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Formation of the Quality Indicators of Hemp (*Cannabis sativa* L.) Seeds Sown under Organic Growing Technology

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ABSTRACT

The oil content of hemp seeds is controlled by the genotype and in the conducted studies did not depend on the growing technology; however, this factor had a synergistic effect with others. The protein content of hemp seeds during the years of research did not depend on weather conditions. Like other quality indicators, it had a slight variation, which indicates the significant role of the genetic characteristics of the varieties. On average, over the years of research, the protein content of the variants grown according to conventional technology was 25.2%, and according to transitional technology, it was 0.03% higher, which was within the limits of statistical error. The organic technology ensured the protein content at the level of 25.3%, and the use of the BioStymix-Niva microbial biodegrader - biodestructor contributed to the further growth of the indicator to 25.4%. The oil content of hemp seeds is not limited by other important characteristics, such as the yield of the hemp stems or the fiber content. Only the Glyana variety showed inverse correlations with plant height, hemp stems and seed productivity, they were of medium strength (r = -0.60 – -0.43). In the Zolotoniski 15 variety, only one inverse relationship was recorded, i.e. plant height (r = -0.57). No correlation was established between protein content and oil content in seeds. Correlations may change depending on other factors of cultivation, including weather conditions, elements of technology, etc., but the evaluation of varieties for cultivation according to these characteristics can significantly increase the efficiency of the production of cannabis products.

Keywords: organic technologies, hemp seeds, oil, protein, correlations.

INTRODUCTION

After many years of unprecedented restrictions, the agricultural culture of ancient civilization is gradually returning to the fields and attracts the deserved attention of science and industry. Not only a narrow approach to the revival of hemp growing, but also a much deeper problem, namely greening of crop production, food and technical industry, improvement of the environment, renewal of raw resources and a large number of aspects of human activity, is gaining special relevance. In this context, the basis is a scientifically based, rational system of hemp cultivation, which is based on the selection of varieties for targeted cultivation as well as development of organic cultivation technologies, taking into account agrobiological and agroecological features. Hemp has the potential for environmentally friendly sustainable cultivation (Small and Marcus, 2002) and the majority of US farmers surveyed, namely 75%, expressed interest in certified production (Dingha, 2019). According to Lithuanian scientists, this crop meets the principles of ecological production as much as possible: it does not deplete the soil, reduces the number of weeds in the field, but in addition, hemp products are also ecologically clean and harmless to the environment. Moreover, the revival of this industry, which began in Lithuania in 2014, has good economic and social prospects, creating new jobs (Jonaitienė et al., 2016). The growth and development of hemp, their productivity largely depend on the supply of plants during the growing season with the necessary productivity factors – heat, moisture, nutrients, etc.

Excessive temperature (daily maximum temperature above 30°C) during the grain filling phase will be one of the main factors affecting seed quality, limiting seed oil accumulation (Baldini et al., 2020). Hemp requires a sufficient amount of moisture during the entire growing season, severe drought can significantly accelerate ripening, plants can have a small height, which will negatively affect the yield and quality of fiber, as well as seed yield (Amaducci et al., 2002).

An important aspect of obtaining the optimal combination of quality and yield is also the density of plants per unit area. In thickened crops, up to 50–60% of plants were lost due to intraspecific competition (Amaducci et al., 2002). There is a debate about the norms and terms of application of nitrogen fertilizers, for example, some researchers say that there is no reaction to the terms of fertilizer use (Finnan and Burke, 2013), and others report the significant effect of post-sowing application (Legros et al., 2013).

