Review article

ACHIEVEMENTS IN THE IMPROVEMENT OF INDUSTRIAL HEMP PRODUCTION IN THE INSTITUTE OF FIELD AND VEGETABLE CROPS, NOVI SAD

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Alternative Crops and Cultivation Practices

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SUMMARY

Program of improving of industrial hemp production at the Institute of Field and Vegetable Crops in Novi Sad started in the middle of the 20th century. During seven decades, several generations of researchers have dedicated their work to develop an assortment that will meet producers' needs in terms of improvement of yield and quality of hemp raw material. Starting from the available germplasm and the requirements of the fiber hemp producers, in the first cycle, an increase in the stalk fiber content by approximately 30% was achieved. Following the development of the market for hemp products in the second cycle, the fiber content was increased by another 10% and a monoecious high-yielding variety with a grain oil content of over 30% was selected. The latest global trends related to healthy food and natural raw materials have initiated the next breeding cycle based on the wide genetic variability of collected and newly developed germplasm. Modern breeding, in addition to yield, is focused to the quality of raw materials i.e. stalk, grain and flower, obtained by widespread production of hemp.

KEYWORDS: breeding of hemp, *Cannabis sativa* L, hemp varieties

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INTRODUCTION

There are no precise data on what kind of hemp was grown in Serbia in the earliest period of introduction and expansion of production. It is certain that over time, local varieties such as 'Futoška', 'Titelska', 'Apatinska', 'Vukovarska', etc. have emerged in some regions of intensive production (Berenji, 1992). Pasković (1939a; 1939b) included the following local varieties as "our most important varieties of hemp": 'Potočka', 'Osječka', 'Beljska', 'Apatinska', 'Leskovačka' and 'Bolognska'. Makendić (1936; 1937) wrote: "Our species that are sown in Bačka and Baranja are very good and can compete with Italian hemp". The comparison with Italian hemp is not accidental, considering that the local varieties were mostly selected from the genetic materials of Italian origin. Local varieties were maintained and propagated by the producers themselves.

One of the most serious problems of hemp growing in this area has long been the unresolved issue of assortment and seed production (Berenji & Sikora, 1996). Therefore, an attempt was made to obtain appropriate domestic varieties by breeding and to make possible the production of quality domestic hemp seed varieties (Berenji, 1992). The Agricultural Experimental and Control Station in Novi Sad, now the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, was the first to start hemp breeding in the then Kingdom of Yugoslavia, today's Republic of Serbia. Since its founding in 1938, according to archival documents, "industrial plants came in the field of plant breeding in importance after small grains, and among them sunflowers and hemp in the first place." Hemp breeding has been especially

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intensified since the establishment of the Hemp, Hop and Sorghum Testing Station in Bački Petrovac, now the Department for Vegetable and Alternative Crops of the Institute of Field and Vegetable Crops Novi Sad, in 1952. The most important results of hemp breeding in Novi Sad is the cultivar 'Novosadska', also known as 'Hemp NS', 'Novi Sad', 'Novi Sad Fleischmann' or 'Fleischmann'. It has been on the national variety list as 'Novosadska' since 1967, and its author was Professor Milenko Lazić. The famous hemp breeder Rudolf Fleischmann's 'F-hemp' served as the starting material for the selection. Fleischmann was in the period 1906-17 the manager of the Ruma estate, where he also dealt with the selection of various cultivated plants, e.g. he is the author of famous corn variety 'Rumski zuban'. By moving to a breeding station in Kompolt, Hungary, he began hemp breeding in 1920. From the material "Italian and Provencal hemp" (Fleischmann, 1922) a variety called 'F-hemp' was created, which was officially recognized in Hungary in 1946 under the name 'F-kender'. In addition to 'Novosadska', this selection served as the source material for the Hungarian variety 'Kompolti'. Apparently, Fleischmann started studying hemp during his stay in Ruma.

The next period was characterized by the import of declared varietal hemp seeds from Italy. These were mainly the varieties 'Bologna' and 'Carmagnola' (Lakić, 1966). In addition to imports, these varieties were also multiplied in the ex-Yugoslavia. There were also attempts to domesticate the French monoecious variety 'Fibrimon', but without much success (Starčević, 1966).

