

VARIATION IN CBDA AND CBD CONTENT IN SOME INDUSTRIAL HEMP GENOTYPES

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Abstract

The content of CBDA and CBD was determined in three hemp genotypes, sown in two different seasons. Six experimental variants resulted, V1 (Silvana, S1), V2 (Silvana, S2), V3 (Loja, S1), V4 (Loja, S2), V5 (Finola, S1), and V6 (Finola, S2). The CBDA content varied between $CBDA = 0.43 \pm 0.04\%$ (V4) and $CBDA = 0.70 \pm 0.04\%$ (V5). The CBD content varied between $CBD = 0.52 \pm 0.06\%$ (V1) and $CBD = 0.88 \pm 0.06\%$ (V2). The CBDA/CBD ratio varied between $CBDA/CBD = 0.5774 \pm 0.0688$ (V2) and $CBDA/CBD = 1.0577 \pm 0.0688$ (V2). The CBD/CBDA ratio varied between $CBD/CBDA = 0.0455 \pm 0.1115$ (V1) and $CBD/CBDA = 1.7320 \pm 0.1115$ (V2). Compared to the mean value of the experiment, in the case of the CBDA, variant V5 presented positive differences, and variant V4 negative differences ($p < 0.05$). In the case of the CBD, compared to the mean of the experiment, variant V1 presented negative differences ($p < 0.05$). Based on PCA, PC1 has explained 64.27% of variance, and PC2 has explained 35.134% of variance. Cluster grouping and a ranking of the experimental variants were generated, in relation to the considered indices.

Key words: clustering, cannabidiol (CBD), cannabidiolic acid (CBDA), industrial hemp, principal component analysis (PCA), ranking.

INTRODUCTION

Industrial hemp is a promising plant with high ecological and economic efficiency (Nath, 2022). Recent studies have highlighted the development potential of an industry based on industrial hemp, due to the diversity of resources provided by this plant (Burton et al., 2022). Industrial hemp has ecological importance (carbon sequestration), agronomic importance (crop structure, beneficial effects on the soil), and economic importance (fiber production, seed production), with beneficial effects for farmers and various economic sectors (Burton et al., 2022; Puiu et al., 2023; Visković et al., 2023; Popa et al., 2024).

Products and by-products derived from industrial hemp are used in the food industry, particularly for functional foods with health benefits (Rupasinghe et al., 2020).

Hemp belongs to the category of "gluten-free" plants, producing highly nutritious seeds (similar to soy) that are used in various functional foods. Moreover, it is a fully

utilizable crop across multiple economic sectors (Yano & Fu, 2023).

From the perspective of climate change, which is increasingly affecting agricultural crops, industrial hemp is a plant of interest for fiber and food production due to its high drought resistance (Ahmed et al., 2022; Gill et al., 2023; Kaur & Kander, 2023).

Over the past decade, research on industrial hemp cultivation has focused on aspects such as yield in relation to climatic stress, seed quality (high-quality seeds), and optimal fertilizer doses (Dudziec et al., 2024). At the same time, studies have analyzed bioactive compounds regarding their selective activity or differences between male and female plants.

The active principles in hemp have been studied for their potential use in medicine and pharmaceuticals (Rupasinghe et al., 2020; Hossain & Chae, 2024; Simeu et al., 2024).

The profile and content of active compounds (e.g., CBDA, CBD) in hemp plants are determined by genotype, as well as the interaction of genotype \times environment \times

cultivation technology (De Meijer et al., 2003; Adesina et al., 2020).

In industrial hemp, phytocannabinoids (e.g., CBD, THC) are present in all genotypes but in varying amounts - with some genotypes exhibiting very low THC levels and others showing higher concentrations. Studies have reported that northern-region industrial hemp tends to have lower THC levels and higher CBD content (Leizer et al., 2000; Russo and Taming, 2011).

