

PHENOTYPIC EXPRESSION OF CANNABINOID PRESENCE AND CONTENT UPON SELF-POLLINATION AND DIRECTIONAL SELECTION IN MONOECIOUS HEMP

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It was found that upon targeted selection of initial cannabis plants without cannabidiol, tetrahydrocannabinol and cannabinol their contents decreased to complete absence in the process of self-pollination. The stability (homozygation) of lines was achieved in $I_2 - I_6$ and depended on the genotype of a particular variety. Self-pollinated lines of these generations should be used as parents in crossing. The ability to segregate cannabinoid-free families as early as in I_1 is a characteristic feature of the hemp varieties under investigation. There were strong positive correlations between the cannabinoid contents, which makes selection for reduced contents of all cannabinoid compounds and easier, but at the same time significantly complicates breeding for increased cannabidiol content with concurrent reduced tetrahydrocannabinol content. The correlations between the contents of cannabinoid compounds in self-pollinated lines were weaker than those in the initial forms, which allows using closely related reproduction in breeding aimed at tetrahydrocannabinol elimination and increasing non-psychoactive cannabinoid contents.

Key words: *hemp, breeding, inbreeding, self-pollinated lines, inheritance, cannabinoids, correlation*

Introduction. The presence of cannabinoid compounds is one of the main biological features of cannabis (*Cannabis sativa* L.), which is constantly monitored at all stages of breeding and seed production: from creation of starting material to production of certified seeds and cultivation on an industrial scale. The need to stabilize the absence of cannabinoid compounds and monoeciousness significantly complicates breeding for increased productivity, improved fiber and oil quality, resistance to pests, diseases and abiotic factors.

Review of literature, problem statement. Cannabinoids are specific substances in cannabis that belong to the class of aromatic compounds and are synthesized and accumulated mainly in glandular trichomes (hairs) [1–5]. It is believed that the cannabinoid biosynthesis occurs on the plasma membrane surface or in the cell wall at the border with secretory cavities. Cannabinoids are found not only in excretory tissue cells. This attests to the fact that genes for the synthesis of these compounds can be expressed in all plant cells; however, it is glandular trichomes that specialize in the synthesis of high amounts of cannabinoids, in other tissues the contents of these substances are much lower [6]. Cannabinoids are toxic for plant cells, as they change the permeability of mitochondrial membranes, cause DNA degradation, which ultimately leads to apoptosis [7, 8]. To avoid damage and cell death, an adaptation, which consists in the fact that cannabinoids are accumulated and stored in the glandular trichome cavities (specialized secretory tissues), evolved. It is glandular trichomes that are the site of the last stage of the biosynthesis of these compounds [2, 6]. Probably, cannabinoids play a protective role in the plant, while reduction in cannabinoid levels and in the number of glands in technical (industrial) hemp do not change these physiological functions; the synthesis of these substances in small quantities by other cells of the plant is sufficient [6].

The most common cannabinoids in the glandular trichomes of cannabis are tetrahydrocannabinol acid (THCA), cannabidiol acid (CBDA) and cannabigerolic acid (CBGA). Bioactive cannabinoids (neutral chemical compounds) – tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG) and others – are produced as a result of the decarboxylation under the influ-

ence of external conditions. Decarboxylated derivatives – cannabichromen (CBC) and cannabinol (CBN) are found in small quantities [9]. About 120 phytocannabinoids have been identified in cannabis [10, 11]. Currently, cannabinoid compounds are classified by their chemical structure, mainly, there are 11 subclasses, which include: 1) seven types of CBG; 2) five types of CBC; 3) five types of CBD; 4) basic psychoactive Δ^9 -THC in nine different forms, including its precursor Δ^9 -THCA (acid), Δ^8 -THC, which is the most stable isomer of Δ^9 -THC, but 20% less active; 5) three types of cannabicyclol (CBL), 6) five different forms of cannabielsoin (CBE); 7) seven types of CBN, which is the final product of the synthesis (oxidation) of Δ^9 -THC; 8) cannabitrinol (CBT); 9) cannabidivarin (CBDV); 10) tetrahydrocannabivarin (THCV); 11) others [11–13]. Cannabimovon (CBM) has recently been identified and is being actively studied [14].

The biosynthesis pathways of major cannabinoid compounds in hemp have been discovered relatively recently. Cannabinoid precursors are produced in two different biosynthetic pathways: polyketide, which produces olivetolic acid, and plastid, which produces geranyl diphosphate, from which CBGA is synthesized by prenyltransferase, which is the key precursor of at least eight different cannabinoids. It has been proved that enzymes can convert CBGA even under experimental hydrophobic imitation of trichomes [3]. Specific synthases that ferment a certain cannabinoid compound have been identified and characterized [17–19], in particular THCA-synthase converts CBGA to THCA [20, 21], accordingly CBDA-synthase – to CBDA [22] and CBCA-synthase – to CBCA [23].

It is customary to distinguish several chemical phenotypes (chemotypes) of cannabis based on the ratio of certain cannabinoids. Given the inheritance patterns of chemical phenotypes, genes encoding THCA-synthase and CBDA-synthase are considered codominant alleles in one locus. This codominance is due to two alleles for different isoforms of the same synthase, which shows different specificity to the conversion of CBGA-precursor to CBDA or to THCA, respectively [24], while the gene encoding CBCA-synthase is in an independent locus. In other studies, a variety of sequences for THCA- and CBDA-synthase was observed, which may be attributed to the presence of several linked loci containing these genes [25]. RAPD analysis showed that the ability to accumulate THC is not a dominant feature, and the primary synthesis of non-psychoactive cannabidiol is associated with high activity of CBDA-synthase [26]. In general, the genetic mechanisms of regulating the cannabinoid presence and contents are quite complex and still being studied.

