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Dry matter content variation in seeds of hemp for fiber - Comparative study in some genotypes

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Abstract
The study analyzed the dry matter content of industrial hemp seeds. The experiment took place within ARDS Lovrin, Timis County, Romania. Six hemp genotypes for fiber were considered, noted in the study with codes FHG1 to FHG6. The studied genotypes were cultivated in four repetitions, non-irrigated system. The dry matter content (DM, %) was determined in seed samples at physiological maturity. The mean values recorded were DM = 93.60±0.22% in the case of FHG1, DM = 93.12±0.02 in the case of FHG2, DM = 92.16±0.05% in the case of FHG3, DM = 93.31±0.05 in the case of FHG4, DM = 92.93±0.01% in in the case of FHG5, and DM = 91.78±0.08% in the case of FHG6. The FHG1 genotype recorded statistically significant differences compared to the FHG2 genotype (p = 0.44, *), the FHG5 genotype (p = 0.005, **), and the FHG6 genotype (p<0.001, ***). The FHG2, FHG2, FHG4 and FHG5 genotypes presented differences in terms of statistical safety compared to the FHG6 genotype (p<0.001, ***). Compared to the mean value per experiment (DM_m = 92.98±0.007%), the FHG1 genotype (p<0.001) and the FHG4 genotype (p<0.05) were highlighted with positive differences. The cluster analysis generated the dendrogram of association of genotypes based on similarity (Coph.corr. = 0.964).

Keywords: Cluster analysis, comparative analysis, dry matter, hemp for fibers, industrial hemp seeds

Introduction

The content of dry matter in different parts of plants represents a characteristic of plant species and genotypes, an evolutionary ability of plants, and varies in relation to different growth factors [16]. The dry matter content of the seeds and the quality index values are determined by genetic factors and environmental factors [2], and in the case of cultivated plants, the crop technology also intervenes in the complex interaction "genotype x environment x technology".

The influence of water and salt stress was analyzed in beans [1]. The authors recorded the variation in the weight of the beans under the study conditions and formulated models for the variation of the dry matter in the beans. The models describing the simulated dry matter, compared with the recorded dry matter, showed statistical reliability (R^2 = 0.85, and R^2 = 0.89).

Dry matter estimation models were reported for different plant species, herbaceous plants cultivated (hydroponically, sand) and herbaceous and woody species collected from field conditions [16]. The authors recorded specific variations of the dry matter content, in relation to the analyzed plant species. Prediction models of the dry substance content were obtained, under statistical safety conditions.

Atta et al. (2004) [2] evaluated the variation of dry matter and protein content in six pea genotypes. The authors communicated the differentiated variation of the protein and dry matter content in relation to the genotype, and in relation to the position of the pods and seeds on the plant, from the base to the upper part of the plants. Kakiuchi and Kobata (2006) [7] recorded different values of dry matter content in soybean seeds and shoots. The authors recorded the variation of the distribution rate of dry matter between shoots and seeds depending on the number of pods. The authors of the study did not record variations regarding the number of seeds and the individual weight of the seeds in relation to the density or shading of the plants.

The behavior of some bean genotypes (36 genotypes) was analyzed in different humidity conditions, respectively water supply and drought [13]. The authors of the study have determined different indices with reference to the plants' response to the growing conditions. Based on the results, the authors recorded that drought stress (in the case of some genotypes) was positively associated with water use efficiency, the increase of dry matter in the production of pods and seeds. The authors concluded that the pod harvest index, which also includes dry matter, can be confirmed as a criterion for the selection of valuable genotypes in common bean breeding programs.

In some studies on cotton, Pabuayon et al. (2020) [12] reported a higher percentage of dry matter in modern varieties compared to an older variety in the comparative study. Associated with the dry substance, the authors also recorded the variation in the content of mineral elements and the fiber quality. Optimizing the allocation of fertilizers could contribute positively to yield and production quality, according to the authors' conclusions.

The vegetation conditions of cultivated plants, associated with climate changes, generate changes regarding the accumulation of dry matter, its distribution / distribution in plant organelles, the yield and quality of many crop plants [3]. The water deficit in the soil influences the physiological processes of plants, the accumulation of dry matter, the distribution of dry matter in the plant, the yield of crops [3]. A sigmoid model was considered to describe the accumulation of agricultural crop biomass [3]. The author considered that dry matter production can be considered an indicator / estimator of the degree of adaptation of cultivated genotypes to environmental conditions.