The common catalog includes many hemp genotypes, but recent studies indicate that the cannabinoid content is not yet well known for all varieties (Glivar et al., 2020). HPLC analysis has been used by researchers to investigate the profile and content of 13 cannabinoids in a collection of 15 hemp varieties. Under uniform cultivation conditions, the tested genotypes showed significant variations in cannabinoid content, according to the authors.

The CBD profile and essential oil content were also investigated in wild hemp genotypes (nine accessions) and 13 registered genotypes, breeding lines, and CBD-rich hemp strains (Zheljazkov et al., 2020). The authors reported variations in CBD profile and oil content depending on the genotypes studied.

Due to the growing importance and interest in CBD, studies have been conducted to identify high-CBD genotypes, as well as research on cultivation technologies that ensure high CBD yield (Chiluwal et al., 2023). Depending on the region and eco-climatic conditions, the sowing date has been evaluated for its influence on CBD production in different hemp varieties (Chiluwal et al., 2023).

For fiber hemp cultivation, high plant density is favorable for fiber production, whereas for seed and CBD production, a wider spacing between plants (between rows) is recommended, as it promotes branching and flower production (Adesina et al., 2020).

Regarding sowing date, a higher CBD content has been associated with earlier sowing (Chiluwal et al., 2023). The authors recorded CBD content variations between genotype groups, depending on their origin (Colorado, Kentucky).

Cannabidiol (CBD), derived from industrial hemp, is a promising bioactive compound for the food industry (functional foods) and the pharmaceutical and medicinal industry (pharmaceutical products and medications) (Deguchi et al., 2020; Charles et al., 2024).

This study evaluates the content of cannabidiolic acid (CBDA) and cannabidiol (CBD) in three industrial hemp genotypes, sown at two different sowing dates under uniform cultivation conditions, in the plains of Western Romania.

MATERIALS AND METHODS

The research was conducted at ARDS Lovrin during the 2023–2024 agricultural year. The field experiments were carried out on Plot 12, under chernozem soil slightly gleyed, medium clay loam with moderate fertility. The climatic conditions for the 2023-2024 agricultural year were recorded at the Meteo Station within ARDS Lovrin (Figure 1).

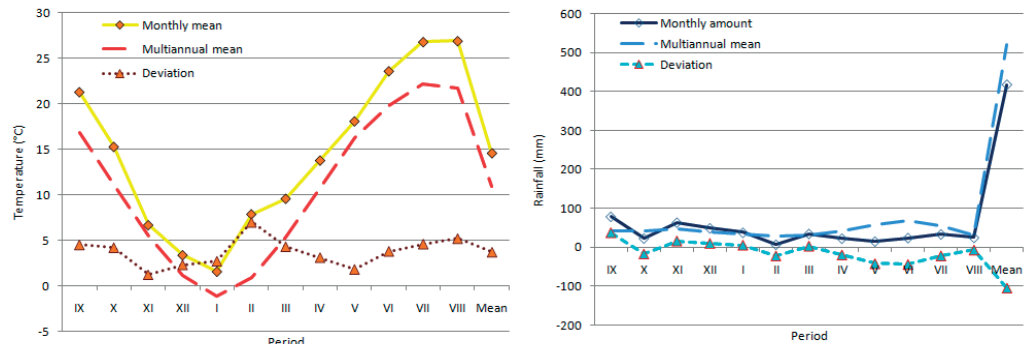


Figure 1. Climatic conditions, 2023-2024 agricultural year, ARDS Lovrin

Three genotypes were cultivated (Silvana, a variety developed at ARDS Lovrin, and Loja and Finola, foreign varieties) in two sowing periods (April 29, 2024, and May 15, 2024) and at four different row spacings (12.5 cm, 70 cm, 100 cm, and 150 cm).

Due to the lack of precipitation in the spring months, plant emergence was uneven. Emergence was recorded starting on May 17, 2024, for the first sowing period and on June 3, 2024, for the second sowing period.

The experimental variants analyzed for CBDA and CBD content were at 1-meter row spacing: conditions, characterized by medium fertility.

