninskij r-n, Penzenskaja obl., Russian Federation.
[2]Penza State Agrarian University, 440014, 30, Botanicheskaja St., Penza, Russian Federation

ABSTRACT

This paper represents the results of four-year cycle of scientific research on studying effect of growth regulators - gibberellin, auxin, cytokinin, chlorcholine chloride, sodium selenite – on the process of cannabinoids synthesis in the plants of cannabis sativa. Observed data was obtained regarding the level of basic cannabinoids accumulation – tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN) in the plants of two-purposed cannabis sativa of Surskaya variety. The differentiated reaction of plants to the plant growth regulators depending on the hydrothermal conditions during the interphase period of vegetation "shoots - budding" which is the critical period of plant growth and development is established. In the conditions of moisture deficit and increased mean daily temperature background in the interphase period of vegetation "shoots - budding" (hydrothermic coefficient is less than 0.5) significant increase by 1.4-1.7 times of total content of basic cannabinoids was noticed, including tetrahydrocannabinol content increase by 1.4-1.6 times. Also reliable differences in the level of cannabinol and cannabidiol accumulation in plants was stated in drought conditions depending on the variant of growth regulator treatment. In the conditions of sufficiently moisturized interphase period of vegetation "shoots - budding" (hydrothermic coefficient is more than 1.1) it was noticed that the treatment influence on basic cannabinoids is not significant except for cannabinol. In the conditions of moderately moisturized interphase period of vegetation "shoots - budding" (hydrothermic coefficient is 0.9-1.0) treatments also didn't affect the content of basic cannabinoids.

Keywords: cannabis sativa, nonnarcotic variety, cannabinoids, tetrahydrocannabinol, cannabidiol, cannabinol, plant growth regulators, auxins, cytokinins, gibberellins, chlorcholine chloride, sodium selenite.

Corresponding author

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INTRODUCTION

Endogenous effect of plants growth regulators at different stages of its ontogenesis. Influence of growth regulators on plant body is especially effective during the transition phase from vegetative growth to generative development. For cannabis plants transition period starts from three pairs of leaves. In this development period apexes of plant body transform from vegetative to pregenerative state and obtain the possibility of response to the change of ambient conditions, due to this use of environmental factors during this period may have multifunctional effect [8, 9, 11].

The effect of growth regulators in monoecious cannabis on functional activity of vegetative organs of monoecious nonnarcotic cannabis plants is underexplored. Mainly the study covered aspects of the influence of endogenous phytohormones on morphophysiological and yield indicators, as well as hormonal regulation of the process of gender differentiation of technical cannabis plants [2-4, 12-15].

That is why the research on the determination of influence of growth regulators (gibberellin, auxin, cytokinin, chlorocholine chloride and selenium) on the specific process of cannabinoid formation in cannabis is of undoubted practical interest when they influence the plant body in the juvenile phase of its development. Previously such researches of nonnarcotic varieties of technical cannabis have never been conducted in Russia.

Thus, research of character and degree of growth regulator influence on the process of cannabinoid formation in plants of nonnarcotic monoecious cannabis characterizes the timeliness of this paper.

MATERIALS AND METHODS

The research was conducted in [1]FSBSI Penza Research Institute of Agriculture in years 2013-2016. Study object is variety of nonnarcotic monoecious cannabis of Middle Russian ecotype Surskaya (two-purposed). Seed reproduction is OS (original seeds).

Complex of scientific and research works was conducted in field experiment using general agrotechnics and at natural length of daylight. Experiment is one-factor, plot allocation is systematic. Experimental seeding was conducted by seeding machine SN-16 with disc coulter in four-row variant.

Total area of plot is 30 m^2, declared area is 25 m^2. Seeding rate is 0.8 million pcs./ha. Replication is fourfold. Number of variants is 6. Experiment area is– 840 m^2. Forecrop is complete fallow.

Experimental design: spraying of plants with solutions of gibberellic acid (GA) in concentration of 30mg/l; solutions of auxin (IAA) in concentration 15 mg/l, cytokinin (CTK -6-benzylaminopurine (BAP)) is concentration 10mg/l; chlorocholine chloride (CCC) in concentration 6mg/l; sodium selenite (Na_2SeO_4) in concentration 3 mg/l. Operational fluid consumption made up 3 liters per 100 m^2 (300 l/ha). Operational liquid concentration and consumption are matched according to preliminary study of complex reaction of cannabis plants to the influence of the wide range of concentrations of mentioned growth regulators.

