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NEW VARIETY OF *CANNABIS SATIVA* LYUDMILA

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ABSTRACT

A characteristic of a new variety of monoecious *Cannabis sativa* Lyudmila is presented. Potential selection number is ГП-5/012м, which participated in competitive crop variety testing in 2017-2019 and demonstrated significant superiority over the standard variety regarding the characteristics "male hemp plants content", "straw yield", "fiber total yield", "fiber total harvest", "combed fiber breaking force", "combed fiber flexibility". In 2020, an application was submitted for the inclusion of a selection number in the State Register of selection inventions of the Russian Federation under the name "cannabis sativa variety Lyudmila". A distinctive feature of the variety is the absence of male hemp plants segregation in a crop and the reduced content of tetrahydrocannabinol in plants in relation to already existing varieties of cannabis sativa of Middle Russian ecotype. At present time, the variety is undergoing the State examination and is at the stage of accreditation for approval for use on the territory of the Russian Federation. Based on the introduction of a new selection achievement, it is planned to transfer the scientific results into various regions of hemp sowing of the Russian Federation agro-industry in order to increase the pace of its economic development, increasing the competitiveness of manufactured products and ensuring import substitution.

Keywords: *Cannabis sativa*, non-narcotic monoecious variety, Middle Russia ecotype, tetrahydrocannabinol, economic character, fiber total yield, long fiber yield.

Introduction

In the history of *Cannabis sativa* L. selection, several characteristic stages were noted: the first one is the creation of dioecious varieties with a high fiber content; the second one is the cultivation of highly productive varieties of monoecious hemp; the third one is the selection of yielding varieties without narcotic properties. The result of each stage was new varieties with improved quantitative and qualitative characteristics that met the requirements of processing industry technologies (Virovets *et al*., 1994).

The basis of selective work with the species *Cannabis sativa* L. is the intravariety hybridization and family-group selection according to the complex of economic traits and characters (Senchenko, 1978).

The method of directed hybridization is of paramount importance for hemp because this botanical species has ecotypes that are contrasting in economic characters (northern, Middle Russian and southern), the hybridization between which is quite effective (Senchenko, 1959).

Selection varieties and/or hybrids of domestic and foreign origin, natural populations of foreign area origin (native varieties), less commonly ruderal forms of different geographical zones, selfed lines, artificial mutants, and polyploids are used as the parent material for breeding (Davidyan, 1971).

Depending on objectives and tasks, solved by breeders, different types of hybridization are used. In the hemp selection practice, the most widespread were intervarietal and intravarietal hybrids and hybrids of geographically distant forms. The result is a diverse hybrid material that has great genetic diversity in terms of quantity and quality (Zhatov, 1971; Senchenko, 1977).

The next stage of the cannabis sativa selection process, which follows hybridization, is the evaluation of the characteristics of the offspring obtained from directed breeding, allocation of the best species, creation of the maximally pronounced characteristic homogeneity of the breeding population (Senchenko *et al*., 1980).

Further work with breeding material consists of its reproduction using massive (negative or improving) selection to obtain the original seeds of new varieties (Senchenko, 1971)

The success of activity on creating varieties is critically dependent on the diversity of the source material. The basis for creating new varieties is the material obtained in the previous selection. The main source of genetic diversity for selection is funds of world culture collection. Based on the study of the collection material, the hybridization program is drawn up, obtaining and evaluation of hybrid material, directions of selection inside of hybrid populations, the potential of individual varietal samples as candidates for approval for use in a particular region (Grigoriev *et al*., 2001; Senchenko, 1977).

Systematic breeding of cannabis sativa deployed in our country in 1931, continues to the present day. Selection achievements of domestic breeders made it possible to form

the necessary variety multiplicity, introduce highly productive varieties in the main hemp sowing regions, increase yield and gross harvest of hemp products. (Senchenko *et al*., 1982; Serkov, 2004; Serkov *et al*., 2004; Stepanov *et al*, 2004; Sukhorada, 2009; Sukhorada *et al*, 2010).