Three hemp genotypes were cultivated at two different sowing times (sowing time 1 and sowing time 2), with a row spacing of 1 meter: Silvana, sowing time 1 (V1), Silvana, sowing time 2 (V2), Loja, sowing time 1 (V3), Loja, sowing time 2 (V4), Finola, sowing time 1 (V5), Finola, sowing time 2 (V6).

To determine the CBDA and CBD content, plant samples were collected on July 19, 2024, from inflorescences. The harvested samples were placed in paper bags and sent for analysis. The plant samples were analyzed at Chromatec Plus Laboratory, a nationally certified SRAC and internationally certified IQNet facility. The CBDA and CBD content was determined using accredited laboratory methods, employing an HPLC system. Multiple replicates were prepared and analyzed for each sample, and the results were reported as mean values.

Based on the obtained CBDA and CBD indices, the CBDA/CBD and CBD/CBDA ratios were calculated.

The experimental results were analyzed to assess differences among experimental variants. Statistical analyses were performed using ANOVA, regression analysis, and multivariate analysis, conducted in Excel and PAST software (Hammer et al., 2001; Wolfram Alpha, 2020).

RESULTS AND DISCUSSIONS

The industrial hemp plant samples (inflorescences) were analyzed, and the CBDA and CBD content values were obtained (Table 1). Based on these values, the CBDA/CBD and CBD/CBDA ratios were calculated (Table 1). CBDA content ranged between $0.43\pm0.04\%$

(V4) and $0.70\pm0.04\%$ (V5), CBD content varied between $0.52\pm0.06\%$ (V1) and $0.88\pm0.06\%$ (V2), The CBDA/CBD ratio recorded values between 0.5774 ± 0.0688 (V2) and 1.0577 ± 0.0688 (V1), The CBD/CBDA ratio ranged between 0.9455 ± 0.1115 (V1) and 1.7320 ± 0.1115 (V2). The reliability of the experimental values was confirmed by the results of the ANOVA test (Table 2).

Table 1. CBD, CBDA values, and calculated ratios in industrial hemp

Experimental variant	CBDA	CBD	CBDA/CBD	CBD/CBDA
	(%)	(%)	Ratio	Ratio
V1	0.55	0.52	1.0577	0.9455
V2	0.51	0.88	0.5774	1.7320
V3	0.65	0.84	0.7698	1.2990
V4	0.43	0.59	0.7247	1.3798
V5	0.70	0.80	0.8792	1.1374
V6	0.52	0.77	0.6753	1.4808
SE	±0.04	±0.06	±0.0688	±0.1115

Table 2. ANOVA Test

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.9906	3	0.6635	19.843	3.29E-06	3.098
Within Groups	0.6688	20	0.0334			
Total	2.6594	23				

For each parameter, the position of the variants was analyzed in comparison with the mean value.

In the case of CBDA, the results are presented in Table 3. Compared to the mean (CBDA = 0.56%), variant V4 presented negative differences, and variant V5 positive differences ($p<0.05$).

Table 3. Significance of differences in CBDA for industrial hemp variants

Variants	Given mean	Difference and Significance	95% conf. interval	t	p (same mean)
V1	0.55	-0.01 ^{ns}	(-0.093677 0.11368)	0.2479	0.814
V2	0.51	-0.05 ^{ns}	(-0.053677 0.15368)	1.2397	0.270
V3	0.65	0.09 ^{ns}	(-0.013677 0.19368)	-2.2315	0.076
V4	0.43	-0.13 ^o	(0.026323 0.23368)	3.2233	0.023
V5	0.70	0.14 [*]	(0.036323 0.24368)	-3.4712	0.018
V6	0.52	-0.04 ^{ns}	(-0.063677 0.14368)	0.9918	0.367

The results for CBD content are shown in Table 4. The mean CBD value across all variants was $\overline{\text{CBD}} = 0.73\%$. V1 showed negative deviations compared to the mean ($p < 0.05$).

