

## RECENT PROGRESS IN MONOECIOUS HEMP VARIETY FOR SEED, OBTAINED IN ROMANIA

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### ABSTRACT

Industrial hemp (*Cannabis sativa* L.) is a multifunctional crop that can be processed to obtain many different products like food, feed, cosmetics and pharmaceuticals, recyclable and compostable biomaterials, energy, while having a positive effect on the environment. As the world market for industrial hemp seeds and oil is on rise, research has been carried out in the climatic conditions of Central Moldova in order to obtain a new genotype of monoecious hemp in accordance with the requirements of the European market and beyond. Olivia is a new monoecious hemp variety for seed, created at Agricultural Research and Development Station Secuieni Neamț, being homologated in 2020. It was patented in 2021 and is registered in the Official European Catalog of Varieties. It was obtained by repeated hybridization (dioecious form Chi65 - female partner and monoecious form Z59 - male partner), isolation in space and repeated selection. This new variety is characterized by stems with a length of 2.6-3.2 m. The yield is of 9.5-11.0 t·ha<sup>-1</sup> of stems and 1600-2000 kg·ha<sup>-1</sup> of seed.

**Keywords:** monoecious hemp, new variety, repeated hybridization, selection, yield.

### INTRODUCTION

*Cannabis sativa* L. is a species cultivated for its fiber and seeds (var. *sativa*) or for its high content in cannabinoids (var. *sativa* and var. *indica*) (Soler et al., 2017).

Although *Cannabis* is usually dioecious, hermaphroditism occurs in some cultivars and the breeding programs have resulted in some monoecious cultivars, primarily restricted to hemp (Spitzer-Rimon et al., 2019).

According to the sexual composition, monoecious hemp represents a complex population comprising up to 20 different types of plants. The ratio between female and male flowers is very varied, including a wide range of intersex (Stepanov, 1974, cited by Șandru et al., 1996).

Due to the heterozygosity character, after acquiring the sex, in monoecious hemp the phenomenon of permanent segregation of ordinary male plants is manifested. Their presence in the hemp population leads to the loss of monoicity, because under conditions of free pollination, the female flowers of monoecious hemp plants prefer for fertilization the pollen from ordinary male plants, which leads to the return to dioecious hemp (Găucă, 1995; Meijer, 1995).

In hemp, sex is determined by the whole genotype (Grisko, 1937, cited by Șandru et al., 1996). Thus, the mechanism of sex determination depends not only on the distribution of the sex chromosomes during the reduction division, but also on the action

exerted by the whole genome on the sex cells that unite in the process of fecundation.

The existence of both tendencies in the same plant, explains both the appearance of different degrees of monoicity and the great diversity of intersex (Ceapoiu, 1958; Șandru et al., 1996). Following the numerous crosses between normal hemp and intersexed forms, but also from the intersection of intersexes between them, Sengbusch (1952) established that a series of alleles participate in determining the sex, which depending on the amount in which they are found determines sex. Thus, the appearance of monoecious hemp forms depends on an unlocated polyallel gene, which results from a mutation that occurs in a dioecious female plant (Găucă and Paraschivoiu, 1991; Găucă and Berea, 1997).

Hemp varieties have high ecological plasticity, which is determined by the richness of the varieties, in biotypes with various physiological characteristics, by the hybrid nature of the varieties and by the plasticity of the various characters and characteristics (Ceapoiu, 1958).

Some morphological and anatomical features, as well as some biochemical and physico-mechanical properties can change depending on the agrotechnical factors, without affecting their heredity.

Therefore, in the breeding processes, it is especially important to know how to transmit the different characters and features.

Currently, the cultivation of hemp varieties with multifunctional capacity, both for seed production and for obtaining stems and fiber, has opened new challenges in many research sectors such as breeding (Baldini et al., 2018).

The monoecious hemp breeding program from ARDS Secuieni has had as a priority the increase of the yielding capacity associated with the reduction of the THC content (Popa et al., 2019). Thus, the purpose of this paper is to present a new monoecious hemp variety, obtained at ARDS Secuieni, from a morphological, physiological and agroproductive point of view.

## MATERIAL AND METHODS

The breeding method of the Olivia monoecious hemp variety consisted in repeated hybridization processes, isolation in space and repeated individual selection. The parents of this variety were represented by the monoecious form Z59 as male partner and the dioecious form Chi65 as female partner, which were chosen from the genetic resources of the monoecious hemp breeding laboratory of the ARDS Secuieni (Popa et al., 2021). Following the process of cross-breeding and selection, a new genotype of monoecious hemp was obtained, intended for seed processing and not only, different from the morpho-physiological and agroproductive point of view from the other creations of SCDA Secuieni and which is in according with the requirements of the world market.

