Protein Content of Several Industrial Hemp Genotypes – Comparative Analysis

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Abstract. *The study aimed to compare the protein content of seeds in a collection of industrial hemp genotypes. The field experiment was located within the ARDS Lovrin, agricultural year 2024 – 2025. The comparative crop was located on a plot with chernozem soil, in a non-irrigated system. Ten hemp genotypes were cultivated, designated IHG 1 to IHG 10 (IHG – industrial hemp genotype). Sowing was done manually, in nests, at a distance of 70 cm between rows and 50 cm between plants per row, with three rows on each experimental plot and an area of 12.6 m^2 for each variant (genotype). The experiment was organized in randomized blocks, in three replications. At physiological maturity, samples of inflorescences with seeds were taken on the experimental variants and replications, and the seeds were extracted. After seed conditioning (selection, drying), subsamples were taken from each experimental variant and the protein content (PRO, %) was determined in the ARDS Lovrin laboratories. The mean values of protein content ranged from PRO = 22.81±1.17% (IHG 4) to PRO = 25.47±0.55 (IHG 7). ANOVA Test confirmed the reliability of the experimental data and the presence of variance in the data set ($p<0.05$). Compared to the average value at the experiment level ($\overline{PRO}$ = 24.19%), three genotypes presented values above the average, with differences at the $p<0.001$ level (IHG 7, ***), $p<0.01$ (IHG 1, **), respectively $p<0.05$ (IHG 5, *), and one genotype with values below the average, at the $p<0.001$ level (IHG 4, ^{OOO}). The ANOVA test (t Test) and the Kruskal-Wallis Test confirmed significant differences between the mean and median values, and the Dunn's post hoc Test confirmed differences between the genotypes tested.*

Keywords*: clustering, comparative analysis, functional foods, industrial hemp seeds, protein crops*

INTRODUCTION

Sustainable food systems require food resources with adequate mineral and nutrient intake, quality proteins, and a high digestibility index (Adhikari et al., 2026; Clinthorne et al., 2026). Protein sources are sought for different food systems, food balance, and consumer categories (Lonnie et al., 2019; Pikosky et al., 2022; Ortega et al., 2024).

From an environmental balance perspective, alternative sources to animal proteins are sought (Lonnie et al., 2019; Lima et al., 2022; Kaur et al., 2025). Vegetable proteins, obtained from agricultural crops, represent a viable alternative for food protein resources and in animal feed (De Ron et al., 2017; Kakabouki et al., 2025; Kaur et al., 2025; Singh et al., 2025).

Within agricultural crops, legumes for grains, pseudocereals, but also other categories of plants, are of high interest due to the protein content in their seeds (De Ron et al., 2017).

Legumes, through a consistent category of crop plants (common beans, faba beans, peas, green peas, chickpeas, cowboy chickpeas, soybeans, peanuts, lupins, lentils) represent a relevant source of vegetable proteins, both for human consumption and for animal feed (De Ron et al., 2015; De Ron et al., 2017; Zhang et al., 2024).

Legumes also present a series of agronomic and ecosystem benefits for the sustainability of farms and farmers (Stagnari et al., 2017; Yanni et al., 2024; Kokkini et al., 2025; Muir et al., 2025).

At the same time, agricultural production in the world is highly specialized, and is based on a limited number of agricultural crops, which provide a large share of resources for the food industry and for processing (Lenné and Wood, 2025, 2026). From the perspective of climate trends, and economic, ecological and social aspects, agricultural diversity can ensure better conditions for the future (Martin et al., 2019; Waha et al., 2022; Knez et al., 2024).

In the context of agricultural diversity, industrial hemp has multiple valences, with a history steeped in the structure of crop plants, but at the same time with multivalent perspectives (Nath, 2022; Yano and Fu, 2023; Michels et al., 2025). In addition to fiber production, ecosystem functions, agronomic, economic and social importance, industrial hemp seeds represent a valuable resource for the food, medical and pharmaceutical fields through the content of active principles they present (Mygdalia et al., 2025; Hossain et al., 2026).

The present study evaluated the protein content of a collection of industrial hemp genotypes, in comparative cultivation in a non-irrigated system, under the climate and soil conditions specific to the Western Plain of Romania, within the ARDS Lovrin.