Since the 1970s, the domestic breeding and genetic studies of hemp has been gradually reoriented to the creation of monoecious high-yielding varieties and hybrids with low cannabinoid contents. Subsequently, almost cannabinoid-free varieties were developed; however, this feature requires further stabilization via eliminating the physiological and biochemical functions of cannabis to synthesize cannabinoids. To accelerate the breeding work, as early as at the initial stages of breeding it is necessary to select such source plants that would not have the ability to synthesize undesirable compounds in the offspring. We assume that closely related reproduction can serve as an effective method, in particular its extreme variant - self-pollination, which allows to differentiate a complex heterozygous population of the cross-pollinated crop into a number of relatively homozygous lines with stable expression of the vast majority of breeding traits, and subsequent development of heterozygous hybrids that would be homogeneous in terms of the absence of cannabinoid compounds from these self-pollinated lines. Self-pollination can be also used in the medical cannabis breeding (which is currently being actively developed), i.e. in the creation of varieties with high contents of non-psychoactive CBD, CBG, CBN, CBC with concomitant absence or very small amounts of THC.

Previous studies have demonstrated that it is possible to obtain self-pollinated cannabis lines, which are valuable because of morphological characteristics of the stem (total and technical length, diameter), fiber characteristics (stem and fiber weight, fiber content), seed productivity, sex composition, etc. [27].

Purpose and objectives. The purpose was to reveal peculiarities of the inheritance of the traits of cannabinoid compound presence and contents upon self-pollination in monoecious hemp. The objectives were to establish peculiarities of the inheritance of the traits of cannabinoid pres-

ence or absence in plants of self-pollinated lines from different generations and varieties developed as a result of targeted selection for complete absence of such compounds; to determine expression levels of the “cannabinoid contents” traits in self-pollinated lines (if starting forms had these traits); to compare the pair correlation coefficients between the contents of major cannabinoid compounds in initial genotypes and self-pollinated lines; and to evaluate the effectiveness of self-pollination of cannabis plants as a breeding method to stabilize (increase the homozygosity level) of starting material by the non-psychoactivity trait.

Material and methods. The study was carried out at the Institute of Bast Crops of the National Academy of Agricultural Sciences (Hlukhiv, Sumy Oblast, Ukraine) in 2009–2019. Inbred lines of industrial hemp varieties Hlukhivski 58, Hlesiia, Mykolaichyk, and Hlukhivski 46 belonging to the Central European eco-geographical type and variety Zolotoniski 15 belonging to the Southern eco-geographical type were taken as test objects. Self-pollination of plants (with and without cannabinoids) was carried out under individual agrotexile bags in a greenhouse. The offspring were grown in an evaluation nursery. Plants were sampled for determination of cannabinoid contents during phase BBCH 87 [28] (20 plants per family – offspring of a self-pollinated plant). Analysis of cannabinoid compounds were conducted by thin-layer chromatography as follows: extraction medium - ethanol, solvent mixture "petroleum ether 65-90°C - diethyl ether" (40:10), staining - durable blue B dye, calibration reference sample with known cannabinoid contents (variety US 9), 10-point rating scale. In all the varieties and inbred lines under study, the THC content did not exceed 0.08%; that is the standard allowed by the current legislation of Ukraine. Data were statistically processed with calculating arithmetic mean, sampling mean error, pairwise correlation coefficients, and curvilinear regression in compliance with the field experimentation methodology [29].

Results and discussion. Plants with the 0-point contents of CBD, THC and CBN were selected from variety Hlukhivski 58 to obtain self-pollinated lines belonging to the Central European eco-geographical type. I_0 plants were obtained from sown seeds, which segregated by the studied trait, indicating their heterozygosity (in a broad sense). The average CBD content was 0.06, THC – 0.01 and CBN – 0.04 points. Subsequently, plants without cannabinoids were self-pollinated, however, in I_1 and I_2 the cannabinoid contents increased: the CBD content was found to be 0.21, THC – 0.15 and CBN – 0.13 points in I_1 , and 0.18, 0.24, 0.17 points in I_2 , respectively. This indicates that genes determining the expression of cannabinoid content genes were converted into a homozygous state, and that mutant plants with cannabinoids segregated as a consequence of the genotypic environment effect on persistent changes in the hereditary apparatus upon the extreme variant of closely related reproduction. The semi-quantitative assessment by thin layer chromatography showed that the CBD content increased by 3.5, THC – by 15.0, and CBN – by 3.2 times in the 1st generation from self-pollination, respectively by 3.0, 24.0 and 4.2 times in the second generation after self-pollination. In I_3 derived from Hlukhivski 58, the CBD content decreased to the level of I_0 , and the contents of the other two studied compounds – to the level of I_1 (Fig. 1).

A characteristic feature of the response of the studied variety to closely related reproduction was that in the 1st generation the THC content increased the most of all, and in the 2nd generation after self-pollination the accumulation persisted. The cannabinoids were completely eliminated in I_4 , and no plants with these compounds appeared in later generations up to I_{10} ; only in I_5 from Hlukhivski 58 there were few specimens with weak trace contents of the cannabinoids and traces of nonpsychoactive CBD (Table 1), but the average content of this substance was 0.00 points.

Biological features of variety Zolotoniski 15 belonging to the Southern eco-geographical type were quite different from those of the above-described variety belonging to the Central European eco-geographical type: the response to closely related reproduction and the inheritance patterns of cannabinoid compounds as a result of self-pollination differed.

