OBTAINING NEW MONOECIOUS HEMP CULTIVARS AT SECUIENI AGRICULTURAL RESEARCH STATION

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Because the new trend in hemp domain is obtaining specialized varieties in fibre or seed production, S.C.D.A. Secuieni propose to create a new monoecious hemp variety small in size, precocious and with a higher seed production. The new variety was obtained by cross breeding the dioecious line F7-3FIN314 as female partner with the monoecious line Z11-4 SCM44 as male partner, followed by two backcrosses for the monoecious character to be stabilized. The F_0 generation was characterized by the small Size of F7-3FIN314 line and a higher seed production. After the two backcrosses the size of the plants stabilized at 200-250 cm, the average plant production is 81 g and the THC content is under 0,06%. The new line is process of testing at I.S.T.I.S. Romania.

***Key words**: hemp, monoecious, production, seed, cross breeding 5*

Hemp cultivars (*Cannabis sativa L. Ssp culta*) are formed by dioecious varieties, monoecious varieties and unisexual female hybrids. Those may be specialized for seed production or fiber production (Finland) or used for both tipes of production (Romania, France).

After 1989 in Est Europe countries hemp crops were reduced in surface, but it's importance grew in West Europe, Canada, Austria, were it was banned after 1940, being considered a drug. Hemp was accepted in this countries as long as the THC level will not exceed 0.3% wich is the international standard, wile in EU the maximum limit is 0.2% THC. World wide there are concerns regarding the improvement of fiber and seed production, decrease of THC level but also for legalization of drug hemp for medicinal purposes.

"Le Mans Institute" in France created monoecious hemp cultivars with a level of THC under 0.001%.

In Russia " Vavilov Institute of Plant Industry" has in view a low THC level and increase of the fiber content (over 34%) as well as obtaining high leveled THC cultivars(15- 20%) for medicinal use.

The same concerns exist in Germany at the "Humboldt University of Berlin" and USA through " Hemp Industries Association"

In Romania there are two research stations that work in the hemp domain : SCDA Secuieni- Neamt with activities in creating and exploiting monoecious hemp cultivars and SCDA Lovrin which deals with dioecious hemp cultivars.

Because the varieties created in our country are mixed varieties SCDA Secuieni wanted to create a new monoecious hemp variety specialized in seed production (1500-2000 kg/ha) characterized by a low level of THC (under 0.1%), precocious, small in size (1.5-1.8m) and suitable to mechanized harvest.

MATERIAL AND METHOD

For selecting the parental forms who contributed to the creation of the new variety analyses, qualitative and quantitative determinations were done over 42 lines and sources. Two lines were selected, a monoecious one used as male partner and a dioecious one used as female partner.

The cross breeding field was isolated from other hemp crops at a distance of minimum 5 km, having an aria of 300 m^2 . The lines were seeded alternatively, four rows of dioecious line (female partner) with two rows of monoecious line (pollinator).

The selection was made in conformity with the proposed objectives, after field and laboratory analysis were done.

RESULTS AND DISCUSSIONS

The study of physiological and morphological characteristics of some hemp lines from the existing collection of SCDA Secuieni for creating the initial material.

For selecting the parental forms which contributed to the creation of the new genotype analyses, qualitative and quantitative determinations were done over 42 lines and sources.

For the harvested elites from each line the following test were done:

- Total stem length, technical length, number of opposite nodes, number of tem striation, total stem weight, fiber weight determined by biometrical measurement and weighing. The fiber content was chemical determined with the modified Bredemann method, witch consist in analyzing the probes after 24 hours in the drying closet at 105°C , and then weighing. The obtained weigh represents the dry mass. After, the probes were boiled in sodium hydroxide (Na OH) 1,5% for 2-4 hours and rinsed with water. The fibers were easily separated under this conditions, well rinsed and dried in the drying closet for dry mass determination and fiber content.
- 1000 seeds mass and plant seed production.
- THC level determination was done with the fast colorimetric method. This method allows the analysis of hundreds of plants daily, the THC content being evaluated with an interpretation scale standardized initially with the chromatographic method. From the 42 initial lines were selected for the cross breeding field two lines:

- Z11-4 SCM-44 monoecious line characterized by a small size, 120 dais vegetation period, high seed production, low THC level. It was used as male partner.

- F7-3 FIN314, dioecious line characterized by a small stem length (1.8m), 90-100 dais vegetation period, high seed production, low THC level. It was used as female partner.

Cross breeding the selected parental lines for obtaining F_0 generation

The cross breeding field was isolated at a distance of 5 km from other hemp fields. It's area was 300 m^2.