MATERIAL AND METHODS

The study was conducted within the ARDS Lovrin, agricultural year 2024 – 2025, under the pedo-climatic conditions specific to the Western Plain of Romania.

The biological material was represented by ten industrial hemp genotypes, noted in the study as IHG 1 to IHG 10.

The field experiment was located in flat terrain conditions, with chernozem soil. The comparative industrial hemp crop was in non-irrigated system. The size of an experimental plot was 12.6 m^2, with dimensions L = 6.0 m, w = 2.1 m (L – length, w – with). The experiment was organized in randomized blocks, with the variants placed in three repetitions.

Sowing was done manually, in nests, at a distance of 70 cm between rows and 50 cm between plants per row, with three rows on each experimental plot. Aspects of the experimental field are presented in Figure 1.



(a) (b) (c)

Figure 1. Aspects from the experimental field; (a) – experimental variants with young plants; (b) – experimental variants with mature hemp plants; (c) – inflorescence with seeds

Specific crop technology for industrial hemp was provided, under uniform conditions for all variants.

The values of the climatic parameters for the period of the agricultural year 2024 – 2025 are presented in Figure 2. The thermal regime presented higher values, compared to the multiannual average, except for May. In the case of the pluviometric regime, it was assessed as deficient, in the months of April, June and August, compared to the multiannual average.

At physiological maturity, seed inflorescence samples were taken from the experimental variants and replicates, and the seeds were extracted. After seed conditioning (selection, drying), subsamples were taken from each experimental variant and the protein content (PRO, %) was determined in the ARDS Lovrin laboratories.

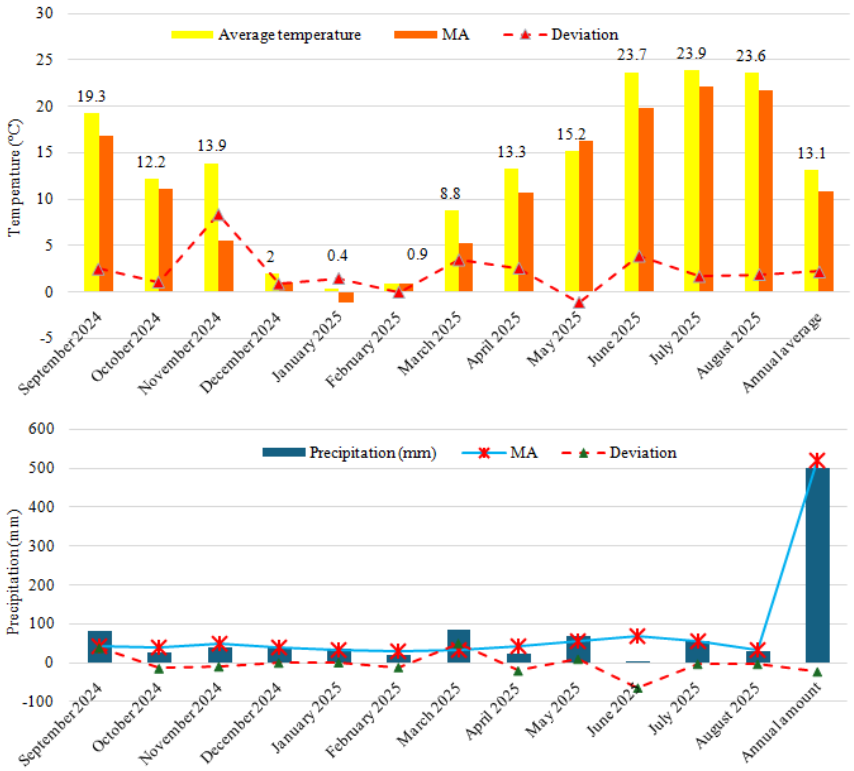


Figure 2. Climatic data for the experimental period, agricultural year 2024 – 2025 (ARDS Lovrin Weather Station)

The experimental data for protein content (PRO, %) were analyzed mathematically and statistically to comparatively evaluate the performance of the genotypes for protein content. The PAST software v.4.14. (Hammer et al., 2001) was used, in relation to the purpose of the study.