The variation of dry matter content and distribution in soybean plants was studied under high rainfall conditions [14]. Through defoliation treatments (three treatments, 15%, 30%, 45%), the authors recorded an increase in dry matter accumulation and translocation in the reproductive area by up to 8% in the case of 15% defoliation. The increase in the number of pods and the number of grains was recorded. The content of dry matter and the rate of accumulation was studied in seed material, elite and pre-elite seeds, in potato [18]. The authors recorded the variation of the dry matter accumulation rate in the pre-elite seeds compared to the certified ones.

The variation of the dry matter content was studied in soybean in relation to the sowing date [9]. The authors obtained a mathematical model, in the form of a modified regression equation, which described the variation of the dry matter in relation to the sowing date, and the decrease of the seed yield in the soybean crop. The authors considered the regression equation useful, as a model/indicator of seed yield under soybean cultivation conditions with irrigation possibilities.

The present study analyzed the dry matter content in the seeds of six genotypes of hemp for fibers, cultivated in a non-irrigated system.

Material and Method

The study was organized within ARDS Lovrin, Timis County, Romania. Six genotypes of hemp for fiber (FHG) were considered in the study, which represented the biological material of the study. Genotypes were denoted FHG1 to FHG6. The hemp genotypes were grown on chernozem soil, non-irrigated culture conditions. Each genotype was cultivated in four repetitions. A standard hemp culture technology for fiber was provided. At physiological maturity, according to BBCH code [11], seed samples were collected for each genotype and repetition. Laboratory samples were taken from the seed samples (field samples) to determine the dry matter (DM, %), figure 1.

Figure 1. Industrial hemp seed samples (original picture, by Florin Sala)

The dry matter was determined by drying at 105°C for 6 hours in an oven with forced air circulation SLW 53 (POL-EKO-Aparatura, Wodzisław Śląski, Poland) [10]. The determination of the dry matter was done in three repetitions for each genotype. The recorded values were analyzed mathematically and statistically in an adequate way, in order to compare the genotypes with each other, as well as compared with the average value at the experiment level (DM_m).

Data processing and analysis was done in EXCEL (statistical calculation module) and with the dedicated application [6]. The interpretation of the results of the comparative analysis of the genotypes was based on established statistical parameters ($p<0.05$; $p<0.01$; $p<0.001$). The significance of the differences was marked by the symbol "*" in the case of positive differences, and by the symbol "o" in the case of negative differences.

Results and Discussion

The seed samples, of hemp for fiber, were analyzed to determine the dry matter content (DM, %). The results were the values for the six genotypes (FHG1 to FHG6), in three repetitions. Based on the primary data, the mean value was calculated for each genotype, DM = 93.60±0.22% in the case of FHG1, DM = 93.12±0.02 in the case of FHG2, DM = 92.16±0.05% in the case of FHG3, DM = 93.31±0.05 in the case of FHG4, DM = 92.93±0.01% in the case of FHG5, and DM = 91.78±0.08% in the case of FHG6. The ANOVA test confirmed the safety of the data and the presence of the variant in the experimental data set, table 1.

Table 1. ANOVA Test for the DM parameter in industrial hemp seeds

Cases	Sum of Squares	df	Mean Square	F	p
Trial	5.993	5	1.199	40.268	< .001
Residuals	0.357	12	0.030		

A comparative analysis was made between genotypes, regarding the content of dry matter (DM, %). The values from table 2 resulted, which represent the differences between the genotypes (Mean Difference), the confidence interval (95%), statistical safety parameters and the significance of the differences.

Table 2. Comparative analysis of hemp genotypes, dry matter content in seeds (DM)

Genotypes and Mean Difference			95% CI for Mean Difference		Statistical safety parameters		
Fiber hemp genotypes		Mean Difference	Lower	Upper	SE	t	p_{tukey}
FHG1	FHG2	0.483 *	0.010	0.957	0.141	3.431	0.044
	FHG3	0.443 ^{ns}	-0.030	0.917	0.141	3.147	0.071
	FHG4	0.297 ^{ns}	-0.177	0.770	0.141	2.106	0.346
	FHG5	0.673**	0.200	1.147	0.141	4.780	0.005
	FHG6	1.827***	1.353	2.300	0.141	12.967	< .001
FHG2	FHG3	-0.040 ^{ns}	-0.513	0.433	0.141	-0.284	1.000
	FHG4	-0.187 ^{ns}	-0.660	0.287	0.141	-1.325	0.767
	FHG5	0.190 ^{ns}	-0.283	0.663	0.141	1.349	0.754
	FHG6	1.343***	0.870	1.817	0.141	9.536	< .001
FHG3	FHG4	-0.147 ^{ns}	-0.620	0.327	0.141	-1.041	0.895
	FHG5	0.230 ^{ns}	-0.243	0.703	0.141	1.633	0.595
	FHG6	1.383***	0.910	1.857	0.141	9.820	< .001
FHG4	FHG5	0.377 ^{ns}	-0.097	0.850	0.141	2.674	0.152
	FHG6	1.530***	1.057	2.003	0.141	10.861	< .001
FHG5	FHG6	1.153***	0.680	1.627	0.141	8.187	< .001