The research was conducted according to Methodical instructions for conducting field and vegetation experiments with cannabis and Methodical instructions for studying the cannabis collection of cannabis [5, 7].

Selection of plant material samples for analysis for cannabinoids content was conducted in accordance with the actual methodical instructions [5, 10]. Picking of top 15 cm of inflorescences was conducted during the beginning of blossom.

Identification and definition of quantitative content of basic cannabinoids (CBN, CBD, THC) was conducted by the method of gas-liquid chromatograph (GLC) analysis according to methodical recommendations Identification of narcotic substance type obtained from cannabis and poppy. [10]. Preparation of samples was conducted by drying tops of inflorescences at 110°C until the constant weight, grinding, taking weighting batch with mass of 0.1 g and flooding with 1 ml ofmethyl stearate with known concentration (1 ml) in ethanol, boiling, cooling, aging for 30 minutes at room temperature and conducting chromatography run.

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Cannabinoids were extracted with 96-% ethanol. Division of cannabinoids was conducted with temperature programming on gas-liquid chromatographic complex Crystal 2000M. Capillary column ZB-1, length is 30m. 0.5-% solution of methyl stearate in ethanol was used as internal standard.

Statistic processing of experimental data with the use of analysis of variance was conducted according to methods of Dospekhov B.A. [1].

THE EXPERIMENTAL PART

Main agro-climatic values during experimental period varied according to the regime of moistening and heat resources.

Vegetation period in 2013, 2016 was sufficiently moisturized (hydrothermic coefficient is 1.3 and 1.2 respectively), year 2015 was moderately moisturized (hydrothermic coefficient is 1.0), year 2014 was under moisturized (hydrothermic coefficient is 0.6).

Levels of basic cannabinoids accumulation in plants in general showed indifferent reaction to treatment with different types of growth regulators (table 1).

Table 1 – Average parameters of basic cannabinoid content in plants depending on the variant of growth regulators treatment, years 2013-2016

Variant	Cannabinoids content, %			
	CBD	THC	CBN	Σ
1. control	1.707	0.058	0.096	1.861
2. CTK 10	1.986	0.068	0.094	2.148
3. CCC 6	1.992	0.068	0.127	2.187
4. Se 3	2.028	0.070	0.125	2.223
5. IAA 15	1.796	0.060	0.089	1.945
6. GA 30	1.899	0.064	0.095	2.058
HCP_{05}	NS	NS	NS	NS

But in the aspect of certain vegetations during the experimental period some peculiarities of cannabinoid formation were discovered.

In 2013 hydrothermic coefficient of interphase period was characterized by the conditions of sufficient moisturizing during critical for plants growth and development period i.e. mass budding- beginning of blossom (hydrothermic coefficient is 1.2).

The evaluation of sum of basic cannabinoids showed that this value varied insignificantly in different plants and was within the range of 1.483 to 2.223%. Control plants were characterized by 1.699% of sum content of cannabinoids. Reliable differences for variants are not stated (table 2).

Table 2 – Content of basic cannabinoids in plants, 2013

Variant	Cannabinoids content, %			
	CBD	THC	CBN	Σ
1. control	1.610	0.057	0.032	1.699
2. CTK 10	1.835	0.062	0.039	1.936
3. CCC 6	1.992	0.068	0.127	2.187
4. Se 3	2.028	0.070	0.125	2.223
5. IAA 15	1.413	0.045	0.025	1.483
6. GA 30	1.971	0.064	0.047	2.082
HCP_{05}	NS	NS	NS	NS

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In 2014 hydrothermic background was satisfactory during critical for plants growth and development period i.e. mass budding- beginning of blossom. hydrothermic coefficient is 0.85 (moderate moisturizing)

The evaluation of sum of basic cannabinoids showed that this value varied slightly in plants and was within the range of 2.218 to 2.522%. Control plants were characterized by 2.293% of sum content of cannabinoids. Reliable differences for variants are not stated (table 3).

Table 3 – Content of basic cannabinoids in plants, 2014

Variant	Cannabinoids content, %			
	CBD	THC	CBN	Σ
1. control	2.061	0.063	0.170	2.293
2. CTK 10	2.245	0.077	0.148	2.470
3. CCC 6	2.050	0.071	0.167	2.287
4. Se 3	2.270	0.077	0.174	2.522
5. IAA 15	2.018	0.067	0.133	2.218
6. GA 30	2.035	0.065	0.152	2.251
HCP_{05}	NS	NS	NS	NS

Parameter “THC content” variety range was 0.063-0.077% and was the smallest in the control variant. Reliable differences for variants are also not stated.