RESULTS AND DISCUSSIONS

Industrial hemp genotypes presented differentiated values of protein content, according to the genetic accumulation potential. Average values were recorded ranging from PRO = 22.81±1.17% (IHG 4) to PRO = 25.47±0.55 (IHG 7), Table 1. The data series presented statistical reliability and variability, according to ANOVA Test (Alpha = 0.05), Table 2.

Table 1. Descriptive statistical analysis results

Statiatical parameters	IHG 1	IHG 2	IHG 3	IHG 4	IHG 5	IHG 6	IHG 7	IHG 8	IHG 9	IHG 10
Min	24.77	23.15	23.52	20.54	23.39	23.29	24.61	22.32	23.16	22.54
Max	26.38	24.96	23.87	24.47	26.44	24.98	26.49	27.10	24.46	25.36
Mean	25.33	23.93	23.74	22.81	24.83	23.87	25.46	24.19	23.66	24.12
Std. error	0.53	0.54	0.11	1.17	0.88	0.56	0.55	1.47	0.40	0.83
Variance	0.83	0.86	0.04	4.14	2.35	0.92	0.91	6.52	0.49	2.07
Stand. dev	0.91	0.93	0.19	2.03	1.53	0.96	0.95	2.55	0.70	1.44
Median	24.83	23.69	23.84	23.42	24.66	23.34	25.29	23.15	23.37	24.45
25 prcntil	24.77	23.15	23.52	20.54	23.39	23.29	24.61	22.32	23.16	22.54
75 prcntil	26.38	24.96	23.87	24.47	26.44	24.98	26.49	27.10	24.46	25.36
Skewness	1.72	1.10	-1.69	-1.23	0.49	1.73	0.79	1.53	1.56	-0.99
Kurtosis	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33	-2.33
Geom. mean	25.32	23.92	23.74	22.75	24.80	23.86	25.45	24.10	23.66	24.09
Coeff. var	3.60	3.88	0.82	8.92	6.17	4.03	3.74	10.56	2.95	5.97

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Table 2. ANOVA Test results

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	52.8994	9	5.8777	3.0718	0.0033	1.9991
Within Groups	153.0744	80	1.9134			
Total	205.9738	89				

The comparative analysis of genotypes was done based on mean data (t Test), with the results in Table 3. Compared to the mean value at the experiment level ($\overline{PRO}$ = 24.19%), four genotypes with statistically significant differences were recorded. Three genotypes showed values above the mean, with differences at the p<0.001 level (IHG 7, ***), with differences at the p<0.01 level (IHG 1, **), and with differences at the p<0.05 level (IHG 5, *), respectively. One genotype showed values below the mean, with differences at the p<0.001 level (IHG 4, ^{OOO}). The graphical distribution of the differences is presented in Figure 3.

Table 3. Positioning of industrial hemp genotypes compared to the experimental mean value – differences and meanings

Genotype	Given mean	Sample mean	95% conf. interval	Difference	95% conf. interval	t	p	Significance
IHG 1	25.33	24.19	(23.616 24.772)	1.136	(0.55756 1.7144)	-4.4426	0.0016	**
IHG 2	23.93			0.264	(-0.31444 0.84244)	1.0324	0.3288	ns
IHG 3	23.74			0.454	(-0.12444 1.0324)	1.7755	0.1096	ns
IHG 4	22.81			1.384	(0.80556 1.9624)	5.4125	0.0004	ooo
IHG 5	24.83			0.636	(0.057559 1.2144)	-2.4872	0.0346	*
IHG 6	23.84			0.354	(-0.22444 0.93244)	1.3844	0.1996	ns
IHG 7	25.46			1.266	(0.68756 1.8444)	-4.9510	0.0008	***
IHG 8	24.19			0.004	(-0.57444 0.58244)	0.0156	0.9879	ns
IHG 9	23.66			0.534	(-0.044441 1.1124)	2.0883	0.0664	ns
IHG 10	24.12			0.074	(-0.50444 0.65244)	0.2894	0.7788	ns