There are conflicting data regarding the norms and terms of nitrogen application, as well as possible undesirable consequences of excessive nitrogen fertilizer application, and therefore it is recommended to study the reaction of varieties to fertilizers under specific environmental conditions where hemp is planned to be grown (Papastylianou et al., 2018). High application rates of nitrogen fertilizers increase the protein content in seeds (Adamovics et al., 2016). Depending on the growing conditions, nitrogen application rates from 60 to 200 kg/ha were found to be the most effective, which can increase plant height, stem diameter, seed yield, and biomass production for dual purpose varieties (Tang et al., 2017; Aubin et al., 2016; Vera et al., 2004). Food products and medicinal products require organic production (Caplan et al., 2013). The application of the organo-mineral fertilization system was most effective when 100 kg ha^{-1} of nitrogen and 20 tons ha^{-1} of cow manure were used. The highest oil content was obtained when applying the maximum N rate of 50 kg ha^{-1} without using phosphorus. 30 tons ha^{-1} of manure and 100 kg ha^{-1} of nitrogen increased leaf yield index and decreased seed yield index. Thus, hemp responds well to the combined application of nitrogen fertilizers and animal manure, while its response to P application was limited (Laleh et al., 2021). When using urea for fertilization, it is advisable to combine its application with nitrogen inhibitors, which ensure a higher yield of straw and seeds, as well as significantly reduce the rate of use of nitrogen fertilizers (Kakabouki et al., 2021).

A comprehensive research program in the Netherlands has concluded that hemp is a potentially profitable crop with unique traits and characteristics to fit harmoniously into a sustainable farming system (Werf et al., 1996). Organic strategies require special adaptation to the environment, whereas conventional strategies do not meet the requirements or principles of organic farming at all. The norm in many European countries is the cultivation of varieties suitable for multipurpose use, in particular fiber and seeds (Carus et al., 2013; Tang et al., 2016). Global food security and sustainability can be improved by increasing the production and use of seeds, which not only provide healthy sources of protein and oil, but the whole plant can be used for fiber (Schultz et al., 2020; Trovato et al., 2023). Hemp oil is rich in the nutrients that have nutritional and functional benefits for the human body. The content and quality of the oil are influenced by both varietal properties and agrotechnical methods (Marzocchi and Caboni, 2020).

The most interesting property of hemp oil is the content of polyunsaturated fatty acids, i.e. linoleic (C18:2, ω-6) and α-linolenic (C18:3, ω-3). In addition, it is noted that the unsaponifiable fraction has an anti-inflammatory, antimicrobial effect and lowers cholesterol. It is assumed that these oils can be used for the production of dietary supplements with a high content of ω-6 and ω-3 of plant origin (Da Porto et al., 2015). Similar data were also obtained by Ukrainian scientists (Sova et al., 2018).

Oil content and quality are largely determined by varietal characteristics (Blasi et al., 2022; Frassinetti et al., 2018). The differences between varieties can be a significant interval. In the studies of Golimowski et al., it was established that the yield of oil from seeds, depending on varietal properties, was 30.45 ± 0.85, 29.47 ± 0.84, and

31.03 ± 1.03% for Finola, Earlina 8FC, and S. Jubileu varieties, respectively, while the oil differed in quality (Golimowski et al., 2022). Thus, the selection of varieties for cultivation needs due attention depending on the conditions (Pavlovic et al., 2019). Hemp seeds are a good source of plant-based protein, for example, 2–3 tablespoons of hemp seeds provide almost 11 grams of protein, containing methionine, lysine and cysteine. Seeds of hemp are a rich source of protein, (it contains about 20–25%), the biological value of which is similar to the hen's egg white (House et al., 2010; Mikulec et al., 2019). In addition, it also contains 25–35% lipids, 20–30% carbohydrates, 10–15% insoluble fiber and minerals such as phosphorus, potassium, sodium, magnesium, sulfur, calcium, iron and zinc (Deferne and Pate 1996; Callaway, 2004; Galasso et al., 2016).

Thus, hemp seed proteins and hydrolysates can be used to make functional food products (Švec and Hrušková, 2015; Chen et al, 2023). Hemp flour can influence the protein content and antioxidant properties of bread (Kladar et al., 2021), be used for the production of gluten-free bread or reduce the consumption of wheat bread (Frassinetti et al., 2018). An important aspect is also the statistically significant relationship between seed yield, oil content and its chemical composition with the amount of nitrogen (Stafecka et al., 2016), which formulates the problem of providing this element in organic farming or in the case of biologicalization of growing technologies (Łochyńska and Frankowski, 2019).

The aim of the work was to determine the influence of varietal properties and growing technologies on the indicators of seed quality – oil and protein. In addition, an attempt was made to determine the dependence between indicators of the yield of stems and the quality of hemp seeds as well as to establish the limiting indicators of each other.