For the characterization of the Olivia variety, we used the experimental results regarding the seed and stem production, the total and the technical length of the stem and the thousand kernel weight of the seeds. Those results were obtained in the centers of the State Institute for Varieties Testing and Registration (SIVTR) in the period 2018-2019, but also at ARDS Secuieni Neamț, in the same period of time. Also, analyzes were performed on the profile of the main psycho- and non-psychoactive compounds (THC, CBD, CBDA, CBDT) of the new genotype. The results were compared with those of the Zenit monoecious hemp variety, the genotype used as a control in the tests performed.

## RESULTS AND DISCUSSION

### Morphophysiological characters

The length of the plant in the crop is between 2.6 and 3.2 m. The stem is green-yellowish and has a number of 4-6 longitudinal striations (Figure 1). The leaf is palmate-fidate, medium pubescent, consisting of 9-11 leaflets, and the petiole has a reddish tinge. The inflorescence

is a scorpioid top, with the male flowers arranged at the base, and the color of the flowers at 10 days after emergence is white. The fruit is an ovoid nut with light gray mosaic (Figure 2).

The vegetation period varies between 100-110 days in the fiber culture, and in the seed culture between 130-140 days, being a late variety (Figure 3).

The flowering period is 20-25 days.



Figure 1. Olivia monoecious hemp variety



Figure 2. Seeds at the Olivia monoecious hemp variety



Figure 3. Appearance of the crop during the vegetation period

### Production capacity

In yield trials carried out during 2018-2019 period in the SIVTR network and 2018-2019 period at ARDS Secuieni, in a number of experiments with a high variability in environmental conditions, the Olivia variety achieved higher yields than the Zenit control.

Analyzing the seed production presented in Table 1, by test centers and by years, it was observed that the hemp variety Olivia showed higher yields than the control variety Zenit in all the test centers, except the centers from Sibiu

This variety is resistant to low spring temperatures, heat, fall, fusariosis and broomrape.

The delta9-tetrahydrocannabinol (THC) content is, in average, 0.019% (2018-2020), according to the analysis bulletins issued by the Regional Laboratory for Quality Control and Wine Hygiene Blaj.

Due to its high ecological plasticity, it is suitable for cultivation in areas with different eco-pedo-climatic conditions.

and Luduș, in 2018, where it achieved 97% and, respectively, 88% of the yield of the control variety and of the centers from Sibiu and Negrești, in 2019, where it achieved 93% and, respectively, 96%. The yield of the Olivia variety varied between 917 kg·ha<sup>-1</sup> in Luduș in 2018 and 2305 kg·ha<sup>-1</sup> in Șimleu Silvaniei in 2019. On average, over the two years of testing, the monoecious hemp variety Olivia recorded yields between 1232 kg·ha<sup>-1</sup> (Luduș) and 2109 kg·ha<sup>-1</sup> (Șimleu Silvaniei).

Table 1. Seed yield of the monoecious hemp varieties tested in SIVTR network

Test centre	Variety	Seed yield				Average (2018-2019)	
		2018		2019			
		kg·ha <sup>-1</sup>	%	kg·ha <sup>-1</sup>	%	kg·ha <sup>-1</sup>	%
Șimleu Silvaniei	Zenit (control)	1442	100	1695	100	1569	100
	Olivia	1913	133	2305	136	2109	135
Average		1678		2000		1839	
Sibiu	Zenit (control)	1891	100	2152	100	2022	100
	Olivia	1828	97	1990	93	1909	95
Average		1860		2071		1966	
Satu Mare	Zenit (control)	1920	100	1694	100	1807	100
	Olivia	2069	108	1938	114	2004	111
Average		1995		1816		1906	
Negrești	Zenit (control)	1200	100	1533	100	1367	100
	Olivia	1282	107	1469	96	1376	102
Average		1241		1501		1372	
Luduș	Zenit (control)	1037	100	1344	100	1191	100
	Olivia	917	88	1546	115	1232	103
Average		977		1445		1212	
Average	Zenit (control)	1498	100	1684	100	1591	100
	Olivia	1601	107	1850	110	1726	108
Average		1550		1767		1659	

Regarding the thousand kernel weight (TKW), it was observed that the Olivia hemp variety showed higher values than the Zenit control variety in all test centers (Table 2). Thus, in 2018, the TKW for the Olivia variety had values between 22 g (Sibiu, Luduș) and 26 g (Satu Mare), and in 2019, the TKW

values for the Olivia variety varied between 19 g (Negrești) and 23 g (Satu Mare, Luduș). On average over the two years of testing, the Olivia monoecious hemp variety recorded TKW values between 21 g in the testing center in Sibiu and 25 g in the center from Satu Mare.