Table 4. Significance of differences in CBDA for industrial hemp variants

Variant	Given mean	Difference and Significance	95% conf. interval	t	p (same mean)
V1	0.52	0.21°	(0.061449 0.36522)	3.6106	0.015
V2	0.88	0.15 ^{ns}	(-0.0052178 0.29855)	-2.4823	0.056
V3	0.84	0.11 ^{ns}	(-0.045218 0.25855)	-1.8053	0.131
V4	0.59	0.14 ^{ns}	(-0.0085511 0.29522)	2.4259	0.060
V5	0.80	0.07 ^{ns}	(-0.085218 0.21855)	-1.1283	0.310
V6	0.77	0.04 ^{ns}	(-0.11522 0.18855)	-0.6206	0.562

In the case of the CBDA/CBD ratio, V1 showed a positive difference (0.2770), $p = 0.0101$, while V2 showed a negative difference (-0.2033), $p = 0.0318$ (Figure 2). In the case of the CBD/CBDA ratio, V1 showed a negative difference (-0.3836), $p = 0.0184$, while V2 showed a positive difference (0.4029), $p = 0.0153$.

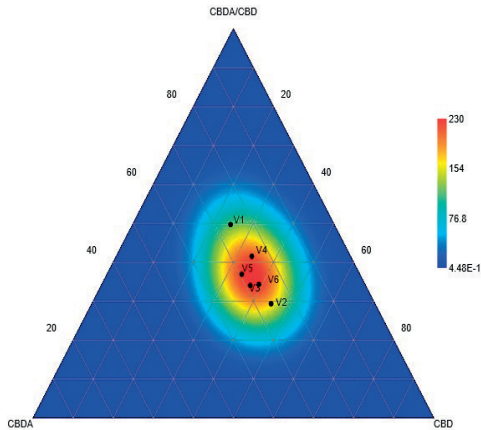


Figure 2. CBDA/CBD ratio in relation to primary parameters

The variation of the CBDA/CBD ratio, depending on the basic indices (CBDA, CBD), was described by the equation (1), $R^2 = 0.998$, $p < 0.001$, with the graphical distribution shown in Figure 3, and Figure 4. A divergent

contribution of CBDA and CBD to the calculated ratio values was observed.

Based on the coefficients of equation (1) as well as the 3D distribution (Figure 3), it was found that the CBDA parameter had a higher amplitude in forming the ratio (CBDA/CBD) compared to CBD.

Additionally, a "scissor-like" interaction of the CBDA and CBD indices in forming the calculated ratio was observed.

$$\text{CBDA/CBD} = ax^2 + by^2 + cx + dy + xy + f \quad (1)$$

where: CBDA/CBD – the calculated ratio;
 x – CBDA content; y – CBD content;
 a, b, c, d, e, f – coefficients in equation (1);
 $a = -0.45245034$; $b = 1.49325136$;
 $c = 3.41182257$; $d = -2.18829850$;
 $e = -2.00205901$; $f = 0.62478504$

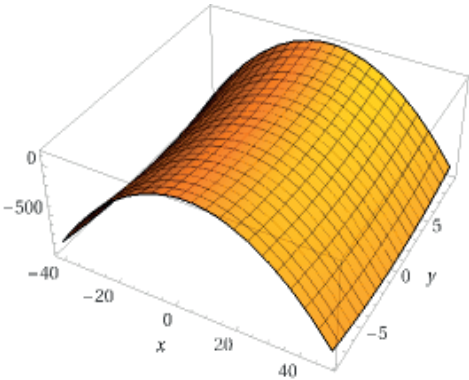


Figure 3. 3D Model for the distribution of the CBDA/CBD ratio, industrial hemp

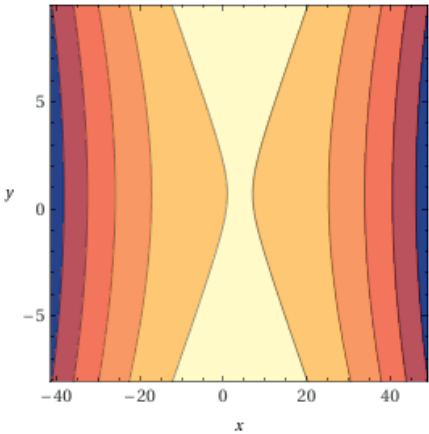


Figure 4. Isoquant model for the distribution of the CBDA/CBD ratio, industrial hemp

The multivariate analysis explained the PCA loadings, in relation to the determined parameters and principal components, as shown in Table 5.