Of the domestic and domesticated foreign varieties of hemp, six varieties were registered in 1967 (Čučković & Dančević, 1967). With the revision of this list at the Fifth Plenary Session of the Commission for Giving Proposals for Recognition of Newly Created and Approving the Introduction of Foreign Varieties of Field Crops held in 1985, only two of the six varieties were left: 'Fleischman's' and 'Novosadska'. The 'Fleischman' variety is identical to the 'F-hemp' from Hungary. According to archives of the Institute of Field and Vegetable Crops, 'Novosadska' was reported to the State Commission for the Recognition of Varieties in 1956. Lakić (1966) noted: "In 1960 and 1961, the Institute for Agricultural Research in Novi Sad set up varietal experiments with the seeds of 'Carmagnola', 'Bologna' and 'Novosadska'. In these experiments, the best results were given by the variety 'Novosadska'. Agro-biological properties as well as the reaction of 'Novosadska' to various agro-technical measures were studied in detail by Starčević (1966; 1972; 1978; 1992).

After the recognition of varieties from Hungary, almost the entire production of hemp in Serbia was based on imported seeds from Hungary. The national variety list included four Hungarian varieties, of which the most imported seeds were 'Kompolti hybrid TC' and 'UNIKO-B'.

In 1992, the hemp research program was reactivated at the Institute of Field and Vegetable Crops, which continues to this day. The program includes hemp breeding, seed production and development of hemp production technology for various purposes. From the first breeding cycle, three varieties emerged: dioecious 'Marina', monoecious 'Helena' and hybrid variety 'Diana'. The authors of these varieties are Janoš Berenji and Vladimir Sikora, and they were entered on the national variety list in 2002. From 2020, the varieties 'Helena' and 'Marina' have been registered in the European Union.

Hemp for fiber

The yield of hemp stalk grown for fiber is determined by the method of sowing (Amaduci et al., 2002), which at the time of expansion of this production in the middle of the last century was done by hand or with small grain seeders, with sowing density of about 300 seeds per square meter. Given the traditionally applied production technology, which was considered appropriate, the primary goal of the improvement was to increase the fiber content in the stalk. The starting material for the first selection cycle were populations locally grown in the region of South Bačka, in which the fiber

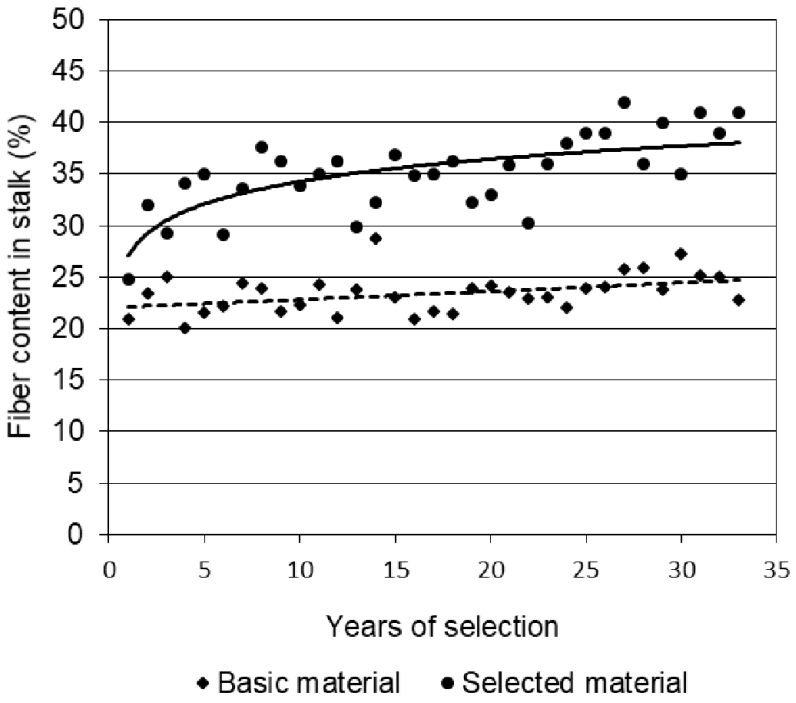

Figure 1. Fiber content in basic and selected hemp material

content in the stalk ranged at the level of 20-25% (Figure 1).

In the first step, individual selection of female plants based on phenotype was done from this material. In selected plants, the fiber content was determined using the Bredeman's method (Bredeman, 1942), and for further reproduction, the seeds of plants with the highest fiber content were sown according to the plant/row principle. After several selection cycles, an increase in fiber content of 30-35% was achieved. This material was used in mercantile production under the name 'Novosadska' until the end of the last century.

Using wider genetic variability (Sikora et al., 2011a) from the crossing of 'Novosadska' and the Italian variety 'Carmagnola', a hemp population which became the basis for further increase in fiber content was created. By applying positive selection of male and female plants based on the Bredeman's method (Berenji & Sikora, 2000), an increase in the fiber content in the stalk of 35-40% was achieved in several years.