* $p < .05$, ** $p < .01$, *** $p < .001$; ^{ns} – non significance

In the case of the FHG1 genotype, statistically significant differences were recorded in the case of the comparative analysis with the FHG2 genotype (p = 0.44, *), of the comparative analysis with the FHG5 genotype (p = 0.005, **) and in the case of the comparative analysis with FHG6 genotype (p<0.001, ***). In the case of the FHG2, FHG3, FHG4 and FHG5 genotypes, differences were recorded in terms of statistical safety, in the case of the comparative analysis with the FHG6 genotype (p<0.001, ***). In the other cases, the differences resulting from the analysis and calculations did not show statistical certainty.

The mean values calculated for each genotype were analyzed compared to the mean value calculated at the level of the experiment (DM_m = 92.98±0.007%), with the graphic distribution in figure 2.

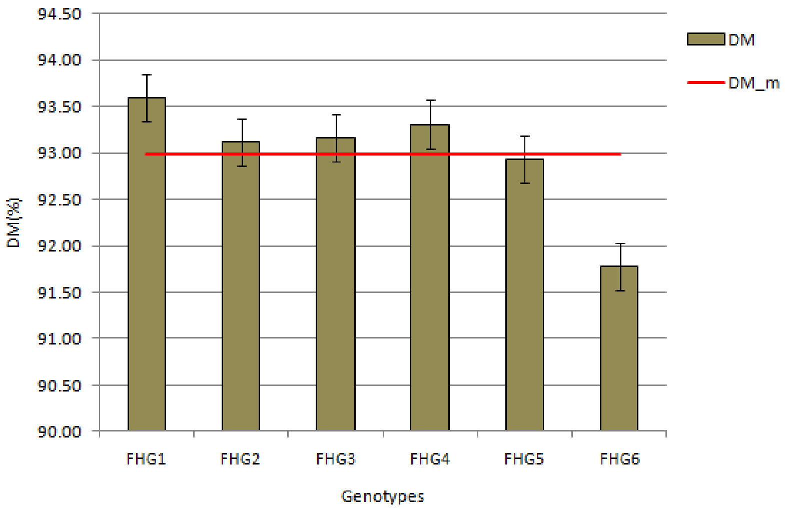

Figure 2. The graphic distribution of DM means values in relation to the mean value per experiment, hemp genotypes for fiber

From the analysis of the graphic distribution of the mean values (DM, %), it was found that the genotypes FHG1, FHg2, FHG3 and FHG4 presented values above the mean, and the genotypes FHG5 and FHG6 presented values below the mean.

Statistical analysis was done to determine the safety of the differences for dry matter (DM, %) in the case of each genotype, compared to the mean value of the experiment (DM_m). The results were the values presented in table 3. The DM differences calculated for each genotype in relation to the mean value (DM_m) are represented in figure 3.

Table 3. The statistical certainty of the differences between the DM values related to the studied genotypes and the mean value at the level of the experimenter (DM_m)

Statistical parameter	Fiber hemp genotypes studied					
	FHG1	FHG2	FHG3	FHG4	FHG5	FHG6
Given mean:	93.600	93.120	93.160	93.310	92.930	91.780
Sample mean (DM_m):	92.983	92.983	92.983	92.983	92.983	92.983
95% conf. interval:	(92.679 93.287)	(92.679 93.287)	(92.679 93.287)	(92.679 93.287)	(92.679 93.287)	(92.679 93.287)
Difference:	0.617	0.137	0.177	0.327	0.053	1.203
95% conf. interval:	(0.31329 0.92116)	(-0.16671 0.44116)	(-0.12671 0.48116)	(0.023286 0.63116)	(-0.25116 0.35671)	(0.89884 1.5067)
t :	-4.2845	-0.95255	-1.2302	-2.2715	0.36636	8.3492
p (same mean):	0.0005	0.3542	0.2354	0.0364	0.7186	2.03E-07
Means are significantly different	***	ns	ns	*	ns	ooo

The dry matter content (DM) of the FHG1 genotype showed a positive difference compared to the calculated average value ($p<0.001$). In the case of the FHG4 genotype, the calculated difference was above the average, under conditions of $p<0.05$.