The PCA diagram is presented in Figure 5. Variants V3 and V5 were positioned in association with CBDA and CBD, while variants V6 and V2 were positioned in association with the CBD/CBDA ratio. V1 and V4 displayed an independent position.

Table 5. PCA loadings based on indices for industrial hemp

	PC 1	PC 2	PC 3	PC 4
CBDA	-0.19722	0.79964	-0.22482	0.52072
CBD	0.44635	0.58765	0.30894	-0.59999
CBDA/CBD	-0.61747	0.06373	0.78398	0.00675
CBD/CBDA	0.61693	-0.10575	0.48928	0.60730

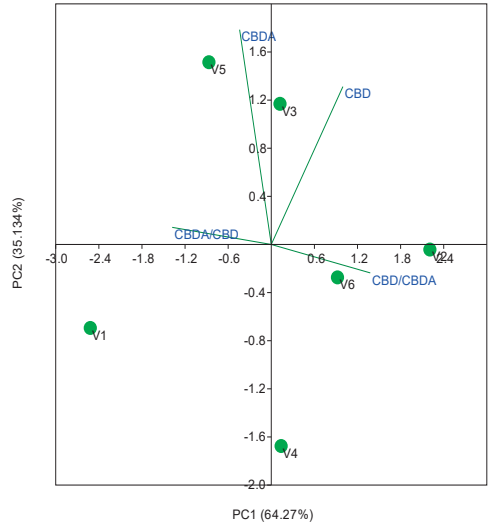


Figure 5. PCA diagram, industrial hemp parameters

The cluster analysis facilitated the grouping of variants based on similar values of the indices and calculated ratios (Coph. corr. = 0.723) (Figure 6).

Variant V1 was positioned independently. The other five variants were grouped into a subcluster, with different associations: V2 was positioned separately, while the other variants were grouped into two subclusters: (V3, V5) and (V4, V6).

The calculated similarity level is presented in Table 6.

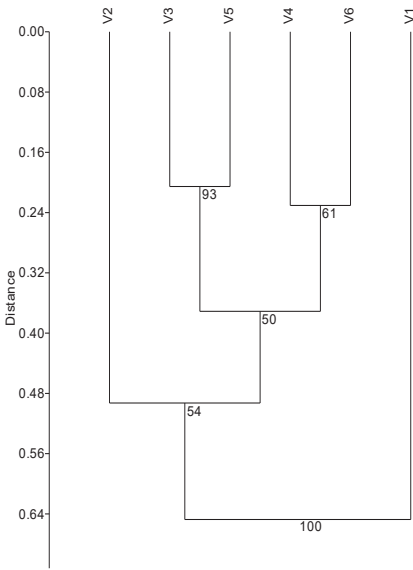


Figure 6. Cluster dendrogram of industrial hemp variants

Table 6. SDI values, industrial hemp variants

	V1	V2	V3	V4	V5	V6
V1		0.9902	0.5659	0.5646	0.4118	0.7044
V2	0.9902		0.4957	0.4861	0.6980	0.2914
V3	0.5659	0.4957		0.3456	0.2054	0.2526
V4	0.5646	0.4861	0.3456		0.4468	0.2305
V5	0.4118	0.6980	0.2054	0.4468		0.4391
V6	0.7044	0.2914	0.2526	0.2305	0.4391	

The influence of row spacing on CBDA and CBD content

The study considered a row spacing of 1 meter for the analyzed variants, but the specialized literature suggests that a greater row spacing can favor more intense branching and an increase in the number of inflorescences, which could explain the observed variations in CBDA and CBD content.