Table 2. The thousand kernel weight (TKW) of the monoecious hemp varieties tested in SIVTR network

Test centre	Variety	TKW				Average (2018-2019)	
		2018		2019			
		g	%	g	%	g	%
Șimleu Silvaniei	Zenit (control)	18	100	18	100	18	100
	Olivia	24	133	22	122	23	128
Average		21		20		21	
Sibiu	Zenit (control)	18	100	18	100	18	100
	Olivia	22	122	20	111	21	117
Average		20		19		20	
Satu Mare	Zenit (control)	17	100	19	100	18	100
	Olivia	26	153	23	121	25	139
Average		22		21		22	
Negrești	Zenit (control)	15	100	16	100	16	100
	Olivia	24	160	19	119	22	138
Average		20		18		19	
Luduș	Zenit (control)	16	100	16	100	16	100
	Olivia	22	138	23	144	23	144
Average		19		20		20	
Average	Zenit (control)	17	100	17	100	17	100
	Olivia	24	141	21	124	23	135
Average		21		19		20	

The vegetation period of the hemp varieties tested in the SIVTR network varied according on the climatic conditions of the year and the test center. Thus, in 2018, the vegetation period of the Olivia monoecious hemp variety varied between 126 days in Luduș and 158 days in Satu Mare, and in 2019

between 119 days in Luduș and 155 days in Negrești (Table 3).

On average over the two years of testing, the vegetation period of the Olivia variety had values between 123 days in the testing center in Luduș and 156 days in the one in Satu Mare.

Table 3. The vegetation period of the monoecious hemp varieties tested in SIVTR network

Test centre	Variety	Vegetation period (days)		Average (2018-2019)
		2018	2019	
		Days	Days	Days
Șimleu Silvaniei	Zenit (control)	139	147	143
	Olivia	141	149	145
Average		140	148	144
Sibiu	Zenit (control)	137	140	139
	Olivia	136	141	139
Average		137	141	139
Satu Mare	Zenit (control)	127	154	141
	Olivia	158	154	156
Average		143	154	149
Negrești	Zenit (control)	105	155	130
	Olivia	136	155	146
Average		121	155	138
Luduș	Zenit (control)	125	118	122
	Olivia	126	119	123
Average		126	119	123
Average	Zenit (control)	127	143	135
	Olivia	139	144	142
Average		133	144	139

During the experimentation period, the performed tests showed higher seed yields obtained by Olivia than the control variety, in all experimental variants. The seed production, for the Olivia variety, had values between 2535 kg·ha<sup>-1</sup> in 2019 and 2730 kg·ha<sup>-1</sup> in 2018.

On average over the two years of testing, the Olivia monoecious hemp variety had recorded a production of 2633 kg·ha<sup>-1</sup>, with production increase of 12% compared to the control (Table 4).

Table 4. Seed yield of the Olivia variety tested at ARDS Secuieni

Distance between rows (cm)	Variety	Seed yield				Average (2018-2019)	
		2018		2019		kg·ha <sup>-1</sup>	%
		kg·ha <sup>-1</sup>	%	kg·ha <sup>-1</sup>	%		
12.5	Zenit (control)	2530	100	2174	100	2352	100
	Olivia	2730	108	2535	117	2633	112
Average		2630		2355		2493	

Regarding the stem yield, it varied for the Olivia variety between 10810 kg·ha<sup>-1</sup> (2019) and 11270 kg·ha<sup>-1</sup> (2018). On average over the two years of experimentation, the Olivia variety

recorded a strain yield of 11040 kg·ha<sup>-1</sup>, with a production increase of 29% compared to the control (Table 5).

Table 5. Stem production of the Olivia variety tested at ARDS Secuieni

Distance between rows (cm)	Variety	Stem production				Average (2018-2019)	
		2018		2019			
		kg·ha <sup>-1</sup>	%	kg·ha <sup>-1</sup>	%	kg·ha <sup>-1</sup>	%
12,5	Zenit (control)	8500	100	8710	100	8605	100
	Olivia	11270	133	10810	124	11040	129
Average		9885		9760		9823	

On average over the two years of testing, the Olivia variety showed a total stem length superior to that of the control variety, of

310 cm and respectively 320 cm, by approximately 19% more than the control variety Zenit (Table 6).

Table 6. The stems total length of the Olivia variety tested at ARDS Secuieni

Distance between rows (cm)	Variety	Total length (cm)				Average (2018-2019)	
		2018		2019			
		cm	%	cm	%	cm	%
12.5	Zenit (control)	260	100	270	100	265	100
	Olivia	310	119	320	119	315	119
Average		285		295		290	

Regarding the technical length, it varied for the Olivia variety between 170 cm (2018) and 180 cm (2019). On average over the two

years of experimentation, the Olivia variety recorded a technical stem length of 175 cm (Table 7).