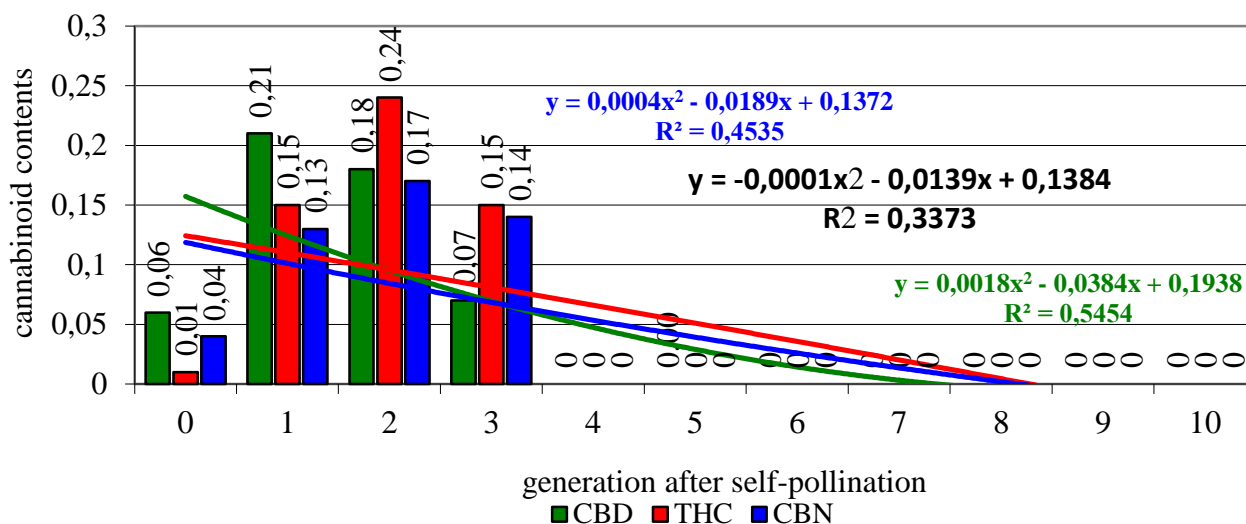


Fig. 1. Reduction of the CBD, THC and CBN contents upon self-pollination of variety Hlukhivski 58 and selection for cannabinoid complete absence (average, 2009–2018)

Table 1

Inheritance of the “complete absence of cannabinoid compounds” trait in plants of self-pollinated lines derived from the hemp varieties as a result of directional (average, 2009–2019)

Com- pound	Number of plants (%) without cannabinoid in generations										
	I ₀	I ₁	I ₂	I ₃	I ₄	I ₅	I ₆	I ₇	I ₈	I ₉	I ₁₀
	Hlukhivski 58										
CBD	88,2	81,3	75,4	80,0	100	97,5	100	100	100	100	100
THC	96,7	90,9	81,7	87,5	100	100	100	100	100	100	100
CBN	96,7	91,9	90,8	88,3	100	100	100	100	100	100	100
	Zolotoniski 15										
CBD	83,6	81,1	81,2	96,7	96,7	92,5	100	100	100	100	100
THC	93,9	93,6	93,8	98,3	100	98,3	100	100	100	100	100
CBN	91,1	93,6	93,1	98,3	100	98,3	100	100	100	100	100
	Hlesiia										
CBD	100	97,4	96,2	98,0	100	100	100	100	100	–	–
THC	100	99,7	100	100	100	100	100	100	100	–	–
CBN	100	99,7	100	99,0	100	100	100	100	100	–	–
	Mykolaichyk										
CBD	91,7	97,8	100	100	100	100	100	–	–	–	–
THC	100	97,8	100	100	100	100	100	–	–	–	–
CBN	91,7	97,8	100	100	100	100	100	–	–	–	–
	Hlukhivski 46										
CBD	100	99,0	100	100	100	100	100	–	–	–	–
THC	100	99,0	100	100	100	100	100	–	–	–	–
CBN	100	99,0	100	100	100	100	100	–	–	–	–

First, higher contents of cannabinoid compounds were observed in I₀ from Zolotoniski 15, although seeds were sown from plants with CBD 0, THC 0 and CBN 0 points. On average, CBD 0.10, THC 0.07 and CBN 0.14 points were identified. In I₁, there was an increase in the contents of test compounds, namely: CBD and THC – up to 0.15 points, CBN – up to 0.25, or by 1.4, 2.1 and 1.8 times, respectively, i.e. the contents of all the three compounds increased similarly. Secondly, starting from I₂ the cannabinoid contents began to decrease (in contrast to I₂ from Hlukhivski 58): as early as in I₃ from Zolotoniski 15 the levels these compounds were lower than those in I₀, which is a characteristic feature of this variety. It should be noted that in I₄ the canna-

binoid contents decreased to almost zero, and in I₅ there was a sharp increase: on average CBD 0.08, THC 0.06 and CBN 0.01 point were detected. The complete absence of cannabinoids and stabilization (homozygation) occurred starting with I₆ (Fig. 2).