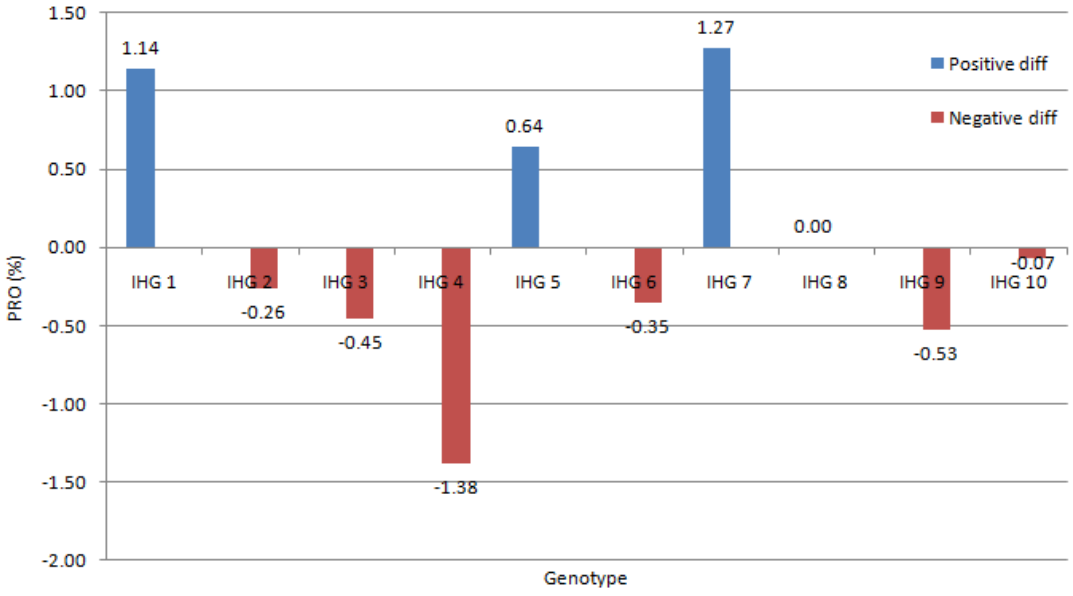


Figure 3. Graphical distribution of differences for studied hemp genotypes

The ANOVA test (Several-sample test) was applied to comparatively evaluate the ten hemp genotypes based on the protein content values. The statistical parameter values were presented in Table 4, which confirmed significant differences at the level of mean values (p<0.05). The distribution of residual values is shown in

Figure 4, with normal distribution type.

Table 4. ANOVA several-sample Test results

Statistical parameters	Statistical parameters values				
	Sum of sqrs	df	Mean square	F	p (same)
Between groups	52.8994	9	5.8777	3.0750	0.0032
Within groups	152.9290	80	1.9116	Permutation p (n=99999)	
Total	205.8280	89	0.0029		
Components of variance (only for random effects)					
Var(group)	0.4407	Var(error)	1.9116	ICC	0.1873
omega^2	0.1718				
Levene´s test for homogeneity of variance, from means	p (same)	1.86E-05			
Levene´s test, from medians	p (same)	0.0004			
Welch F test in the case of unequal variances	F=5.6, df=30.68, p=0.0001416				
Bayes factor	5.775 (substantial evidence for unequal means)				

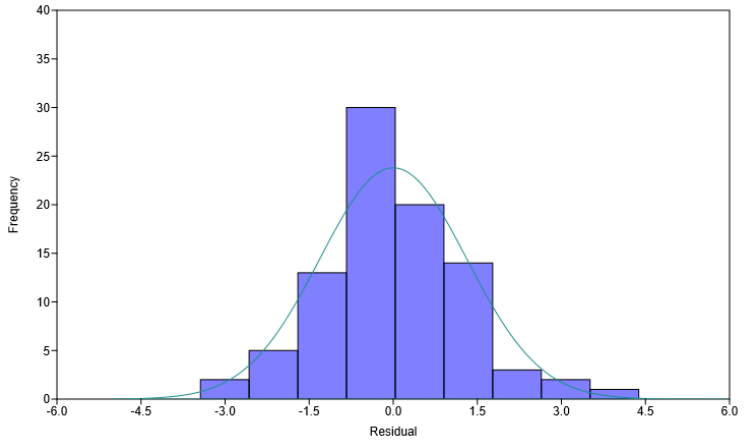


Figure 4. Histogram of residual values

The Kruskal-Wallis test also indicated a significant difference between the median values, with H(chi^2) = 25.04, H_c(tie corrected) = 25.05, p = 0.0029.