The modern assortment of hemp for fiber is represented by the dioecious variety 'Marina', in which varietal purity and fiber yield parameters are maintained by producing of elite seeds from plants in which the fiber content in the stalk exceeds 40%.

Hemp for grain

During the long period of its cultivation, hemp grain was used primarily to establish a new field, while the remaining quantities were used for poultry feeding. Given the potential of hemp as an oilseed (Berenji et al., 2001), the increase of the oil content in the grain was defined as one of the goals of selection. Given the increasing demand for cold-pressed hemp oil in the Western European market, and taking into account the oil content in the existing assortment (Figure 2), the ideotype was a monoecious variety with grain yield up to 1000 kg per hectare and the oil content in the grain over 30%.

The selection began with manual crosses of monoecious Ukrainian and French varieties. From the obtained variable F2 population, material of satisfactory yield and oil content was stabilized by individual selection for next 6 generations (Figure 2). The selected monoecious variety from this program is commercially available under the name 'Helena'.

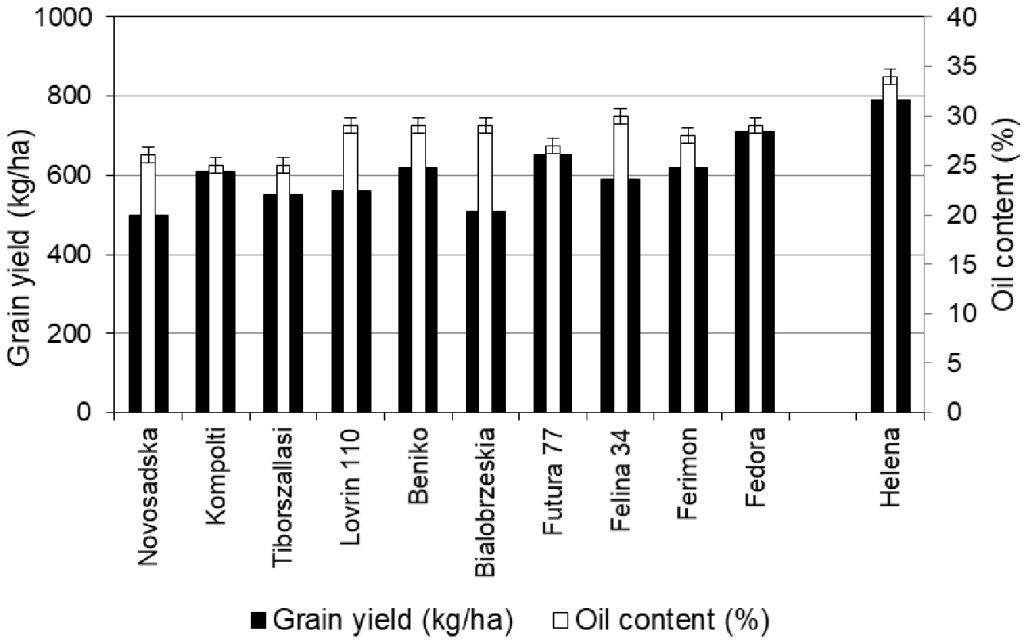

Figure 2. Seed yield and oil content in commercial varieties and selected hemp material (Bački Petrovac, average for 2015-2017 growing seasons)

Contemporary trends in hemp breeding

Modern market trends such as a healthy diet and the use of natural raw materials caused the renaissance of hemp as a multipurpose plant at the beginning of the new century. When designing the current breeding cycle, the starting point was the fact that in terms of stalk yields (over 15 tons per hectare) and fiber content in the stalk (approx. 40%), the contemporary assortment fulfill the needs of the market.