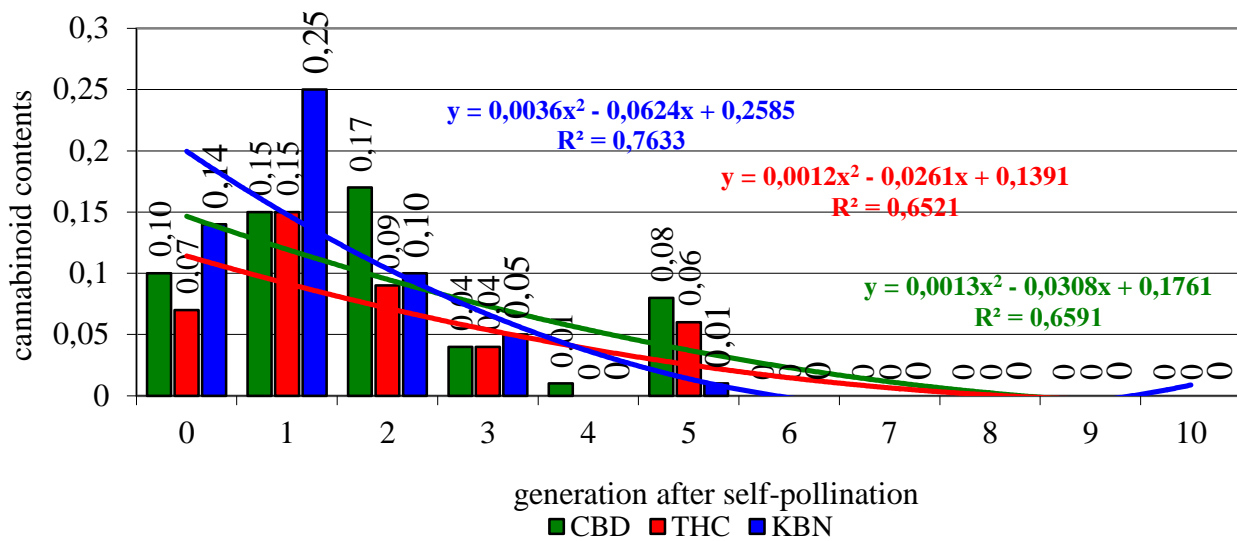


Fig. 2. Reduction in the contents of CBD, THC and CBN upon self-pollination of variety Zolotoniski 15 and selection for their complete absence (average, 2009–2018)

Thus, variety Zolotoniski 15 is more heterozygous for the studied traits and requires involvement of the later generations of self-pollinated lines to create starting breeding material, for example via hybridization. At the same time, a notable feature of self-pollinated lines derived from Zolotoniski 15 is a larger number of plants without cannabinoids at all in I₁–I₃, compared to the self-pollinated lines derived from Hlukhivski 58. Thus, 81.1% of plants I₁ from Zolotoniski 15 contained no CBD, 93.6% of plants – no THC, and 93.6% – no CBN, i.e., as a rule, more in comparison with I₁ from Hlukhivski 58, where these indicators were 81.3, 90.9 and 91.9%, respectively. In I₂ from Zolotoniski 15, these values were 81.2, 93.8 and 93.1%, respectively; compared with 75.4, 81.7 and 90.8% in I₂ from Hlukhivski 58, respectively; in I₃ from Zolotoniski 15 - 96.7, 98.3 and 98.3%, respectively, compared with 80.0, 87.5 and 88.3% in I₃ from Hlukhivski 58 (see Table 1).

Variety Hlesia appeared to be quite stable in terms of absence of cannabinoid compounds in general and nonpsychotropy (no THC). No specimens with cannabinoids were detected among I₀ plants. In I₁ from Hlesia, the CBD content was 0.03 points and CBN – 0.01; in I₂, the CBD content averaged 0.02 points; in I₃, plants with CBN segregated, and the CBN content averaged 0.01 points. THC was not detected at all. From I₄ to I₈, there were no cannabinoid compounds at all.

In contrast to varieties Hlukhivski 58 and Zolotoniski 15, variety Hlesia is characterized by an extremely high level of homozygosity for the absence of cannabinoids upon close related reproduction, since few plants with cannabinoids segregated. This fact is confirmed by the numbers of plants without the three studied compounds among the families of self-pollinated lines, which were very high (only few specimens were able to accumulate them), namely, 97.4–99.7% in I₁, 96.2–100.0% in I₂ and 98.0–100.0% in I₃, i.e. the range (limits) of segregation by these traits was narrow (see Table 1).

In the offspring from original cannabinoid-free plants of variety Mykolaichyk (I₀), although plants expressing the studied traits (CBD 0.17 points and THC 0.04) were observed, in I₁ their contents reduced to 0.01 points. Despite the fact that plants with THC appeared (0.01 points), as early as in I₂ the “no cannabinoids” and “nonpsychotropy” traits became homozygous, which persisted to I₆ inclusively (see Table 1). Obviously, the pattern of cannabinoid inheritance depends on the variety genotype, valence of genes determining the biosynthesis of cannabinoids, quantity of mutant genes in the recessive state, which in the process of self-pollination become homozygous and expressed in phenotypes, breeding purity and type of material, which depend on

the methodological conditions of cannabinoid tests and compliance with the rules of selection for these traits before anthesis in breeding nurseries.

Variety Hlukhivski 46 also proved to be highly stable: only single plants with minor amounts of CBD and CBN segregated. 99.0% of the specimens in the test sample contained none the three compounds at all. From I₂ to I₆, no cannabinoids were detected (see Table 1).

Self-pollinated lines from original plants with cannabinoids (within the limits allowed by the current legislation) also contained these compounds. It is noteworthy that phenotypes such as CBD/+THC-/CBD - ("+" – presence, "-" – absence), CBD/+ THC-/CBD+, CBD+/THC+/CBD- with varying contents of CBD, THC and CBN (in case of their presence) have plants with cannabinoids in their offspring, in addition, there is a significant variability in their contents. The absence of a certain compound in the initial form can give a sharp increase in its content in the offspring from self-pollination (Tables 2–4).

Table 2

Expression of the cannabinoid contents trait in I₁–I₂ self-pollinated lines derived from Hlukhivski 58, provided that the initial forms contained these cannabinoids (average, 2009–2011)

Cannabinoid contents in I ₀ , score	Cannabinoid contents in I ₁ –I ₂ the self-pollinated lines, score					
	CBD		THC		CBN	
	$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families	$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families	$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families
I ₁ Hlukhivski 58						
CBD 0,25–1	0,16±0,097	0,02–0,41	0,10±0,080	0–0,56	0,15±0,131	0–0,40
THC 0						
CBN 0						
CBD 0,5–1	1,18±0,306	0,92–1,44	0,72±0,280	0,51–0,92	1,76±0,669	1,22–2,32
THC 0						
CBN 0,5						
CBD 0,5	2,15±0,456	2,15	2,24±0,680	2,24	4,98±0,830	4,98
THC 0,5						
CBN 0						
CBD 0,5–2	1,32±0,397	0,52–2,00	0,99±0,383	0,30–2,32	2,45±0,895	1,30–3,69
THC 0,25–1						
CBN 0,25–2						
CBD 5–6	4,00±0,243	2,36–5,51	2,82±0,300	2,10–5,60	7,36±0,542	5,74–9,50
THC 4–5						
CBN 6						
CBD 4	5,95±0,050	5,95	9,85±0,150	9,85	10,00	10,00
THC 4						
CBN 10						
I ₂ Hlukhivski 58						
CBD 0,5–1	1,88±0,510	1,32–2,44	2,44±0,840	1,61–3,28	2,21±0,824	1,34–3,08
THC 0,25						
CBN 0,25						
CBD 3	3,20±0,408	3,20	4,45±0,773	4,45	4,90±0,675	4,90
THC 3						
CBN 5						