However, to this day, the variety of monoecious hemp completely without the male hemp plants in population hasn't been created – it's an undesirable trait, which gradually leads to the reversal of monoecious hemp to dioecious without the use of special high-cost methods (multiple manual cleaning), which make up to 20% of original seeds cost value (Serkov *et al.*, 2019).

The THC content in the plants of hemp selection variety also can be reduced to lower values and, in the future, can be almost completely eliminated from plants by method of directional selection (Senchenko 1976; Rumyantseva, 1989).

Thus, quantitative and qualitative economic traits and characters of hemp plants may be improved by selection methods. Breeding of cannabis sativa forms with the invariable trait of monoeciousness, which doesn't require the resource-consuming techniques in the reproduction of seeds, which possess the THC content reduced to the level of trace value (less than 0.05%), characterizes the significance of this research work.

Research objective. For the successful development of the domestic breeding, it's significant to form the variety multiplicity of cannabis sativa and create the new sample varieties with improved parameters of economic traits in relation to existing varieties for timely varietal change. In this regard, the research objective was the creation of a new variety of non-narcotic monoecious cannabis sativa, adaptive to the agro-ecological conditions of the Middle Volga region and with the high parameters of quality fiber content (more than 30%), invariable trait of monoeciousness during reproduction, and THC content in plants less than 0.1%.

Research tasks

- To allocate the samples with optimal parameters of economic traits and characters following the assessment of selective material (selfed lines I_5-I_6) for the complex of economic traits and characters;
- To conduct breeding between the allocated samples, to obtain the hybrid material of monoecious hemp;
- To study the selection characteristics of hybrid combinations and allocate the high-potential samples for creating new highly productive varieties of monoecious hemp of the Middle Russian ecotype for various economic uses;
- To conduct a competitive variety test of potential samples with recognized varieties and, based on the results of a comparative assessment, to recommend the best selection numbers for including into the State Register of selection inventions of the Russian Federation.

Materials and Methods

Research work was carried out in the field and laboratory conditions in the period between 2012-2019.

The soil of the test plot – leached black soil, middle loamy, medium power, pH 5.6–6.0, humus content 6.2–6.9%, hydrolyzable nitrogen – 78-86 mg/kg of soil, labile phosphorus–139-145 mg/kg of soil, exchangeable potassium – 158-167 mg/kg of soil.

The techniques and schematic models generally accepted in breeding research institutions were used in the experiments. R&D method - intravariety hybridization and multiple selections on the complex of valuable selection traits and properties. The main limiting selection feature is THC content in the upper parts of inflorescences not more than 0.1%.

The research was conducted in space isolated seed-plots: hybridization (2012), tests of hybrid combinations 1-2 years (2013-2014), preliminary multiplication and selection of elite plants (2015), control (2016) and competitive variety testing (2017-2019). Research objects are the monoecious hemp varieties of cannabis sativa of Penza Scientific Research Institute of Agricultural Sciences (Surskaya, Vera, Nadezhda) and potential selection numbers, allocated as a result of consecutive stages of selection.

Securing of seed-plots and study of selection material was conducted in accordance with methodology guidelines (Senchenko *et al.*, 1980; Rumyantseva, 1989). The method of hybridization seed-plots sowing and assessment of new hybrid combinations is manual, under the guide mark with a row spacing of 50 cm on single or double row plots without repetition. The method of sowing of the control seed-plot and preliminary multiplication is manual, on four-row plots with row spacing of 50 cm without repetitions. The method of seed-plot sowing for competitive variety testing is mechanized with the seeder SN-16 in a four-row option with row spacing of 50 cm in four repetitions.

Identification and quantitative determination of basic cannabinoids content was performed by GLC analysis in the gas-liquid chromatographic complex Crystal 2000M according to recommendations (Sorokin *et al*., 1995).

Chromatograms were quantitatively processed by peak areas using the Chromatek Analytic 2.5 software. Quantity of analysis samples is 2. The calculation of quantity content of tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) was conducted by the internal standard method. 0.5% solution of methyl stearate in ethanol was used as the internal standard.