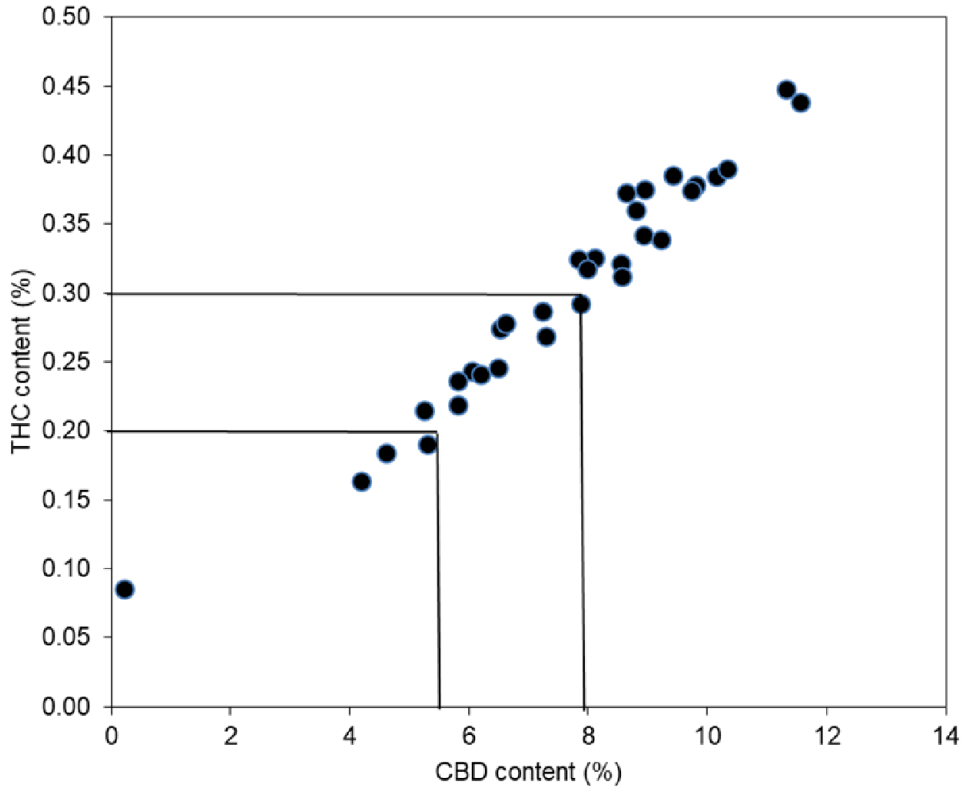

Figure 3. Cannabinoid content in hemp experimental lines (Bački Petrovac, growing season 2020)

Table 1. Composition of essential oil of fiber hemp 'Helena' according to Zheliazkov et al. (2020)

Compound	LRI*$_{lit}$	LRI*exp	%	Compound	LRI*$_{lit}$	LRI*exp	%
Heptanal	902.0	902.3	0.07	*α-Terpineol*	1,189.7	1,189.5	0.03
α-Pinene	936.0	933.5	9.63	*Myrtenol*	1,194.1	1,195.6	0.04
Camphene	950.3	947.4	0.27	*α-Ylangene*	-	1,370.1	0.04
Sabinene	973.0	972.7	0.05	*α - Caryophyllene*	1,406.5	1,405.3	0.42
β-Pinene beta	977.7	976.7	3.08	*β -Caryophyllene*	1,420.1	1,420.2	18.36
Myrcene	989.2	992.1	9.66	*α-Bergamoten, trans*	1,434.5	1,435.3	1.11
α-Phellandrene	1,004.1	1,005.4	0.19	*α-Humulene*	1,453.1	1,452.9	6.49
3-Carene	1,011.3	1,010.6	0.17	*β-Farnesene*	1,445.9	1,457.7	2.27
α-Terpinene	1,017.1	1,016.3	0.15	*γ-Curcumene*	1,480.3	1,479.0	0.08
p-Cymene	1,024.3	1,023.6	0.07	*β-Selinene beta*	1,486.1	1,484.3	1.56
Limonene	1,029.5	1,027.8	1.13	*α-Selinene*	1,493.4	1,493.1	1.38
1,8-Cineole	1,031.8	1,030.3	0.12	*α-Bulnesene*	1,504.1	1,504.3	0.34
α -Ocimene	1,037.8	1,035.9	0.64	*β-Bisabolene beta*	1,508.4	1,508.3	0.26
β -Ocimene	1,047.7	1,047.0	5.90	*Sesquiphellandrene*	1,523.5	1,523.1	0.26
γ-Terpinene	1,059.7	1,057.2	0.17	*Selina-3,7-diene*	1,540.5	1,539.2	0.19
Cis-Sabinene hydrate	1,066.5	1,065.3	0.03	*α-Bisabolene E*	1,540.3	1,543.1	0.06
Terpinolene	1,086.9	1,088.6	4.41	*Nerolidol E*	1,560.9	1,563.9	0.50
Linalool	1,099.0	1,099.9	0.05	*Caryophyllene oxide*	1,580.6	1,581.7	6.27
Nonanal	1,103.3	1,104.3	0.09	*Ledol*	1,566.8	1,600.0	0.20
Pinene hydrate trans	1,121.2	1,120.4	0.04	*Humulene epoxide II*	1,604.7	1,606.9	2.52
Pinocarveol trans	1,140.0	1,137.3	0.07	*Caryophylla-4-dien-5-beta-ol*	1,640.3	1,634.3	1.61
Pinocarvone	1,160.6	1,161.0	0.06	*α-Selin-11-en-4-ol*	1,654.9	1,651.9	0.13
Borneol	1,166.2	1,164.0	0.06	*CBD*	-	2,424.4	7.80
4-Terpineol	1,177.1	1,175.7	0.19				
Total identified							88.61
Total monoterpens							36.30
Total sesquiterpens							44.45