Table 3

Expression of the cannabinoid contents trait in I₁ self-pollinated lines derived from Hlesia, provided that the initial forms contained these cannabinoids (average, 2009–2011)

Cannabinoid contents in I ₀ , score		Cannabinoid contents in self-pollinated lines, score					
		CBD		THC		CBN	
		$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families	$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families	$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families
I ₁ Hlesia							
CBD	0,25	0,04±0,016	0–0,09	0	0	0	0
THC	0						
CBN	0						
CBD	0,5	1,14±0,426	0,18–2,12	1,38±0,653	0–2,75	3,50±1,450	3,40–3,60
THC	0						
CBN	0,5–3						
CBD	0,5	0,71±0,240	0,12–1,30	0,99±0,532	0,10–1,88	0,73±0,411	0,11–1,35
THC	0,5						
CBN	0,5						
CBD	1	2,53±0,385	1,85–3,70	4,61±0,721	2,91–8,20	4,62±0,798	3,35–7,10
THC	0,5–4						
CBN	3–5						
CBD	6	3,18±0,506	1,90–4,45	5,94±0,719	3,80–8,80	6,92±0,772	5,15–9,45
THC	8–10						
CBN	10						

Table 4

Expression of the cannabinoid contents trait in I₁–I₂ self-pollinated lines derived from Zolotoniski 15, provided that the initial forms contained these cannabinoids (average, 2009–2011)

Cannabinoid contents in I ₀ , score		Cannabinoid contents in self-pollinated lines, score					
		CBD		THC		CBN	
		$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families	$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families	$\bar{x} \pm s_{\bar{x}}$	Min–Max within the families
1	2	3	4	5	6	7	8
I ₁ Zolotoniski 15							
CBD	0,25–	0,32±0,104	0,05–0,60	0,10±0,055	0–0,19	0,11±0,078	0–0,22
THC	0,5						
CBN	0						
CBD	1	0,98±0,354	0,98	0,68±0,391	0,68	1,08±0,585	1,08
THC	0,5						
CBN	0						
CBD	0,5–2	1,37±0,726	0,98–1,58	1,30±0,694	0,78–1,65	2,59±1,027	2,00–3,28
THC	0,25–1						
CBN	1						
CBD	3	3,25±0,898	3,25	4,52±1,536	4,52	8,90±0,823	8,90
THC	8						
CBN	10						

1	2	3	4	5	6	7	8
I ₂ Zolotoniski 15							
CBD	0,5–1	0,76±0,341	0,72–1,64	0,66±0,414	0,25–1,08	1,62±0,778	0,55–2,68
THC	0,25						
CBN	0,25						
CBD	2	0,84±0,334	0,84	6,15±0,930	6,15	3,06±0,864	3,06
THC	5						
CBN	2						

Such expression indicates significant relationships between the traits of presence and contents of different cannabinoid compounds, location of genes determining their biosynthesis and biochemical transformations in the plant in close linkage groups, and synthesis of different substances from a common precursor. Genetic determination of the traits of presence and contents of different cannabinoid compounds is quite complex, they appear to be inherited polygenically by nuclear-cytoplasmic mode. For example, in I₁ derived from original Hlukhivski 58 plants with indicators of CBD 0.25–1 points, THC 0 and CBN 0, on average CBD 0.16 points, THC 0.10 and CBN 0.15 were obtained, i.e. the compounds that were absent in I₀, were expressed. In contrast to these data, the I₁ offspring from of a self-pollinated Hlesia plant with a similar phenotype only contained CBD at the level of 0.04 points. From plants with the CBD+/THC-/CBD+ and CBD+/THC+/CBD- phenotypes, lines having all the three test substances were obtained (I₁ Hlukhivski 58, I₁–I₂ Hlesia, I₁ Zolotoniski 15) as a result of self-pollination. It has been established that in the early generations from self-pollination the offspring may have values of the cannabinoid contents that are similar to those in the parents, or significantly lower values, or significantly higher values. For example, in I₁ self-pollinated lines derived from Hlesia plants with indicators of CBD 6 points, THC 8–10 and CBN 10, on average CBD 3.18 points, THC 5.94 and CBN 6.92, and in I₁ Glukhivsky 58, obtained from the original form with indicators of 4 CBD, 4 THC and 10 points CBN, on average, these compounds were found to be 5.95, 9.85 and 10 points, respectively.

The first and second generations from self-pollination with various cannabinoid contents are important for breeding, because as a result of closely related reproduction a significant segregation of traits occurs, allowing one to distinguish lines with less close relationships between cannabinoid compounds to create starting material with high contents of nonpsychotropic cannabinoids with a concomitant decrease in THC. This is supported by a considerable range of the trait variation (the difference between the maximum and minimum) within the families of self-pollinated lines.

There are strong positive correlations between the contents of cannabinoid compounds [30], simplifying breeding for reduced contents or absolute absence of all cannabinoid compounds, but being a negative phenomenon when it is desirable to increase the amount of one or more non-psychoactive substances while controlling a low THC content. At the same time, different chemotypes of hemp (at least III and V) can have different degrees of association [31], so it is logical to ask whether there are differences in correlations among varieties and self-pollinated lines, assuming that inbreeding can result in breeding material with relatively weak relationships between cannabinoid compounds.