MATERIALS AND METHODS

The experiments were conducted in the Poltava region, which belongs to the zone of unstable moisture. The soil is leached chernozem, a layer of 0–20 cm, with the following parameters: pH – 6.6, P_2O_5 content – 140.3, K_2O – 87.7 mg/kg. The depth of the humus horizon is 53–100 cm, the humus content is 4.16%. After the main tillage of the soil in the fall (ploughing), spring closing of moisture and pre-sowing cultivation were carried out. Sowing was carried out by a Monosem seeder with a seeding rate for two-sided use – 1.2 million pieces/ha, for greens – 4.0 million pieces/ha of similar seeds in four times. The registered area of the site for green areas and two-way use is 25 m², the total area of the experiment is 0.68 hectares. Before spring tillage, BioStimIx-Niva destructor was applied using a ground sprayer at the rate of 1 l/ha. In the experiment, seed hemp varieties Glyana, Zolotoniski 15, Lara, Globa, Sula were grown according to cultivation technologies: conventional with application of N30P30K30 as a broadcast before sowing; transitional, organic and organic with the use of microbial biodegradation – biodestructor BioStymix-Niva (1 l/ha). The oil content was determined from the defatted residue using a Soxhlet apparatus. Diethyl (sulfur) ether was used as a solvent, 1 g of ground seeds was extracted until the ether was completely decolorized. The protein content in seeds was determined by the Kjeldahl method (House et al., 2010).

RESULTS AND DISCUSSION

The oil content of hemp seeds is controlled by the genotype and, in the studies conducted, did not depend on the growing technology, but this factor had a synergistic effect with others. Actually, the oil content indicator itself also had a weak variation, as did the fiber content. On average, over the years of research, the lowest oil content was contained in the seeds of the 2019 harvest – 29.41% – whereas in the following two years it was slightly higher – 29.45%. The highest oil content, on average over three years of research, was obtained on variants of the Globa variety – 29.73–29.76% (Table 1).

The Lara variety was characterized by a slightly lower content, the oil content in the seeds of which was within 29.72%, the rest of the varieties had an even lower indicator, and the lowest amount of oil was noted in the seeds of the Glyana variety – 29.1–29.16%. The use of the destructor in cultivation technologies did not lead to any significant change in the oil content. The protein content of hemp seeds during the years of research did not depend on weather conditions. Like other quality indicators, it had a very small variation, which indicates the significant role of the genetic characteristics of the varieties. The protein content in the seeds depended on the varietal properties and the interaction of this factor with the growing technology, as well as the combined effect of weather conditions and varietal properties was observed. Indeed, over the years of research, the protein content of the seeds was virtually the same at 25.3%. Therefore, the difference between the options should be sought in the middle of the multifactorial complex.

Over three years of research, the best results in terms of protein content were recorded in the Sula variety, the difference between the options of inorganic technology and organic technology exceeded the NIR value (Table 2). On average, over the years of research, the difference was 0.16%, reaching 0.2% in 2019. The average protein content in hemp seeds of the Sula variety was 25.9% for all cultivation options. Other varieties contained a comparatively smaller amount.

The Globa variety was characterized by an average protein content of 25.5%, in the Lara variety, the amount of protein was in the range of 24.9–25.3%, providing an average value of 25.2%. The Zolotoniski 15 and Glyana varieties had the same content, which did not exceed 29.9%. Thus, the cultivar factor is the most effective and should be taken into account when selecting cultivars for cultivation. The "phenol" variety contains 24.8% protein (Callaway, 2004). On average, over the years of research, the protein content of variants grown according to conventional technology was 25.2%, and according to transitional technology, it was 0.03% higher, which was within the limits of statistical error.

Organic technology provided protein content at the level of 25.3%, and the use of a destructor contributed to further growth of the indicator to 25.4% (Figure 1). From an economic point of view, the difference between varieties and variants of cultivation technology did not have too much influence on the formation of protein content, thus all varieties are suitable for cultivation

Table 1. Oil content in seeds depending on growing conditions (factor A), varietal characteristics and growing technology