LRI = Linear retention indeces on HP5-column

Using the photo-neutral variety 'Finola' as a donor of the early maturity gene, a program of hemp selection for grain was started. The goal of this selection is to create lines of shorter vegetation, grain yield over 1 ton per hectare and plant habitus that allows harvesting with regular harvesters.

Interest in secondary biomolecules, phytocannabinoids and terpenes (Aizpurua-Olaizola et al., 2016), which are synthesized by the hemp plant, has encouraged the definition of the current breeding direction. Of the more than 100 identified phytocannabinoids, cannabidiol (CBD) is particularly interesting due to its therapeutic potential (Grotenhermen & Müller-Vahl, 2012). The design of this program was based on existing genetic material with a CBD level of 1-2% (Sikora et al., 2011b) with the introduction of exotic Chinese germplasm. After crossing domestic varieties with Chinese lines in the *sativa* type, several variable F2 populations were obtained (Figure 3) in which the CBD content is higher, compared to the starting material in the southern hemp type.

The limiting factor in this selection is the content of psychoactive tetrahydrocannabinol - THC (Piluzza et al., 2013), with content regulated by law at the level of 0.2% and 0.3% in the European Union and Serbia respectively. It is expected that in the future, lines of the allowed THC content and increased CBD content will be selected from this material.

Regarding the terpenes content and composition, the current research refers to the evaluation of existing germplasm related to plant material as well as to essential oils, Table 1 (Zheliazkov et al., 2020) and the broadening of genetic variability by introduction of feral hemp and crossings with exotic material, in order to provide a broader basis for selection in the direction determined by market requirements.

CONCLUSION

With a systematic approach to the program of improving hemp production at the Institute of Field and Vegetable Crops, several generations of experts meet the needs of farmers during almost seventy years of continuous work in terms of assortment and scientific achievements related to the technological process of production.

At the beginning of the research, activities were primarily related to hemp for fiber, and depending on the market demand, the focus is now concentrated on other raw materials such as grain and flowers.

Although the modern assortment fully corresponds to the current needs, the market of hemp products is rapidly developing and expanding so that new directions of breeding are conceived. Current work relates primarily to the widening genetic variability for desirable traits, with the introduction of feral hemp and exotic germplasm, and the further selection of genotypes with enhanced desirable traits.

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SAŽETAK

POSTIGNUĆA U POBOLJŠANJU PROIZVODNJE INDUSTRIJSKE KONOPLJE U INSTITUTU ZA RATARSTVO I POVRTARSTVO IZ NOVOG SADA

VLADIMIR SIKORA, ANAMARIJA KOREN

Program unapređenja proizvodnje industrijske konoplje u Institutu za ratarstvo i povrtarstvo u Novom Sadu započet je sredinom dvadesetog veka. Tokom sedam decenija generacije istraživača posvetile su se razvoju sortimenta koji bi zadovoljio potrebe proizvođača u smislu poboljšanja prinosa i kvaliteta konopljinih sirovina. Polazeći od dostupne germplazme i zahteva proizvođača konoplje za vlakno, u prvom je ciklusu postignuto povećanje sadržaja vlakana u stabljici za oko 30%. U drugom ciklusu, nakon razvoja tržišta proizvoda od konoplje, sadržaj vlakana povećan je za dodatnih 10% i selekcionisana je visokoprinosna jednodoma konoplja sa sadržajem ulja u zrnu od preko 30%. Najnoviji globalni trendovi tržišta proizvoda zdrave hrane i prirodnih sirovina pokrenuli su naredni ciklus oplemenjivanja utemeljen na širokoj genetskoj varijabilnosti postojeće i novoproširene germplazme u Institutu. Savremeno oplemenjivanje osim ka povećanju prinosa usmereno je i ka poboljšanju kvaliteta konopljinih sirovina stabljike, zrna, lista i cveta.

Ključne reči: *Cannabis sativa* L., oplemenjivanje industrijske konoplje, sorte industrijske konoplje