Table 7. The stems technical length of the Olivia variety tested at ARDS Secuieni

Distance between rows (cm)	Variety	Technical length (cm)				Average (2018-2019)	
		2018		2019			
		cm	%	cm	%	cm	%
12.5	Zenit (control)	120	100	130	100	125	100
	Olivia	170	142	180	138	175	140
Average		145		155		150	

Regarding the TKW, it was observed that the Olivia hemp variety showed higher values than the Zenit control variety in all testing years (Table 8). Thus, in 2018, the thousand kernel weight at the Olivia variety had a

value of 24.85 g, and in 2019, the value of TKW for the Olivia variety was of 26.10 g. On average over the two years Olivia monoecious hemp variety recorded a TKW value of 25.48 g.

Table 8. The TKW of the Olivia variety tested at ARDS Secuieni

Variety	TKW				Average (2018-2019)	
	2018		2019			
	g	%	g	%	g	%
Zenit (control)	20.04	100	19.90	100	19.97	100
Olivia	24.85	124	26.10	131	25.48	128
Average	22.45		23.00		22.73	

About 113 different cannabinoids can be found in hemp, but the most important and researched are CBD and THC.

CBD has the widest therapeutic application, being currently the most studied phytocannabinoid. Unlike THC, CBD has no psychoactive effect, which is why it is legal in many countries around the world. Currently, it is used in the treatment of many diseases, being used as an anticonvulsant, immunostimulant, antioxidant, antidepressant, anti-psychotic, anti-inflammatory, antitumor, anxiolytic, anti-insomnia.

Although some genetic markers and loci of quantitative traits that determine sexual expression in hemp have been developed, more researches regarding sex-related traits

and QTL mapping are important for achieving the goals of cannabinoid development and regulation (Schlutenhofer and Yuan, 2017).

In terms of cannabinoid content, testing of the Olivia variety and other ARDS Secuieni monoecious hemp varieties was conducted within the "Extremadura" National Agricultural Technological Center. The results of the analyses are presented in Table 9.

Analyzing the results, we can see that the Olivia hemp variety has a higher cannabinoid content compared to the Zenit control variety. Thus, for the Olivia variety, the CBD content was of 0.06%, the CBDA of 0.24% and the CBDT of 0.27%, compared to 0.02% CBD, 0.10% CBDA and 0.10% CBDT as recorded in the control variety.

Table 9. Cannabinoid content (%) at monoecious hemp varieties created at ARDS Secuieni

No	Variety	Cannabidiol (CBD)	Acid cannabidiol (CBDA)	Total cannabidiol (CBDT)
1.	Zenit	0.02	0.10	0.10
2.	Olivia	0.06	0.24	0.27

Regarding the THC content, the testing of the Olivia variety and other monoecious hemp varieties created at ARDS Secuieni, was carried out within the Regional Laboratory for Quality Control and Wine Hygiene Valea Călugărească - Blaj subsidiary. The results of the analyses are presented in Table 10.

After analyzing the results, we can see that no sample contained more than 0.2% THC. In all samples the THC values are below 0.03%, which makes the varieties created at ARDS Secuieni to be particularly valuable and recommends them for cultivation in other European countries.

Table 10. The THC content of the monoecious hemp varieties created at ARDS Secuieni

No.	Variety	Quality test	THC content (%)			
			2018	2019	2020	Average (2018-2020)
1.	Zenit	Leaves + inflorescence peaks	0.028	0.016	0.016	0.020
2.	Secuieni - Jubileu	Leaves + inflorescence peaks	0.015	0.017	0.018	0.017
3.	Olivia	Leaves + inflorescence peaks	0.021	0.017	0.018	0.019

## CONCLUSIONS

A new variety of monoecious hemp for seeds 'Olivia' was created at ARDS Secuieni. It has been included in the Official European Catalog of Varieties and is intended for marketing. The new genotype can be characterized as a late variety, with high seed yields and a seed weight (thousand kernel weight) being the highest in the varietal

conveyor existing on the European market. Moreover, the new variety is richer in CBD, CBDA and CBDT, compared with the other ARDS Secuieni varieties, while the THC content is within the limits of European legislation.

It is recommended to cultivate the new variety to obtain seed for processing, in order to get husked seed, oil and auxiliary products intended for consumption.

It is recommended to be cultivated in main cultivation system, with the “Secuieni Method” application, in order to facilitate the harvesting with the cereal combine directly from the field.

Highly favorable areas include central and northern Moldova, central and northern Transylvania, Banat, but the new variety is suitable for cultivation in other culture areas from Romania and Europe.

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