Varieties (factor B)	Technology (factor C)	Oil content, %			
		2019	2020	2021	Average
Glyana	Conventional	29.12	29.18	29.17	29.16
	Transition*	28.94	29.16	29.20	29.10
	Organic	29.03	29.17	29.19	29.13
	Organic + BioStymix	29.07	29.14	29.18	29.13
Zolotoniski 15	Conventional	29.17	29.21	29.21	29.20
	Transition	29.19	29.25	29.23	29.22
	Organic	29.18	29.22	29.22	29.21
	Organic + BioStymix	29.19	29.22	29.24	29.22
Lara	Conventional	29.71	29.72	29.72	29.72
	Transition	29.73	29.71	29.69	29.71
	Organic	29.70	29.71	29.71	29.71
	Organic + BioStymix	29.70	29.73	29.71	29.71
Globa	Conventional	29.73	29.78	29.78	29.76
	Transition	29.75	29.78	29.77	29.77
	Organic	29.72	29.77	29.76	29.75
	Organic + BioStymix	29.73	29.76	29.70	29.73
Sula	Conventional	29.38	29.37	29.39	29.38
	Transition	29.36	29.36	29.39	29.37
	Organic	29.37	29.38	29.40	29.38
	Organic + BioStymix	29.36	29.39	29.41	29.39
LSD_{05} (factor *A*)					0.01
LSD_{05} (factor *B*)					0.01
LSD_{05} (*AB*)					0.02
LSD_{05} (*BC*)					0.02
LSD_{05} (*ABC*)					0.03

Note: * Transition from conventional to organic.

Table 2. Protein content in seeds depending on growing conditions (factor A), varietal characteristics and growing technolog

Varieties (factor B)	Technology (factor C)	Protein content, %			
		2019	2020	2021	Average
Glyana	Conventional	24.9	24.8	24.9	24.87
	Transition	25.0	24.9	25.0	24.97
	Organic	25.0	24.8	25.0	24.93
	Organic + BioStymix	25.0	24.9	25.1	25.00
Zolotoniski 15	Conventional	24.9	25	25.1	25.00
	Transition	24.6	24.8	25.0	24.80
	Organic	24.8	24.8	25.2	24.93
	Organic + BioStymix	24.8	25	25.3	25.03
Lara	Conventional	24.9	25.1	25.1	25.03
	Transition	25.0	25.2	25.2	25.13
	Organic	25.2	25.3	25.2	25.23
	Organic + BioStymix	25.2	25.3	25.3	25.27
Globa	Conventional	25.4	25.4	25.3	25.37
	Transition	25.5	25.4	25.4	25.43
	Organic	25.6	25.5	25.5	25.53
	Organic + BioStymix	25.7	25.6	25.5	25.60
Sula	Conventional	25.9	25.8	25.6	25.77
	Transition	26	25.9	25.7	25.87
	Organic	26	25.9	25.7	25.87
	Organic + BioStymix	26.1	26.0	25.7	25.93
LSD_{05} (factor *B*)					0.06
LSD_{05} (factor *C*)					0.05
LSD_{05} (*AB*)					0.10
LSD_{05} (*BC*)					0.11

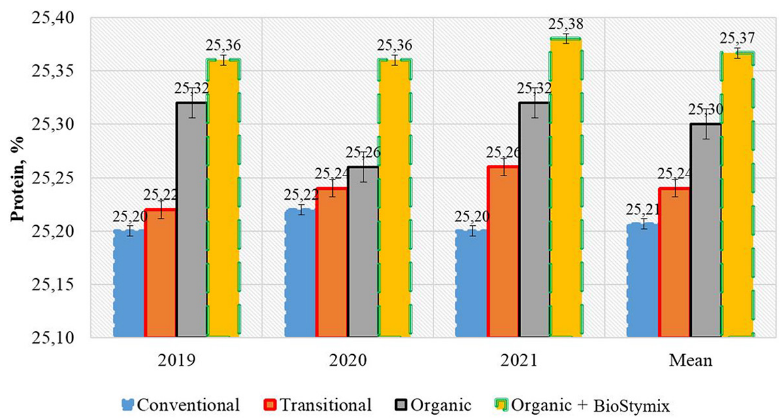

Figure 1. Protein content in seeds depending on the cultivation technology

according to organic technologies. The observed regularity, however, should be taken into account for the selection of the variety, evaluating it also from the point of view of other economic and valuable features. To solve this issue, it was necessary to conduct a correlation analysis of the protein content with other economic value indicators. Varietal properties significantly influenced the formation of dependencies; for example, in the Glyana variety, no correlations that would limit the accumulation of its content in seeds were observed – all of them were within the range of