Thus, in I₁–I₃ self-pollinated hemp lines from Hlukhivski 58 and I₁–I₃ lines from Zolotoniski 15 there was a strong positive correlation between the CBD and THC contents (correlation coefficients ranged 0.72 to 0.79 and 0.71 to 0.90, respectively), a strong or medium positive correlation between the CBD and CBN contents (0.68–0.80 and 0.67–0.82, respectively) and a strong positive correlation between the THC and CBN contents (0.71–0.83 and 0.80–0.85, respectively). At the same time, as compared to the original genotypes (I₀), this relationship was somewhat weaker; the coefficients tended to decrease to I₃, and, importantly for medicinal cannabis breeding, the range of variation of the coefficient correlations sharply broadens, especially in I₁, as a result of self-pollination (Figs. 3, 4). Thus, there are prerequisites for the selection of breeding material, which is characterized by interrupted cannabinoid biosynthesis, although self-

pollinated lines with weak (at least by individual correlation coefficients between different cannabinoids) or medium associations are quite rare. Closely related reproduction provides prospects for the creation of starting material with high contents of non-psychoactive compounds and without THC.

The cannabis self-pollination efficiency as a breeding method of stabilization (increasing the homozygosity level) of starting material in terms of the “no cannabinoid compounds” trait [32] with simultaneous targeted selection was confirmed by several-year inbreeding of unrelated varieties Hlukhivski 58, Hlesia, Mykolaichyk, Hlukhivski 46 (Central European eco-geographical type), and Zolotoniski 15 (Southern European eco-geographical type). Self-pollination only of plants without CBD, THC or CBN can reduce the contents of these cannabinoids to zero in the entire offspring. The inheritance patterns of the “presence and contents of cannabinoid compounds” depend on the genotype of a variety and specific source plant taken for self-pollination. After crossing self-pollinated lines, no heterosis effect for the cannabinoid contents in hybrids is observed [33].

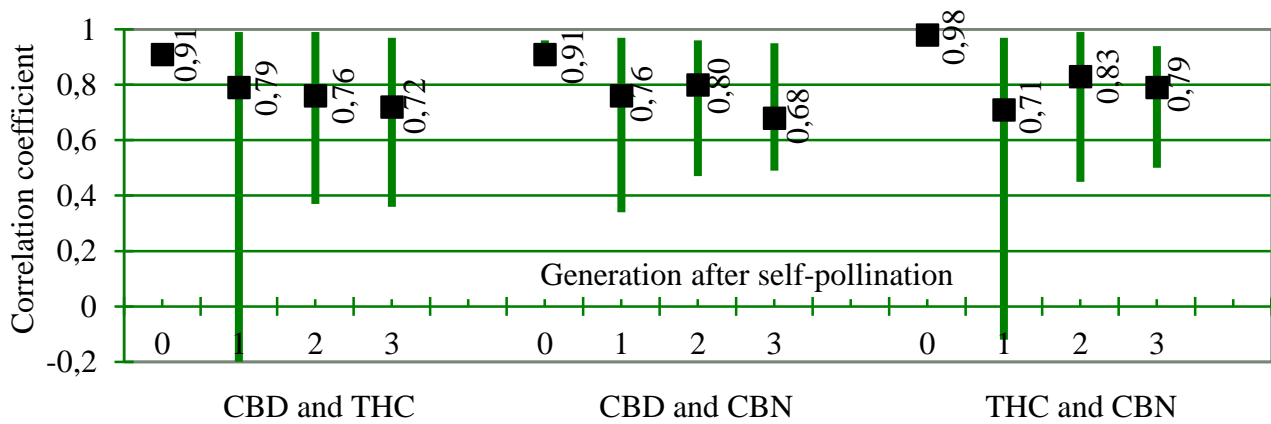


Fig. 3. Average and limit values of pairwise correlation coefficients between the cannabinoid contents in the original genotype and self-pollinated lines from variety Hlukhivski 58 (2009–2012, values are significant at the significance level of 0.05)

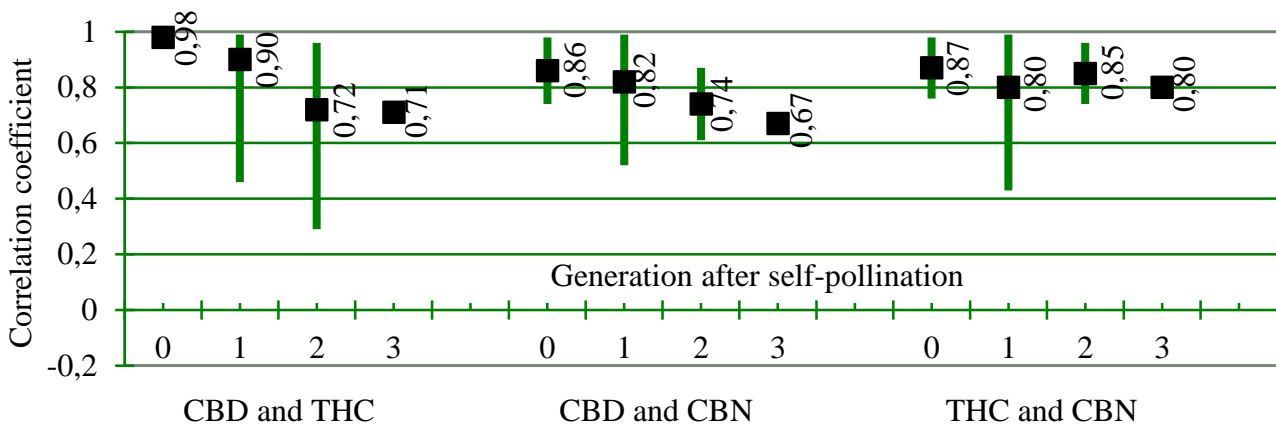


Fig. 4. Average and limit values of pairwise correlation coefficients between the cannabinoid contents in the original genotype and self-pollinated lines from variety Zolotoniski 15 (2009–2012, values are significant at the significance level of 0.05)