The lines were seeded alternatively 4 rows from the dioecious line F7-3 FIN314, used as female partner, and 2 rows from the monoecious line Z11-4 SCM-44 used as male partner. Seeding was done at 70 cm between rows and 50 cm between plants per row, using 5-6 seeds per hole.

After the plants reached the stage with 3-4 pairs of leaves only 3 plants per hole were left. When the floral buttons appeared the male plants from the dioecious and monoecious hemp rows were eliminated.

The purpose of the purification is to ensure that the female plants from the dioecious variety are pollinated only by the monoecious plants, obtaining in this way the first hybrid generation F_0.

Also the small, non typical plants, attacked by disease or pest were eliminated.

Individual field selection of the female form pollinated by the male partner and of the male form for continuing the breeding process

In the cross breeding field,starting with the forming of floral buttons, elite plants from the female partner and male partner were chosen. In the vegetation period observations and notes were made for all plants from monoecious and dioecious rows, as shown in chart nr.1.

After seed maturity 200 elite plants were harvested from the female rows and 100 plants from the male rows.

After selecting 200 elite plants from the hybrid form F_0 (F7-3 FIN 314 x Z11 4SCM-44) and 100 elite plants from Z11 4SCM-44 line analyses and biometrical measurements were done.

- Total stem length, technical length number of opposite nodes, number of stem striations, stem diameter and total stem weight;
- Fiber extraction was done with the Bredemann method, followed by weighing the fibers and determination of the fiber content for each plant;
- 1000 seeds mass and plant seed production;
- THC level determination.

From the 300 examined plants have been selected to continue the breeding program 20 proveniences from the hybrid form F_0 and 12 from the monoecious line as pollinator. The characteristics of those proveniences are showed in charts 2 and 3. All those proveniences were used to obtain the second hybrid generation, B_1 (backcross 1).

To avoid negative influences, once the floral buttons were differentiated (11-14 June) male plants, small, un characteristic plants, attacked by disease or pest were eliminated.

Table 1

Vegetation period observations and notes

No.	Observations	Dioecious line Female partner	Monoecious line Male partner
1	Stem color	Green	Yellowish green
2	No. of stem striations	3-5	5-7
3	Leaf color	Bluish green	Dark green
4	Nr of leaflets per leaf	5-7	7-9
5	Floral buttons date of appearance for the male flowers	July /01/ 2006	July/10/2006
6	Stigmas date of appearance for the female flowers	July/08/2006	July/15/2006
7	End of blooming	July/20/2006	July/25/2006
8	Inflorescence type	?	?
9	Monoecious type	I and II (female and male plants)	III (typical monoecious plants)
10	Seeds technical maturity	August/15/2006	August/25/2006

In order to maintain a low level of THC, plants were tested "in green", before blooming. All plants that exceeded the limit of 0.1 % THC were eliminated.

During the vegetation period the following notes and observations were made: stem and leaf color, number of stem striations, shape and number of leaflets, monoecity type, date of floral buttons appearance at male plants, date of stigmas appearance at female plants, end of blooming, stem length, inflorescence length, date of seed maturity, and vegetation period.

Following the observation 185 plants from the hybrid form B_1, female partner, and 110 plants from the male form (Z11 4 SCM-44) were chosen to continue the breeding program. The characteristics of the chosen plants are shown in chart 4.

In the preliminary multiplication fields, maturity of seeds happened on August/18 for the hybrid form F_0 and August/23 for the father form, those being seeded later than the back cross field. After the necessary purification and eliminating not valuable plants, 5 kg of seeds from the hybrid form F_0 and 3 kg of seeds from the father form were obtained. Those will be used for testing in the orientation comparative fields.

In the back cross field (B_2), created with the purpose to obtain the third hybrid generation (second back cross), all the necessary observations, determination, and purification were made in the field and laboratory.

The observations and notes are presented in chart number 5.

From the back cross field were selected 135 elite plants. For those plants the following biometrical measurements and analyses were made: plant size, stem color, number of stem striations, number of leaflets per leaf, inflorescence form, seed production per plant, 1000 seeds mass and the THC level. Following the analyses 47 elite plants were chosen. The characteristics of those plants are showed in chart number 6. In the preliminary multiplication fields for the B_1 seeds the biological purifications, THC analyses and eliminations were made. The harvest

maturity had place on August/20/2008. 4 kg of STAS seeds were obtained which will be utilized in comparative fields.