If we extrapolate this idea, the higher CBDA values in variant V5 (Finola, first sowing season) may be associated with a more vigorous plant structure, with more active inflorescences, allowing for a greater accumulation of cannabinoid acids.

This hypothesis could be confirmed by correlating plant biomass with CBDA/CBD content, an aspect that was not included in this analysis but is worth investigating.

Similarly, the higher CBD content in V2 (Silvana, second sowing season) suggests a possible effect of sowing time on CBD biosynthesis, which may be more efficient in the second season due to the influence of higher temperatures during the vegetation period.

The impact of climatic conditions on

CBDA \Rightarrow CBD conversion

Based on the presented data, we know that the plants were harvested on July 19, 2024, meaning they were exposed to high temperatures and potentially intense thermal stress during the final weeks of vegetation.

Variant V2 (Silvana, second sowing season), which had the highest CBD content, was sown later, meaning it had a shorter vegetation period but developed under higher temperature conditions.

Previous studies (Chiluwal et al., 2023) have shown that high temperatures can accelerate CBDA \rightarrow CBD conversion by increasing the activity of enzymes responsible for this transformation.

This phenomenon could explain why the CBDA/CBD ratio is lower in variant V2 and why CBD/CBDA is higher. In other words, the sowing time influences not only cannabinoid production but also the balance between CBDA and CBD.

Variation among genotypes and possible correlations with their genetic origin

Looking at the raw data, we observe that Finola (V5 and V6) has the highest CBDA content, while Silvana (V1 and V2) shows the greatest variations in CBDA/CBD ratios. This could indicate that:

- Finola is more stable in CBDA accumulation, regardless of sowing time. This may be explained by its genetic origin, as Finola is known as a northern variety adapted to colder conditions, which favors the accumulation of cannabinoid acids.
- Silvana, a native Romanian variety, is more sensitive to climatic factors, making sowing time a critical factor in optimizing its CBD production.
- Loja (V3 and V4), despite having moderate values, seems to be the most affected by changes in sowing time,

suggesting that this genotype may be less stable and more dependent on environmental factors.

These observations suggest that the analyzed genotypes responded differently to environmental factors, and future selection efforts could consider resistance to climate stress and the stability of cannabinoid biosynthesis.

Possible implications for optimizing CBD production

If the goal is to maximize CBD production, the results suggest that:

- Silvana should be sown later (second sowing season) to achieve a high CBD content, but it needs to be monitored to prevent losses due to the degradation of active compounds.
- Finola provides stable CBDA production, making it an ideal candidate for CBDA-based extracts, but it may require different processing methods to maximize CBD conversion.
- Loja exhibits high variability, suggesting that further studies are needed to determine its optimal cultivation conditions.

These findings provide new insights for optimizing CBD and CBDA production based on genotype selection, sowing time, and climatic conditions.

CONCLUSIONS

Industrial hemp genotypes sown at two different times and cultivated under uniform conditions generated variable content of bioactive compounds (CBDA and CBD)

Variant V5 (Finola, sowing time 1) ensured a higher CBDA content compared to the other variants, with statistical significance ($p < 0.05$) relative to the experiment's mean.

In the case of CBD, variant V2 (Silvana, sowing time 2) exhibited a higher content, but without statistical significance when compared to the experiment's mean.

For the CBDA/CBD ratio, compared to the experiment's mean, variant V1 showed a positive difference (0.2770; $p = 0.0101$), while variant V2 exhibited a negative difference (-0.2033; $p = 0.0318$).

In the case of the CBD/CBDA ratio, compared to the calculated mean, variant V1 showed a negative difference (-0.3836; $p = 0.0184$), while variant V2 showed a positive difference (0.4029; $p = 0.0153$).

A polynomial model ($R^2 = 0.998$, $p < 0.001$) and graphical models (3D, isoquants) described the variation of the CBDA/CBD ratio in relation to the basic indices (CBDA, CBD). A divergent contribution of both indices to the calculated ratio values was observed.

The multivariate analysis highlighted the association of the variants with the indices and the calculated ratios, as well as the grouping of the variants based on similarity.

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