Conclusions. Self-pollination is an effective way to determine the cannabis variety population stability in terms of presence and contents of cannabinoid compounds and a breeding method to create starting material with stable absence or presence of these compounds. Directional selection of source plants without CBD, THC or CBN in the process of self-pollination leads to a reduction in their contents to absolute absence. The stabilization (homozygation) of the lines occurred in

I₂ – I₆ and depended on the genotype of a variety. Self-pollinated lines of these generations should be used as parents in crossing. In addition, a characteristic feature of the studied modern hemp varieties is the ability to segregate families without CBD, THC or CBN at all as early as in I₁. There are strong positive correlations between the contents of cannabinoid compounds, simplifying the selection for reduced the contents of all cannabinoids and considerably complicating the breeding for an increased CBD content with a concomitant reduction in the THC content. The correlations between the contents of cannabinoid compounds in self-pollinated lines are slightly weaker than the original genotypes, which allows one to use closely related reproduction in the breeding for THC elimination and increased contents of non-psychoactive cannabinoids.

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ФЕНОТИПОВИЙ ПРОЯВ ОЗНАК НАЯВНОСТІ ТА ВМІСТУ КАНАБІНОЇДІВ У ПРОЦЕСІ САМОЗАПИЛЕННЯ ОДНОДОМНОЇ КОНОПЛІ ТА СПРЯМОВАНОГО ДОБОРУ

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Мета та задачі дослідження – установити особливості успадкування ознак наявності та вмісту канабіноїдних сполук у процесі самозапилення однодомної коноплі; провести компаративний аналіз значень коефіцієнтів парної кореляції між ознаками вмісту основних канабіноїдних сполук у вихідних компонентах та самозапиленних лініях; дослідити ефективність застосування самозапилення в селекції коноплі.

Матеріали і методи. Дослідження проведено на базі Інституту луб'яних культур НААН (м. Глухів, Сумська обл., Україна) протягом 2009–2019 рр. Об'єктом дослідження були самозапилені лінії сортів промислової коноплі Глухівські 58, Глесія, Миколайчик, Глухівські 46 середньоєвропейського еколого-географічного типу та Золотоніські 15 південного еколого-географічного типу. Самозапилення рослин (за відсутності та наявності канабіноїдів) проводили під індивідуальними ізоляторами з агроволокна в умовах вегетаційного будиночка. Потомство вирощували в розсаднику оцінки, аналіз канабіноїдних сполук проводили методом тонкошарової хроматографії. В рослинних зразках усіх досліджених сортів та самозапиленних ліній вміст тетрагідроканабінолу (ТГК) не перевищував 0,08 % – норми, дозволеної діючим законодавством України. Статистичну обробку даних здійснювали за показниками середнього арифметичного, похибки вибіркової середньої, коефіцієнтів парної кореляції та криволінійної регресії.

Обговорення результатів. За умови спрямованого добору вихідних рослин з відсутністю канабідіолу (КБД), ТГК та канабінолу (КБН) в процесі самозапилення їх вміст знижувався до повної відсутності. Стабільність (гомозиготація) ліній наступала в I₂–I₆ і залежала від генотипу конкретного сорту. Самозапилені лінії саме цих поколінь доцільно залучати як батьківські компоненти в схрещування. Характерною особливістю досліджених сучасних сортів коноплі є властивість давати вже в I₁ сім'ї з відсутністю КБД, ТГК і КБН.

Між ознаками вмісту канабіноїдних сполук установлено сильну позитивну кореляцію, зокрема в I₁–I₃ Глухівські 58 та в I₁–I₃ Золотоніські 15 установлено сильну позитивну кореляцію між ознаками вмісту КБД і ТГК (r від 0,72 до 0,79 та від 0,71 до 0,90), сильну або середню позитивну кореляцію між ознаками вмісту КБД і КБН (0,68–0,80 і 0,67–0,82) та сильну позитивну кореляцію між ТГК і КБН (0,71–0,83 та 0,80–0,85 відповідно). Це спрощує добір на зниження вмісту всіх компонентів канабіноїдів та значно ускладнює селекцію на підвищення КБД і одночасного зниження вмісту ТГК. Кореляція між вмістом канабіноїдних сполук у самозапиленних ліній є більш слабкою в порівнянні з вихідними компонентами, а коефіцієнти мають значний розмах варіації, що дозволяє використовувати близькоспоріднене розмноження в селекції на зниження вмісту ТГК та підвищення вмісту непсихотропних канабіноїдів.

Висновки. Самозапилення є ефективним методом визначення стабільності популяції сорту коноплі за ознаками наявності та вмісту канабіноїдних сполук і селекційним методом створення вихідного матеріалу із стабільною ознакою їх відсутності або наявності.

Ключові слова: конопля, селекція, інбридинг, самозапилена лінія, успадкування, канабіноїд, кореляція

ФЕНОТИПИЧЕСКОЕ ПРОЯВЛЕНИЕ ПРИЗНАКОВ НАЛИЧИЯ И СОДЕРЖАНИЯ КАННАБИНОИДОВ В ПРОЦЕССЕ САМООПЫЛЕНИЯ ОДНОДОМНОЙ КОНОПЛИ И НАПРАВЛЕННОГО ОТБОРА

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Цель и задачи исследования – установить особенности наследования признаков наличия и содержания каннабиноидных соединений в процессе самоопыления однодомной конопли; провести компаративный анализ значений коэффициентов парной корреляции между признаками содержания основных каннабиноидных соединений в исходных формах и самоопыленных линиях; исследовать эффективность использования самоопыления в селекции конопли.