Table 2

Morfophysiological particularytis of F_0 generation

No.	No. elite	Total lenght [m]	Stem diameter [mm]	Total segment weight [g s.u.]	Total fiber weight [g s.u.]	Fiber content [%]	MMB [g]	Seed weight [g]	THC note
1	5	1.33	16.5	50.27	13.17	26.2	20.4	69	1
2	11	1.47	16.2	67.89	16.5	24.3	19.6	70	1
3	23	1.52	15.6	82.31	22.11	26.9	19.2	80	2
4	31	1.44	14.1	46.54	12.86	27.6	20.0	74	3
5	44	1.38	13.5	85.08	21.71	25.5	20.5	69	3
6	47	1.55	15.4	62.92	16.32	25.9	19.4	81	2
7	56	1.46	15.7	77.31	18.62	24.1	21.2	77	2
8	78	1.39	16.1	72.56	18.44	25.4	22.4	66	2
9	80	1.45	14.2	51.37	11.58	22.5	20.9	75	1
10	92	1.53	15.4	57.35	13.53	23.6	20.8	72	2
11	99	1.48	17.3	67.43	18.54	27.5	19.1	83	1
12	106	1.35	14.9	54.16	11.77	21.7	22.5	87	1
13	113	1.39	14.8	95.18	21.18	22.3	19.0	74	1
14	121	1.54	13.2	85.74	22.97	26.8	21.3	78	2
15	154	1.42	12.2	56.74	16.11	28.4	20.4	84	3
16	166	1.51	12.5	54.44	13.45	24.7	19.8	83	1
17	175	1.49	13.7	45.46	10.36	22.8	19.7	89	3
18	180	1.50	14.7	42.63	11.27	26.4	20.6	92	1
19	188	1.36	14.5	46.66	12.24	26.2	21.1	77	1
20	194	1.43	13.6	39.00	9.32	23.9	22.3	86	2

Table 3

Morfophysiological particularytis of Z11 4SCM-44

No.	Elite no.	Total lenght (m)	Stem diameter [m]	Total segments weight [g]	Total fiber weight [g s.u.]	Fiber content [%]	MMB [g]	Seeds weight [g]	THC note
1	210	1.67	13.8	92.64	20.63	22.3	18.5	55	2
2	223	1.70	14.5	126.19	28.97	23.0	19.1	61	2
3	231	1.81	13.9	97.29	21.92	22.5	20.2	69	2
4	246	1.80	13.3	97.07	20.90	21.5	20.5	58	1
5	254	1.76	14.2	96.82	20.23	20.9	19.5	65	3
6	261	1.65	16.6	187.85	33.42	17.8	18.3	70	2
7	266	1.66	12.2	72.89	16.87	23.1	18.9	58	1
8	272	1.74	13.6	119.38	24.05	20.1	19.3	64	1
9	278	1.79	13.4	97.39	22.06	22.7	19.6	67	3
10	281	1.68	15.2	125.17	27.12	21.7	20.7	51	3
11	289	1.73	13.2	98.72	19.72	20.0	21.0	63	2
12	296	1.77	14.9	144.00	30.05	20.9	20.4	71	2

Table 4

Hybrid form plants and male plants morfophysiological particularytis

No.	Character	Notes and observations	
		Female partner B_1 (F_0 XZ11 4SCM-44)	Male partner (Z11 4SCM-44)
1	Stem and leaf color	green	Yellowish green
2	Form and no. Of leaflets/leaf	Lanceolate; 5-7	Lanceolate; 7-9
3	Inflorescence type	Cima scorpioidă semicompact	Cimă scorpioidă compact
4	Monoeicity type	II (female plants)	III (monoecious plants)
5	Floral buttons date of appearance for the male flowers	11-14 june	-
6	Stigmas date of appearance for the female flowers	18-21 june	25-30 june
7	End of blooming	8-10 july	10-12 juliy
8	Plants size(m)	1.30-1.50	1.60-1.80
9	Inflorescence lenght (cm)	40-45	48-55
10	Seed maturity	10 august	14 august
11	Vegetation period (days)	102	107

Table 5

Back cross field observations

No.	Character	Observations	
		Female partner B_1	Male partner Z11 4SCM-44
1	Seeding date	02.05.2008	02.05.2008
2	Data răsăritului	21.05.2008	21.05.2008
3	Stem and leaf color	Green	yellowish green
4	Shape and no. of leaflets	Lanceolate; 5-7	Lanceolate; 7-9
5	Inflorescence type	Cimă scorpioidă, semicompactă	Cimă scorpioidă, compactă
6	Monoeicity type	II (female plants)	III (monoeicious type)
7	Floral buttons date of appearance for the male flowers	-	1-4 july
8	Stigmas date of appearance for the female flowers	7-10 july	5-8 july
9	End of blooming	28-30 july	23-25 july
10	Stem size (m)	1.50-1.80	1.70-2.00
11	Inflorescence type (cm)	50-55	58-65
12	Seed maturity	20.08.2008	25.08.2008
13	Vegetation period (days)	110	115