In 2015 hydrothermic background for vegetation was stabilized on the level of under moisturizing during critical for plants growth and development period i.e. mass budding- beginning of blossom. hydrothermic coefficient is 0.46.

In conditions of a highly droughty interphase period “shoots - budding" treatment with growth regulators significantly increased the content of cannabinoids. Evaluation of the sum content of the basic cannabinoids showed that in plants of different variants this index varied significantly and was in the range from 2.264 to 1.904%. Control plants were characterized by 1.317% of a sum content of cannabinoid (table 4).

Parameter “THC content” variety range was 0.065-0.045%. The control variant showed the least value of this parameter. Thus it was stated the significant increase of the parameter in variants with growth regulators treatment due to rigid hydrotermic conditions of interphase period “shoots - budding”. Nevertheless, the absolute values of the paratmeter were less than the law-permissible level of content (less than 0.1%) by 0.027-0.035%.

Table 4 – Content of basic cannabinoids in plants, 2015

Variant	Cannabinoids content, %			
	CBD	THC	CBN	Σ
1. control	1.195	0.045	0.078	1.317
2. CTK 10	1.874	0.067	0.112	2.054
3. CCC 6	2.055	0.073	0.137	2.264
4. Se 3	1.981	0.072	0.136	2.190
5. IAA 15	1929	0.071	0.122	2.122
6. GA 30	1.731	0.065	0.108	1.904
HCP_{05}	0.387	0.013	0.024	0.432

Parameter “CBD content” varied in variants from 1.195 to 2.055% and also was the smallest in the control variant.

Cannabinol content changed significantly in experimental variants and in all variants exceeded the parameter value if compared with the control variant by 0.030-0.059%.C

In 2016 during critical for plants growth and development period i.e. mass budding- beginning of blossom hydrotermical background was characterized by sufficient moisturizing. hydrothermic coefficient is 1.21.

In conditions of moisturized interphase period “shoots - budding” treatment with growth regulators didn’t affect the total content of cannabinoids. Evaluation of the sum content of the basic cannabinoids showed that in plants of different variants this parameter varied insignificantly and was in the range from 1.956 to 2.127%. Control plants were characterized by 2.130% of a sum content of cannabinoid (table 5).

Table 5 – Content of basic cannabinoids in plants, 2016

Variant	Cannabinoids content, %			
	CBD	THC	CBN	Σ
1. control	1.961	0.066	0.103	2.130
2. CTK 10	1.988	0.064	0.075	2.127
3. CCC 6	1.870	0.059	0.078	2.007
4. Se 3	1.832	0.062	0.066	1.960
5. IAA 15	1.824	0.056	0.076	1.956
6. GA 30	1.859	0.060	0.073	1.992
HCP_{05}	NS	NS	0.022	NS

Parameter “THC content” variety range was 0.056-0.064%. In control variant the value of parameter was 0.066%.

Parameter “CBD content insignificantly varied in variants from 1.824 to 1.988%. CBN content in all variants of experiment was significantly lower than in control variants by 0.025-0.037%.

CONCLUSIONS

In the aspect of cannabinoid formation process the reaction of cannabis sativa plants to growth regulator treatment was registered depending on the character of hydrotermical conditions. In normally moisturized vegetation conditions during the critical period of plants growth and development period i.e. “shoots-budding” insignificant variability of basic cannabinoid content level, including tetrahydrocannabinol, if compared with the control variant was registered.

In conditions of draughty interphase period “shoots-budding” treatment with growth regulators significantly increased the sum content of cannabinoids by 1,4 – 1.7 times, including tetrahydrocannabinol content increase by 1.4-1.6 times. Also reliable differences in levels of cannabinol and cannabidiol accumulation in plants depending on the type of growth regulator treatment was stated in draughty conditions. Nevertheless, the absolute values of the parameter “THC content” were less than the law-permissible level of content (less than 0.1%) by 0.027-0.035%.

Thus, the reaction of plants of monoecious cannabis sativa to growth regulator treatmentdepends rather onthe factor of hydrotermic conditions in the interphase period “budding-beginning of blossom” than on the type of certain growth regulator.

Application of growth regulator treatment for nonnarcotic cannabis sativa plants of monoecious ecotype in order to increase quantitative and qualitative parameters of the yield does not create the risk of THC content increase, as THC is the basic narcotic compound in plants of this culture.

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