In the case of the FHG6 genotype, a negative difference was recorded, under conditions of $p<0.001$. In the case of FHG2, FHG3 and FHG5 genotypes, the differences did not present statistical certainty ($p = 0.137$ in the case of PFG3, $p = 0.177$ in the case of FHG3, $p = 0.053$ in the case of FHG5).

The differences in the content of dry matter (DM, %) in the hemp fiber genotypes studied, in relation to the calculated average value (DM-m) at the level of the experiment, are shown graphically in figure 3. For each genotype, the standard error is also graphically represented.

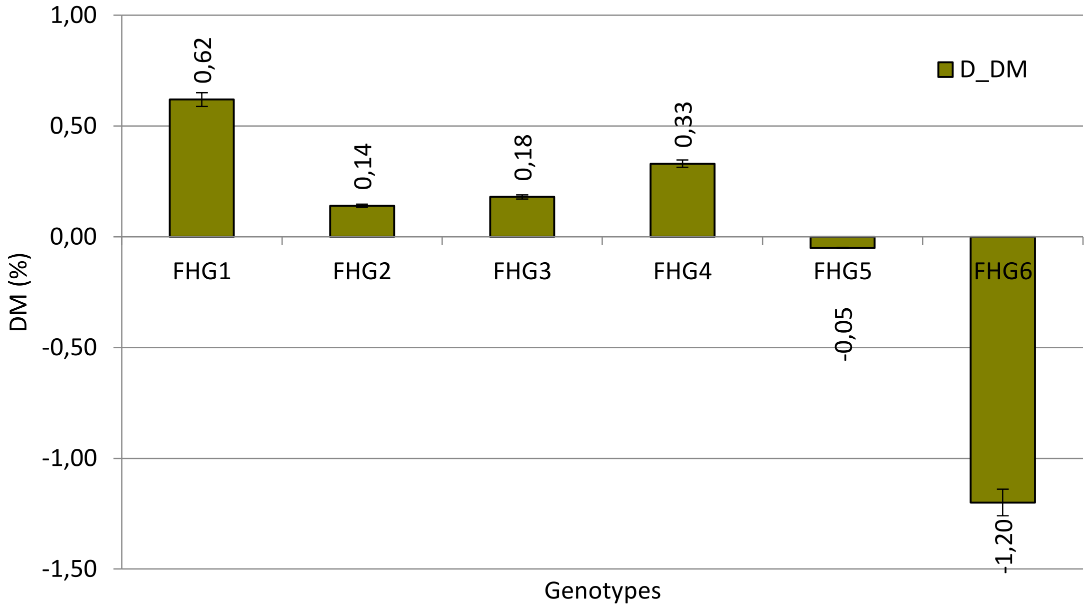

Figure 3. Graphical distribution of differences (DM) in hemp genotypes compared to the average value per experiment (DM_m)

The cluster analysis led to the dendrogram in figure 4 (Coph.corr. = 0.964). The independent positioning of the FHG6 genotype was found, in the case of which the content of dry substance in the seeds presented the lowest value, DM = 91.78%.

The other five genotypes were grouped in a cluster based on similarity. The association of FHG2 and FHG3 genotypes was found, which presented the highest level of similarity, SDI = 0.040, according to data presented in table 4.

The group (FHG2, FHG3) was joined by FHG4 genotypes, followed by FHG5. In this cluster, with a separate position, the genotype FHG1 was positioned, where the highest value of the DM parameter was recorded, table 3.

The separate position of the FHG6 genotype in the deodrogram (Figure 4) was confirmed by the recorded SDI values, table 4. Thus, the FHG6 genotype had the SDI value = 1.820 with the FHG1 genotype (the highest value among the genotypes), the SDI value = 1.340 with the genotype FHG2, the SDI value = 1.380 with the FHG3 genotype, the SDI value = 1.530 with the FHG4 genotype, and the SDI value = 1.150 with the FHG5 genotype.

The content of dry matter in the seeds was appreciated as important, in relation to the yield, with the content of mineral elements and some active principles in the seeds of different crop plants [1], [9].

The dry matter content was associated with tolerant to water stress, and was considered as an important indicator in the selection of genotypes for the breeding program [13].