Following confirmation of the differences between the mean and median values, under conditions of statistical safety, Dunn's post hoc test was applied, with the results in Table 5. Fields marked in green indicate significant differences between genotypes, based on seed protein content.

Table 5. Dunn's post hoc results

	IHG 1	IHG 2	IHG 3	IHG 4	IHG 5	IHG 6	IHG 7	IHG 8	IHG 9	IHG 10
IHG 1		0.0160	0.0083	0.0012	0.3210	0.0143	0.8639	0.0384	0.0045	0.0535
IHG 2	0.0160		0.8180	0.4014	0.1566	0.9676	0.0099	0.7351	0.6682	0.6325
IHG 3	0.0083	0.8180		0.5425	0.0996	0.8497	0.0049	0.5697	0.8427	0.4788
IHG 4	0.0012	0.4014	0.5425		0.0241	0.4246	0.0006	0.2390	0.6814	0.1877
IHG 5	0.3210	0.1566	0.0996	0.0241		0.1451	0.2445	0.2809	0.0650	0.3481
IHG 6	0.0143	0.9676	0.8497	0.4246	0.1451		0.0088	0.7047	0.6980	0.6039
IHG 7	0.8639	0.0099	0.0049	0.0006	0.2445	0.0088		0.0250	0.0026	0.0355
IHG 8	0.0384	0.7351	0.5697	0.2390	0.2809	0.7047	0.0250		0.4431	0.8888
IHG 9	0.0045	0.6682	0.8427	0.6814	0.0650	0.6980	0.0026	0.4431		0.3645
IHG 10	0.0535	0.6325	0.4788	0.1877	0.3481	0.6039	0.0355	0.8888	0.3645	

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Cluster analysis was used to obtain the grouping of genotypes based on similarity in relation to seed protein content. The dendrogram in Figure 5 resulted, under conditions of Coph.corr. = 0.841. The genotypes with performance for seed protein content were grouped in cluster C1 (IHG 5(IHG 7, IHG 1)). The other genotypes, with low performances for protein were grouped in cluster C2, in two subclusters.

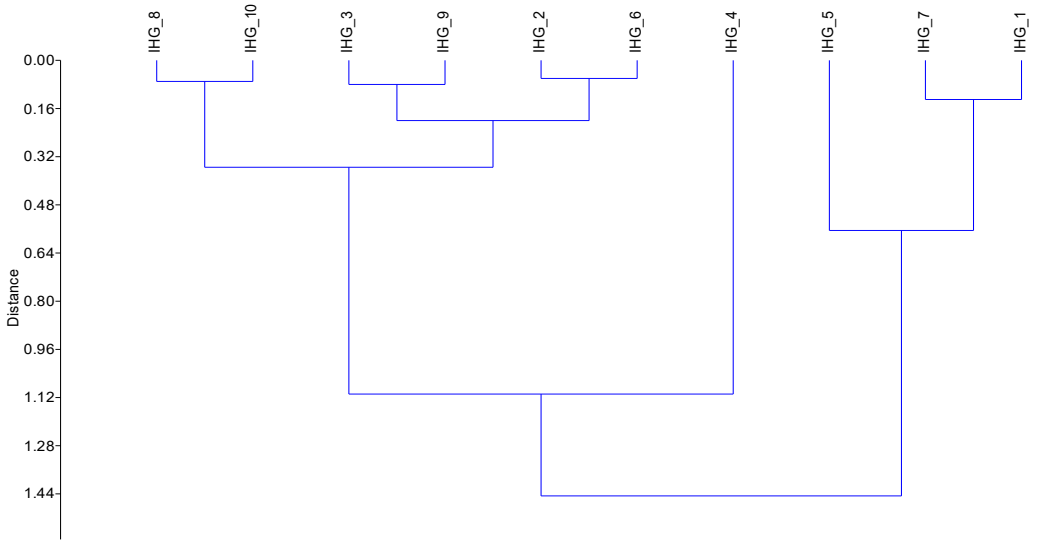


Figure 5. Cluster dendrogram

The SDI values (similarity and distance indices) confirmed the similarity grouping of hemp genotypes in relation to protein content, Table 6. In the group of genotypes with protein performance, a high level of similarity was recorded between IHG 1 and IHG 7 (SDI = 0.13). A detached position was presented by the IHG 5 genotype, which recorded the highest protein content.