Материал и методы. Исследования проведены на базе Института лубяных культур НААН (г. Глухов, Сумская обл., Украина) в течение 2009–2019 гг. Объект исследований – самоопыленные линии сортов промышленной конопли Глуховские 58, Глесия, Миколайчик, Глуховские 46 средневропейского эколого-географического типа и Золотоношские 15 южного эколого-географического типа. Самоопыление растений (с отсутствием и наличием каннабиноидов) осуществляли под индивидуальными изоляторами с агроволокна в условиях вегетационного домика. Потомство выращивали в питомнике оценки, анализ каннабиноидных соединений проводили методом тонкослойной хроматографии. В растительных образцах всех исследуемых сортов и самоопыленных линий содержание тетрагидроканнабинола (ТГК) не превышало 0,08 % – нормы, разрешенной действующим законодательством Украины. Статистическую обработку данных осуществляли по показателям среднего арифметического, погрешности выборочной средней, коэффициентов парной корреляции и криволинейной регрессии.

Обсуждение результатов. При условии целенаправленного отбора исходных растений с отсутствием каннабидиола (КБД), ТГК и каннабинола (КБН) в процессе самоопыления их содержание уменьшалось до полного отсутствия. Стабильность (гомозиготация) линий наступала в I_2 – I_6 и зависела от генотипа конкретного сорта. Самоопыленные линии именно этих поколений целесообразно привлекать как родительские компоненты в скрещиваниях. Характерной особенностью исследуемых современных сортов конопли является способность давать уже в I_1 семьи с отсутствием КБД, ТГК и КБН.

Между признаками содержания каннабиноидных соединений установлены сильные положительные корреляционные связи, в частности в I_1 – I_3 Глуховские 58 и в I_1 – I_3 Золотоношские 15 обнаружена сильная положительная корреляционная связь между признаками содержания КБД и ТГК (r от 0,72 до 0,79 и от 0,71 до 0,90), сильная или средняя положительная корреляционная связь между признаками содержания КБД и КБН (0,68–0,80 и 0,67–0,82) и сильная положительная связь между ТГК и КБН (0,71–0,83 и 0,80–0,85 соответственно), что упрощает отбор на снижение содержания всех компонентов каннабиноидов и значительно усложняет селекцию в направлении повышения КБД и одновременного снижения содержания ТГК. Корреляционные связи между содержанием каннабиноидных соединений в самоопыленных линиях слабее сравнительно с исходными формами, а коэффициенты имеют значительный размах вариации, что позволяет использовать близкородственное размножение в селекции на снижение содержания ТГК и повышение содержания непсихотропных каннабиноидов.

Выводы. Самоопыление является эффективным приемом определения стабильности популяции сорта конопли по признакам наличия и содержания каннабиноидных соединений и селекционным методом создания исходного материала со стабильным признаком их отсутствия или наличия.

Ключевые слова: конопля, селекция, инбридинг, самоопыленная линия, наследование, каннабиноид, корреляция

PHENOTYPIC MANIFESTATION OF THE TRAITS OF PRESENCE AND CONTENT OF CANNABINOIDS IN THE PROCESS OF SELF-POLLINATION IN MONOECIOUS HEMP AND SELECTION

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Purpose and objectives. To reveal peculiarities of the inheritance of the traits of cannabinoid compound presence and content upon self-pollination in monoecious hemp, to compare the pair correlation coefficients between the contents of major cannabinoid compounds in initial genotypes and self-pollinated lines, and to evaluate the effectiveness of self-pollination in hemp breeding.

Material and methods. The study was carried out at the Institute of Bast Crops of the National Academy of Agricultural Sciences (Hlukhiv, Sumy Oblast, Ukraine) in 2009–2019. Inbred lines of industrial hemp varieties Hlukhivski 58, Hlesia, Mykolaichyk, and Hlukhivski 46 belonging to the Central European eco-geographical type and variety Zolotoniski 15 belonging to the Southern eco-geographical type were taken as test objects. Self-pollination of plants (with and without cannabinoids) was carried out under individual agrotexile bags in a greenhouse. The offspring were grown in a nursery. Evaluation and analysis of cannabinoid compounds were conducted by thin-layer chromatography. In all the genotypes under study, the tetrahydrocannabinol (THC) content did not exceed 0.08% that is standard allowed by the current legislation of Ukraine. Data were statistically processed with calculating arithmetic mean, sampling mean error, pairwise correlation coefficients, and curvilinear regression.

Results and discussion. Provided directional selection of initial plants that do not contain cannabidiol (CBD), THC or cannabinol (CBN), self-pollination reduced their contents to complete absence. Stabilization (homozygation) of the lines occurred in I₂–I₆ and was specific to a particular variety. Inbred lines of these generations are recommended to involve as parents in crossing. The ability to segregate CBD-, THC-, and CBN-free families as early as in I₁ is a characteristic feature of the hemp varieties under investigation. There were strong positive correlations between the cannabinoid contents, in particular there was a strong positive correlation was found between the CBD and THC contents ($r = 0.72$ – 0.79 and 0.71 – 0.90 , respectively), a strong or medium positive correlation between the CBD and CBN contents (0.68 – 0.80 and 0.67 – 0.82 , respectively), a strong positive correlation between the THC and CBN contents (0.71 – 0.83 and 0.80 – 0.85 , respectively) in I₁–I₃ Hlukhivski 58 and I₁–I₃ Zolotoniski 15. This makes selection for reduced contents of all cannabinoid compounds and easier, but at the same time significantly complicates breeding for increased CBD content with concurrent reduced THC content or increased contents of non-psychoactive cannabinoids. The correlations between the contents of cannabinoid compounds in inbred genotypes are weaker than those in the original breeding genotypes, with the coefficients ranging significantly, which allows using closely related reproduction in breeding aimed at reducing THC content or increasing non-psychoactive cannabinoid contents.

Conclusions. Self-pollination is an effective method of determination of the hemp population stability in terms of the cannabinoid compound presence and contents and at the same time is a method of creating breeding genotypes with stable traits of cannabinoid absence or presence.

Key words: *hemp, breeding, inbreeding, self-pollinated lines, inheritance, cannabinoids, correlation*