0.29–0.42. In the Zolotoniski 15 variety, an inverse correlation was observed with plant height, and in the Lara variety with fiber content, the correlation coefficients were –0.32 – –0.30. Thus, these varieties can fulfill a dual purpose, but in case of a change of some factor, it is quite possible, as well as weather conditions, that the problem of loss of quality will arise in one of the areas of use. The Sula variety, which had the best yield and seed quality indicators, was also characterized by inverse correlations with plant height, oil content, and fiber yield (*r* = –0.29 – –0.66). However, in the general array of data of the experiment, these reverse correlations dissolved and were actually absent. The close correlation of the protein content with the yield of the hemp stems turned out to be significant – *r* = 0.70, which, in authors' opinion, indicates that up to a certain value of the yield or protein content, there may be a linear relationship between them, which can be transformed into some other.

The obtained research results allow stating that regardless of the biometric aspects of cultivation and the existence of interdependencies between them, organic cultivation technologies do not limit the protein content, but on the contrary, contribute to its increase. Therefore, in organic production, the food direction of growing seed hemp also has significant, if not primary, prospects. As it can be seen from the above-mentioned results, the oil content of hemp seeds is not limited by other important characteristics, such as the hemp stems yield or the fiber content. Only the Glyana variety showed inverse correlations with plant height, hemp stems and seed productivity, they were of medium strength (*r* = –0.60 – –0.43). In the Zolotoniski 15 variety, only one inverse relationship was recorded, i.e. plant height (*r* = –0.57). In the Globa variety, such a correlation was observed with seed yield. It is quite possible that the large number of correlations in the Glyana variety is the reason for its lower yield and somewhat lower quality due to certain limitations of some factors by others.

In general, according to the results of multiple regression analysis, the equation for the formation of oil content can be written in the form presented in Figure 2, which shows the actual and possible dependence of oil content on protein content and seed yield. Thus, the equation of dependence between these indicators will be written by equations shown in Figure 2.

According to the obtained equations, the oil content was inversely correlated with seed yield and directly correlated with protein content. Modeling by the method of nonlinear multiple estimation showed that the highest protein content in hemp seeds can be formed at a seed yield of 0.54–0.60 t/ha. In this case, it is also possible to achieve the highest protein content at the level of 26.5–27.0% under the conditions of organic cultivation with unstable moisture.

The protein content also depended on the yield of seeds, but in addition, this indicator was also influenced by the yield of the crop (Figure 3). A direct relationship was recorded between these traits, but the regression model of non-linear assessment of dependence allowed establishing the possibility of the existence of a feedback relationship with the protein content in the protein system

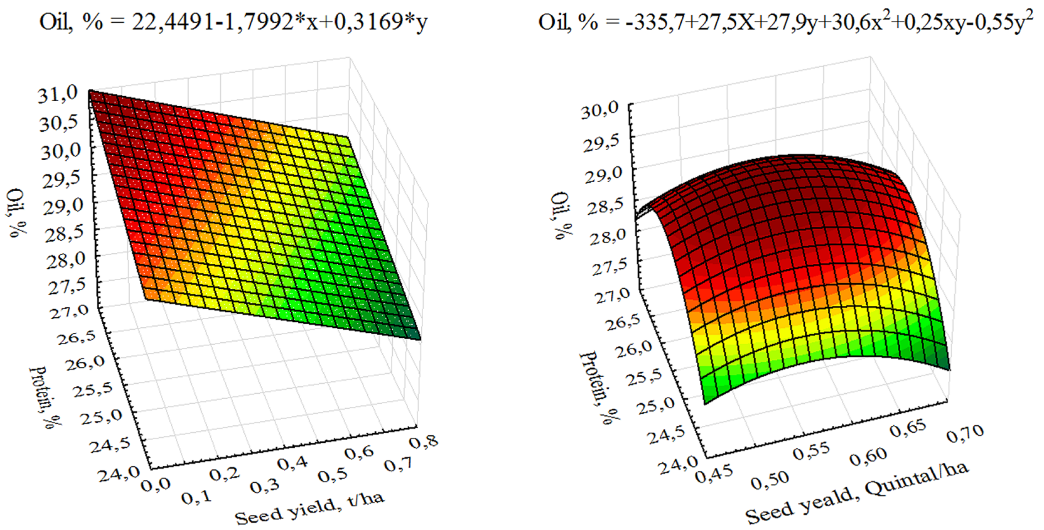

Figure 2. Linear and non-linear model of dependence of oil content on protein content and seed yield