Table 6. SDI values

	IHG 1	IHG 2	IHG 3	IHG 4	IHG 5	IHG 6	IHG 7	IHG 8	IHG 9	IHG 10
IHG 1		1.40	1.59	2.52	0.50	1.46	0.13	1.14	1.67	1.21
IHG 2	1.40		0.19	1.12	0.90	0.06	1.53	0.26	0.27	0.19
IHG 3	1.59	0.19		0.93	1.09	0.13	1.72	0.45	0.08	0.38
IHG 4	2.52	1.12	0.93		2.02	1.06	2.65	1.38	0.85	1.31
IHG 5	0.50	0.90	1.09	2.02		0.96	0.63	0.64	1.17	0.71
IHG 6	1.46	0.06	0.13	1.06	0.96		1.59	0.32	0.21	0.25
IHG 7	0.13	1.53	1.72	2.65	0.63	1.59		1.27	1.80	1.34
IHG 8	1.14	0.26	0.45	1.38	0.64	0.32	1.27		0.53	0.07
IHG 9	1.67	0.27	0.08	0.85	1.17	0.21	1.80	0.53		0.46
IHG 10	1.21	0.19	0.38	1.31	0.71	0.25	1.34	0.07	0.46	

In the group of genotypes with lower protein performance, a high level of similarity was recorded between IHG 2 and IHG 6 (SDI = 0.06). Genotypes IHG 8 and IHG 10 were associated in a subcluster, with the smallest differences from the experiment average. The other four genotypes were grouped in pairs, based on similarity.

Genetic and phenotypic diversity in industrial hemp genotypes was determined based on seed size, mineral content profile and specific markers (Langa et al., 2026). Agronomic parameters, yield and nutritional profile based on bioactive compounds were evaluated in industrial hemp genotypes (Tamang et al., 2025, 2026).

Clustering has been useful in studies on industrial hemp for grouping experimental variants in relation to quality indices (CBDA, CBD) and calculated ratios (CBDA/CBD, CBD/CBDA), recorded in experimental variants given genotypes and sowing dates (Buzna et al., 2025), in relation to morphological parameters and the mineral profile of seeds in some industrial hemp genotypes (Langa et al., 2026), or based on bioactive

compounds in industrial hemp (Eržen et al., 2026).

In the present study, the mathematical and statistical tools used facilitated the comparative analysis of the studied genotypes, highlighting the performance of the genotypes and their classification in relation to the protein content of the seeds. The study will consider for the future the expansion of the genotype collection, the conduct of the study over a longer period, and the inclusion of a larger number of physiological indices, agronomic parameters and quality indices in order to quantify in a more complex way the behavior of industrial hemp genotypes under the study conditions.

CONCLUSIONS

The ten industrial hemp genotypes presented differentiated values of protein content in seeds, with values ranging from PRO = 22.81±1.17% (IHG 4) to PRO = 25.47±0.55 (IHG 7). Compared to the mean value at the experiment level ($\overline{PRO}$ = 24.19%), four genotypes showed differences in statistical safety conditions. Three genotypes performed compared to the mean of the experiment, with differences at the $p<0.05$ level (IHG 5, *), at the $p<0.01$ level (IHG 1, **), respectively at the $p<0.001$ level (IHG 7, ***). One genotype showed values lower than the mean, with differences at the $p<0.001$ level (IHG 4, ^{OOO}).

The ANOVA and Kruskal-Wallis tests indicated significant differences between the mean and median values of protein content achieved by the ten industrial hemp genotypes ($p<0.05$). Dunn's post hoc test confirmed statistically significant differences in 14 comparative analyses between genotypes, out of a total of 45 comparative analyses. Cluster analysis grouped the hemp genotypes based on similarity, in relation to the values of protein content in the seeds.

The results recorded in the study are important for the industrial hemp breeding program, in order to select genotypes based on performance for protein content. At the same time, they are also important from the perspective of promoting for agricultural practice genotypes performing for protein production, complementary to the other agronomic and ecosystem advantages of industrial hemp.

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