Recording of productivity from plots were conducted by method of sheer harvesting. Seed and stem harvest was reduced to the standard humidity (13 and 25% respectively). Analysis of seeds, stems and fiber harvest structure was conducted according to the methods (Senchenko *et al.*, 1980; Rumyantseva 1989).

The determination of the oil content in the seeds was carried out according to the modified Lebedyantsev-Raushkovsky method (Raushkovsky 1959).

Statistical processing of experiment data was conducted using the regression analysis according the methodology (Dospekhov, 1985).

Experimental work was accompanied by related observations and records, including:

- observation of air temperature and precipitation during the growth;
- phenological observations according to the method (Senchenko *et al*., 1980)
- assessment and recording of plant damage by pests and disease damage in a 5-point scale (Rumyantseva, 1989)

Results and Discussion

The main agrometeorological data of the research period varied in moisture regime and heat resources (table 1).

Table 1 : Agrometeorological conditions of hemp growth (2012-2019)

Criteria	Year								
	2012	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**Average**
$\Sigma_{A.T.,}$ °C	2143	2041	2270	2263	2141	2014	2116	1950	2117
Precipitation, mm	291	263	140	230	255	154	63	120	190
Hydrothermal index	1.36	1.29	0.62	1.02	1.19	0.76	0.30	0.62	0.90

Growing season in 2012, 2013, 2016 was well moistened, 2015 was normally moistened, 2014, 2017, 2019 were insufficiently moistened, 2018 was very arid. The contrasting vegetation conditions allowed to compare the adaptive ability of the compared varieties and potential numbers in terms of productivity elements formation of the main types of products and their qualitative characteristics, as well as the levels of THC in plant biomass.

As a result of the work in Penza ISH-branch of FGBNU FNC LK the new variety of monoecious non-narcotic cannabis sativa Lyudmila was created (selection number - ГП-13/012в). It belongs to the Middle Russia ecotype group. The variety was obtained by method of crossbreeding of I-line of generation I_6 (202/06-2-1-1 × 155-2-1-1-1) with multiple family-group selection on the complex of characteristics.

In planting with optimal density the plant of the variety is unbranched (Fig. 1), in spaced it is low-branched. The height of the plants depends on the hydrothermic regime of growth and varies from 220 to 270 cm (tall-growing) (Fig. 2), technical length of the stem changes from 177 to 215 cm. The diameter of the stem in the middle part is 9-12 mm. Number of joints – 12-14 pcs., average length of the joint – 13-19 cm. Content of masculinized morphotypes in population – 0.04-0.06%. Content of male hemp plants – 0%. The population is dominated by ideally monoecious plants (82-85%) with approximately equal number of male and female flowers in a panicle.

Thus, as a result of selection work, a population of monoecious cannabis sativa was created, where at the final stage of selection the segregation of male hemp plants didn't occur.

Inflorescence is compressed (Fig. 3). Seeds are light gray, without a mosaic pattern (Fig. 4). Average weight of 1000 seeds is 14.5-16.9 g, oil content in seeds reaches 30.0%.

Fig. 1 : General view of the plants

Fig. 2 : Height of the plant

Seed leafs are lance-shaped, color is green. During the stage of mass ripening a weak degree of anthocyanin coloration is observed on petiolule of topmost leaves, stems and inflorescences. Variety Lyudmila is mid-season, the duration of the period from mass sprouting to technical ripeness of the fiber is 75-80 days, from mass sprouting to mass seeds ripeness - 118-125 days.

Fig. 3 : Plant panicle

Fig. 4 : Seeds

Practical use of the variety is bilateral (seeds + fiber), but it is recommended mainly for obtaining greenery (per fiber). Economic characteristic of the new variety is given according to the three years experimental data of the competitive variety testing (2017-2019) with agricultural cultivation techniques generally accepted for culture (table 2).