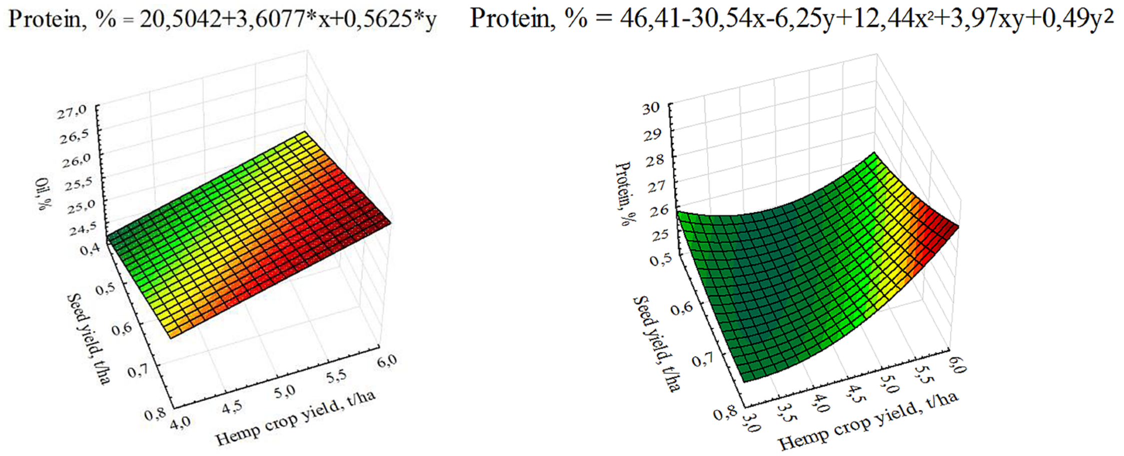

Figure 3. Linear and non-linear model of dependence of protein content on seed yield and crop yield

– seed yield – hemp stems yield. However, this does not exclude the possibility of obtaining the maximum protein content in combination with the maximum yield of hemp stems and seeds.

The given dependencies indicate the absence of a connection between the quality indicators of hemp within the values obtained over the years of the experiment. Similarly to yield properties, qualitative indicators do not deteriorate under the condition of growing hemp according to organic technologies. The obtained results indicate the prospect of managing hemp production processes to achieve maximum performance.

In order to effectively manage the economic and valuable features of hemp agrocenoses, it is necessary to carefully analyze the system of mutually limiting relationships, which may carry the risk of yield failure or deterioration of product quality. Such relationships can be detected using correlation analysis. In production, the use of such a method of evaluating varieties will obviously encounter certain problems, because for objective evaluation it is necessary to conduct accurate observations and records, to have appropriate laboratory and software equipment, as well as trained and highly qualified personnel. Therefore, such a task should be set in the originating institutions.

No correlation was established between protein content and oil content in seeds. The studied varieties were characterized by a different number of correlations (Table 3). The variety Glyana had inverse relationships of fiber content in stalks and oil content in seeds with plant height, hemp stems and seed yield, and the values of correlation coefficients were close to defining these inverse relationships as strong. The protein content in the seeds, on the contrary, had a weak or medium relationship with the indicated indicators. Thus, this variety is better suited for cultivation for purposes related to the use of specific compounds, i.e. proteins.

Similar features of the manifestation of correlations were also observed in the Globa variety; the correlation coefficients showed the average inverse relationship of the oil content with the height of the plants, yield of hemp stems, fiber and seeds. In the Zolotoniski 15 variety, the height of the plants during the harvesting period turned out to be a limiting factor for the oil and protein content; the correlation coefficients were –0.57 and –0.30, respectively. Thus, this variety may be better used for cultivation for technical purposes, to obtain fiber.