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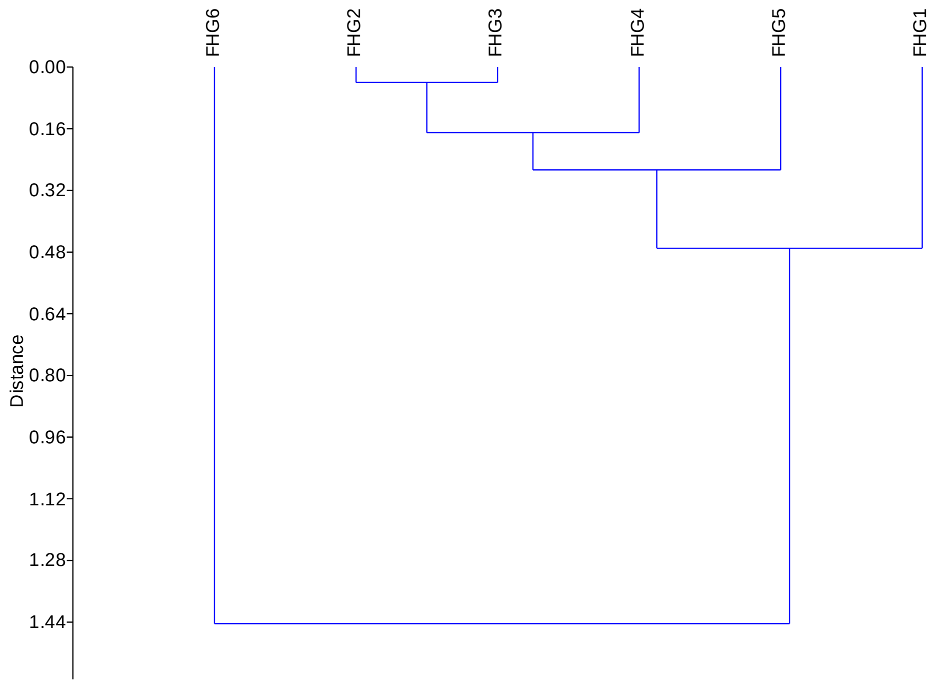

Figure 4. Grouping dendrogram of hemp genotypes for the fiber studied, in relation to DM values (%)

Table 4. SDI values for the hemp genotypes for fiber studied

	FHG1	FHG2	FHG3	FHG4	FHG5	FHG6
FHG1		0.480	0.440	0.290	0.670	1.820
FHG2	0.480		0.040	0.190	0.190	1.340
FHG3	0.440	0.040		0.150	0.230	1.380
FHG4	0.290	0.190	0.150		0.380	1.530
FHG5	0.670	0.190	0.230	0.380		1.150
FHG6	1.820	1.340	1.380	1.530	1.150	

Interest in hemp seeds has been revised, as a result of their specific properties, with importance for human nutrition, through the content of valuable active principles [8], [17].

Among the six genotypes of hemp for fibers studied, genotype FHG6 recorded the lowest value of dry substance content (DM = 91.78±0.08%), followed by genotype FHG5 (DM = 92.93±0.01%). Both genotypes were placed below the average value calculated at the level of the experiment (DM_m = 92.98±0.007%).

The FHG1 genotype presented the highest value of dry matter content (DM = 93.60±0.22%), followed by the FHG4 genotype (DM = 93.31±0.05). Both genotypes were placed above the average value at the experiment level, under statistical safety conditions. Values of the dry substance content above the average value of the experiment were also recorded for the genotypes FHG2, respectively FHG3, but without statistical certainty.

In the crop conditions, non-irrigated system, it can be appreciated that the FHG1 genotype presented a very good adaptation, followed by the FHG4 genotype.

These genotypes provided seeds with a high content of dry matter, under the study conditions. They can be of interest for improvement programs, but also for agricultural production. Industrial hemp seeds represent an important source for the food industry, for functional foods [4], [15], [5].

Genotypes FHG2 and FHG3 are important for agricultural practice, for fiber production and seed production (on a secondary level) and can be considered for improvement programs, associated with other morpho-anatomical and productive attributes that they can present.

Conclusions

The six genotypes of hemp for fiber (FHG1 to FHG6) used in the comparative study, had the production of seeds in which variable content of dry matter (DM, %) was determined.

The FHG1 genotype (DM = 93.60±0.22%) was positioned with a high content, with a difference compared to the other genotypes in terms of statistical safety ($p<0.001$). Also, the dry matter content in the case of the FHG1 genotype was higher than the average value of the dry matter content at the level of the experiment ($p<0.001$).

The FHG4 genotype was also highlighted by the high value of the dry substance content in the seeds, above the average value of the experiment ($p<0.05$).

Two genotypes were positioned below the average value of the experiment, FHG6 ($p<0.001$) and the FHG5 genotype.

The other two genotypes FHG2 and FHG3 presented dry matter content values above the average value of the experiment, but without statistical certainty ($p>0.05$).

The cluster analysis facilitated the cluster grouping of the genotypes, based on similarity, in relation to the dry matter content of the seeds. The SDI values confirmed the level of similarity in the grouping of hemp genotypes for the fiber studied.

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