Table 2 : Comparative characteristics of the new variety of *Cannabis sativa* Lyudmila with the standard variety (2017-2019)

Criteria	Surskaya (standard)	Lyudmila
The stem yield at standard humidity, t/ha	8.4	12.3
The seed yield at standard humidity, t/ha	0.81	1.05
Vegetation period from full sprouts to the end of blossoming, days	82	85
Vegetation period from full sprouts to the full seed ripening, days	117	120
Weight of 1000 seeds, g	16.5	16.7
Oil content, %	31.0	30.0
Oil harvest, dt/ha	0.25	0.32
Fiber total yield, %	30.6	33.2
Total fiber harvest, dt/ha	2.6	4.1
Long fiber yield, %	16.5	21.4
Long fiber harvest, dt/ha	1.4	2.6
Combed fiber breaking force, kgf	18.9	28.6
Combed fiber flexibility, mm	14.6	21.3
THC content, %	0.069	0.025
Content of male hemp plants, %	4.5	0.0
Morbid affection, %, points		
Fusarium blight (*Fusarium oxysporum Schl.f. vasinfectum)*	0, (1)	0, (1)
Stem rotting (*Botrytis cinerea Fr.,Whetzelinia sclerotiorum)*	0, (1)	0, (1)
Gray speck of stems (*Dendrophoma marconii Cav.)*	3-4, (3)	0, (1)
Septoria blight (*Septoria cannabis Sacc.)*	1-2, (3)	0, (1)
Vermin damage, %, points		
Hemp flea (*Psylliodes attenuata Koch.*)	3-5, (3)	3-5, (3)
European corn borer (*Pyrausta nubilalis Hb.*)	3-5, (3)	1-3, (3)

A characteristic feature of variety Lyudmila is a very high fiber total yield (> 30%) and high long fiber yield (130% to st). Qualitative characteristics of the fiber are also of higher levels in relation to the standard, including the breaking force (+9.7 kgf to st) and combed fiber flexibility (+6.7 mm to st).

During the phase of mass sprouting at vegetation the slight population of hemp flea (*Psylliodes attenuata Koch.*) was observed on the plants as well as the slight presence of European com borer (*Pyrausta nubilalis Hb.*) during the phase of mass seed ripening.

The new variety of cannabis sativa Lyudmila meets most of the technological requirements for plant hemp material obtained from this textile crop.

Cannabis sativa is a crop which requires high agricultural technology and compliance with all elements of agricultural technology at cultivation.

Features of the varietal agrotechnology of the new variety: placement in the crop rotation after complete fallow, grain crops (winter and spring), grain legumes or tilled crops, perennial grass; planting of seeds at the soil temperature not less than +10^{o}C at a depth of 3-4 cm in lines (7.5-15 cm) with a sowing rate of 2.6-2.8 million germinating seeds per 1 ha; seed rolling; harvesting of greenery in the phase of full defloration of plants (80%).

Conclusion

As a result of the research, the non-narcotic variety of cannabis sativa Lyudmila was bred, which has a complex of improved economic traits and characters in relation to existing varieties. The variety is distinguished by the absence of the male hemp plants segregation, lowered content of THC and cannabinoids in relation to standard variety and other varieties but with the higher level of traits “total/long fiber content” and “total/long fiber harvest”.

By a quantitative assessment of the THC content in the tops of inflorescences of a plant variety, on average, this psychotropic compound content is 0.044% (or 2.8 times) less than in the standard variety.

Variety Lyudmila outrates the standard variety and other selective varieties of Middle Russian ecotype in stem yield (+3.9 t/ha to st), total fiber content in stems (33.2% or +2.6% to st in average), seed yield (+0.24 t/ha to st), and corresponds to the level of the standard variety by average weight of 1000 seeds.

The variety is designed to produce high-quality fiber for the textile industry as well.

Following the results of the competitive variety testing of potential selective numbers in 2020, the application for the inclusion of a new variety of cannabis sativa Lyudmila into the State Register of selection inventions of the Russian Federation and for the grant of a patent was submitted.

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