The Lara variety was characterized by the smallest number of correlations between economic and valuable traits; only the oil content had an average correlation with the yield of the hemp stems - r = –0.32. This property of the variety indicates its prospects for dual purpose cultivation. The system of correlations in the Sula variety was somewhat different. Fiber content in stems was inversely correlated with length of period to biological maturity and fiber yield. Also, this variety was characterized by the presence of inverse correlations of the protein content with the rest of the economic value characteristics, while the oil content was directly correlated with them.

It should be noted that the correlations may change depending on other factors of cultivation, under particular weather conditions, elements of technology, etc., but the evaluation of varieties for cultivation according to these characteristics can significantly increase the efficiency of hemp production. Therefore, modeling the processes of forming indicators of product quality and yield

Table 3. Correlations of economic and value characteristics depending on the variety

Varieties	Industrial indicators of the plant	Fiber content in green leafy stems, %	Oil, %	Protein, %
Glyana	The height of plants in the period of biological maturity, cm	-0.67	-0.61	0.29
	Duration to biological maturity, days			0.42
	The height of the plants during the harvesting period, cm	-0.61	-0.46	0.29
	Yield of the hemp stems, t/ha	-0.60	-0.55	
	Seed yield, t/ha	-0.73	-0.61	
	Fiber yield, t/ha	0.49	0.48	0.34
Zolotoniski 15	The height of plants in the period of biological maturity, cm		-0.38	
	Duration to biological maturity, days	0.40		
	The height of the plants during the harvesting period,cm		-0.57	-0.30
	Fiber yield, t/ha	0.97		0.44
Lara	Duration to biological maturity, days	-0.32		
	The height of the plants during the harvesting period, cm	0.51		
	Yield of the hemp stems, t/ha		0.37	
Globa	The height of plants in the period of biological maturity, cm	0.43	-0.49	
	Yield of the hemp stems, t/ha			0.33
	Seed yield, t/ha		-0.46	0.38
	Fiber yield, t/ha	0.56	-0.47	
Sula	The height of plants in the period of biological maturity, cm		0.34	-0.44
	Duration to biological maturity, days	-0.43	0.38	-0.29
	The height of the plants during the harvesting period, cm	0.74		0.30
	Fiber yield, t/ha	-0.56	0.44	-0.66

properties is a very relevant issue. As noted by (Tang et al., 2017) modeling methods are promising for the cultivation of dual-purpose hemp. It should be added that hemp has not been of interest for intensive study for almost half a century, and if there is a sufficient amount of information on the influence of environmental factors on the formation of productivity, then it is almost absent on the influence on seed quality (Tang et al. 2016; Amaducci et al., 2015). One of the most important components of modeling is the suitability of varieties for growing for specific purposes under specific conditions. According to (Baldini et al., 2020; Carus et al., 2013), the first models of hemp crops were implemented more than 20 years ago, and later studies considered only the phenology of the crop.

The conducted research largely compensates for the lack or low prevalence of the mentioned information and is aimed at the multi-purpose use of hemp, which is now gaining special relevance (Baldini et al., 2020). The established features of correlation relationships to a large extent specify the selection of varieties for growing under organic production conditions. As it was noted, (Carus et al., 2013; Tang et al., 2016) the combination of dual use of hemp for fiber and seeds, as a rule, leads to the formation of uneven short stems; thus, there is a possibility of the existence of inverse correlations. In the conducted research, such correlations characterized the varieties Zolotoniski 15, Glyana and Globa; the coefficients of oil content with plant height were in the range of –0.61 – –0.38. Lara and Sula varieties did not have such connections, which indicates their better suitability for dual use and growing according to organic technologies.

CONCLUSIONS

The oil content in the seeds is mainly influenced by varietal properties and conditions of the year of cultivation. The choice of cultivation technology did not affect the formation of this indicator; however, an additive effect was observed with other factors: the variety and conditions of the year. Growing hemp using organic technologies contributed to a significant increase in the protein content of seeds. Compared to inorganic cultivation options, organic technology provided protein content at the level of 25.3%, and the use of a destructor contributed to the further growth of this indicator to 25.4%. Each variety had its own characteristics of correlations of this indicator with others, which proves the need for careful selection of varieties for cultivation. To select the varieties for growing for different purposes, it is advisable to use correlation analysis, since they can be characterized by a different number, strength and direction of connections. The definition of such a system can make it possible to more effectively use the potential of agrocenoses of culture.

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