Table 6

Morfophysiological particularyties of B_2 elite plants

No.	Plant lenght [cm]	Stem color	Strations no.	Leaflets no.	Inflorescente form	Seed production [g]	MMB [g]	THC note.	[%] THC
1	250	VG	6	7	Dense	100	17,7	3	0,06
2	210	VG	7	7	Dense	40	18,3	1	0,00
3	210	VG	7	9	Dense	35	21.2	1	0,00
4	250	VG	5	7	Dense	55	17,3	3	0,06
5	240	VG	6	7	Dense	75	17,6	3	0,06
6	210	VG	6	7	Dense	68	19,7	2	0,01
7	210	VG	6	7	Dense	60	20,3	2	0,01
8	250	VG	7	9	Dense	71	18,3	1	0,00
9	240	VG	5	7	Dense	76	17,6	4	0,15
10	230	VG	6	7	Dense	64	16,4	4	0,15
11	210	VG	6	9	Dense	88	18,5	3	0,06
12	230	VG	6	9	Dense	79	18,7	2	0,01
13	200	VG	6	7	Dense	87	19,0	4	0,15
14	240	VG	7	7	Dense	71	19,9	2	0,01
15	220	VG	7	7	Dense	90	19,4	4	0,15
16	250	VG	5	7	Dense	135	18,9	2	0,01
17	240	VG	6	7	Dense	81	17,0	2	0,01
18	230	VG	6	9	Dense	54	20,1	2	0,01
19	240	VG	7	7	Dense	78	18,7	4	0,15
20	210	VG	5	9	Dense	114	18,8	3	0,06
21	200	VG	6	9	Dense	58	17,2	1	0,00
22	240	VG	6	7	Dense	74	21,4	3	0,06
23	240	VG	7	7	Dense	75	18,7	3	0,06
24	230	VG	7	7	Dense	77	19,8	2	0,01
25	210	VG	5	9	Dense	65	18,9	2	0,01
26	230	VG	7	9	Dense	86	18,4	1	0,00
27	210	VG	6	7	Dense	70	21,5	1	0,00
28	250	VG	7	7	Dense	82	20,5	2	0,01
29	240	VG	7	7	Dense	96	19,3	4	0,15
30	230	VG	6	9	Dense	75	17,3	3	0,06
31	210	VG	7	9	Dense	85	17,5	2	0,01
32	240	VG	5	7	Dense	92	18,7	1	0,00
33	230	VG	7	7	Dense	114	19,8	3	0,06
34	210	VG	7	7	Dense	84	18,7	4	0,15
35	220	VG	5	9	Dense	84	19,3	3	0,06
36	250	VG	6	7	Dense	80	19,7	3	0,06
37	250	VG	7	7	Dense	98	18,0	2	0,01
38	240	VG	7	9	Dense	100	18,2	2	0,01
39	200	VG	7	7	Dense	75	22,2	3	0,06
40	230	VG	5	7	Dense	90	19,2	3	0,06
41	220	VG	7	9	Dense	88	20,2	2	0,01
42	240	VG	7	7	Dense	76	19,3	1	0,00
43	250	VG	5	7	Dense	68	18,4	1	0,00
44	240	VG	7	7	Dense	119	20,6	3	0,06
45	250	VG	6	9	Dense	94	17.4	2	0,01
46	240	VG	7	7	Dense	90	18,1	4	0,15
47	210	VG	5	9	Dense	86	17,0	3	0,06

VG= yellowish green

CONCLUSIONS

1. In the breeding and selection program the THC level was maintained under 0.1%.

2. elite plants from the hybrid B2 form are cultivated in the hybrid fields for 2-3 years for consolidating it's characters, uniformity and stability.

3. In the comparative fields the hybrid form had a production of 1294 kg/ha, with a vegetation period of 102-107 dais.

BIBLIOGRAPHY

1. Sandru, I., D., Paraschivoiu, Rodica, Găucă, Constantin, 1996 – *Hemp culture.* Ed. Helicon Timisoara, p. 41.
2. Găucă, C. , 1995 – *Hemp monoecity –, USAMV Iaşi, Lucrăti ştiinţifice vol. 38, Seria Agronomie*, Ed. "Ion Ionescu de la Brad".
3. Rosenthal, Ed., 1997 – *Hemp today*, Quick American.
4. Săulescu, N.A.,Săulescu, 1967 –*Experimental field* , Ed. Agro-Silvică, Bucureşti.