

Results of Efforts by French and Ukranian Breeders to Reduce Cannabinoid Levels in Industrial Hemp (*Cannabis sativa* L.)

P. Holoborodko
V. Virovets
I. Laiko
S. Bertucelli
O. Beherec
G. Fournier

ABSTRACT. Results are given of independent, multi-year efforts by French and Ukrainian breeders to create new, highly productive, monoecious hemp varieties without drug properties, while retaining an innate resistance to pests and diseases.

KEY WORDS. Hemp, breeding, *Cannabis sativa*, monoecious, cannabinoids, drug-free.

Pavlo Holoborodko is the director of the Institute of Bast Crops at the Ukrainian Academy of Agrarian Sciences, 45 Tereschenkiv Street, 41400 Hlukhiv, Sumy region, Ukraine

Viacheslav Virovets and Iryna Laiko are research assistants at the Institute of Bast Crops at the Ukrainian Academy of Agrarian Sciences, 45 Tereschenkiv Street, 41400 Hlukhiv, Sumy region, Ukraine

Olivier Beherec is a hemp breeder and deputy director of the Federation Nationale des Producteurs de Chanvre, 20, rue Paul Ligneul, 72 000 Le Mans, France

Sylvestre Bertucelli is the director of the Federation Nationale des Producteurs de Chanvre and director of the Coopérative Centrale des Producteurs de Semences de Chanvre, 20, rue Paul Ligneul, 72 000 Le mans, France

Gilbert Fournier is professor of pharmacology at the Faculté de Pharmacie de Chatenay Malabry, 5 rue Jean Baptiste Clement, 92 290 Chatenay Malabry, France

INTRODUCTION

Cannabis (hemp) is widely distributed on all continents of the world. Depending on the industrial or herbal uses of the plant by a given culture, and as a result of many years of natural and human selection, two biological types of hemp were formed: *Cannabis sativa* L. (hemp) and *Cannabis indica* Lam. (Indian hemp). Today, the origin, spread, and use of these two divergent lineages are of interest to botanists, taxonomists and hemp breeders. The uncertainty of hemp's evolutionary origin (Dewey, 1913; Heuser, 1933; Vavilov, 1971) has resulted in it being variously assigned to the Moraceae, Urticaceae or Cannabaceae family.

In Europe, Canada, the USA, China, and Australia, hemp is grown as a fiber crop. The largest areas of hemp production are primarily in Europe, where a lot of attention is given to the biological, morphological and technological peculiarities of the crop. Special research institutes were founded in the USSA (now the Institute of Bast Crops at the Ukrainian Academy of Agrarian Sciences), and in France (the Federation Nationale des Producteurs de Chanvre), where a number of scientists are dedicated toward investigating a single crop - hemp.

BREEDING RESULTS

In this article, we describe the results of selection by hemp breeders in Ukraine and France for the purpose of decreasing the psychotropic properties of industrial hemp grown in these two countries. In 1972 in Ukraine, and in 1974 in France, scientists began breeding new hemp varieties without drug properties because of regulations imposed in Ukraine by the Ministers of Health Protection, Internal Affairs and Agriculture, and in France by the Ministers of Health Protection and Agriculture. Their breeding efforts were made in collaboration with the N. I. Vavilov All-Union Scientific Research Institute of Plant Industry (now the All-Russian Institute of Plant Industry, St. Petersburg, Russia), and the All-Union Scientific Research Institute of Internal Affairs (Moscow, Russia). Over a period of 30 years, independent work with the same objectives was undertaken by scientists in France and Ukraine, leading toward the development of different but complementary breeding techniques. A cooperative effort between scientists in these two countries became possible and began only a few years ago.

In Ukraine, the first steps toward breeding for decreased cannabinoid content failed to find starting material having all the traits necessary for use in a breeding program. To accomplish our task, we had to devise new methods or modify and improve classical methods of breeding. These new methods are based on the modernization of controlled selection techniques that exclude the possibility of unintentional cross-pollination in field-grown populations of hemp. This became possible in Ukraine by the development of a method to test individual plants for cannabinoid presence or absence in the field, and in France by the ability to quickly test individual plants for very low or absent tetrahydrocannabinol (THC) content in the laboratory.

Because of the necessity to maintain high productivity while decreasing THC content, plant height, fiber content, monoecious flowering, seed weight, duration of vegetative period, and disease resistance was also controlled. At the time that breeding efforts to achieve these goals were being made, government regulations imposed a continuing decrease in THC content. In Ukraine, the maximum THC content allowed for industrial hemp varieties was 0.3% (dry weight) in 1973, 0.2% in 1980 and 0.15% in 1995. In France (followed by the European Union (EU) in 1989), the maximum THC content was 0.3% at the beginning of the breeding work, and 0.2% in 2001.

In 2006, eleven varieties of monoecious hemp were added to the State Register of Plant Varieties Suitable for Dissemination in Ukraine. They were created at the Institute of Bast Crops UAAS (USO-14, USO-31, Hlukhivs'ki 33, Hlukhivs'ki 46, Hlera), the Zolotonosha Department of Hemp Breeding and Seed-Growing at the Institute of Bast Crops UAAS (Zolotonis'ki 11, Zolotonis'ki 15) and at the Synel'nykivs'ka Research Station at the Institute of Corn Economy UAAS (Dniprovs'ki odnodomni 6, Synel'nykivs'ki odnodomni 3, Dniprovs'ki 14, Dniprovs'ki odnodomni 19).

Between 1997 and 2007, six varieties of monoecious hemp with very low or an absence of THC were added to the French and European catalog of varieties by the Federation Nationale des Producteurs de Chanvre (Fedora 17, Felina 32, Epsilon 68, Futura 75, Santhica 27 and Santhica 70), and one variety was added by the Institute of Bast Crops UAAS (USO-31).

The average THC content of these different varieties is given in Tables 1 and 2.

Table 1 – Mean THC content in Ukrainian hemp varieties tested at the Institute of Bast Crops UAAS, 2004 (maximum allowed level: 0.15% dry weight)

| № | Variety | Mean THC content, % d.w. |
|---|------------------|--------------------------|
| 1 | USO-31, standard | 0.003 |
| 2 | Hliana | 0.003 |
| 3 | Hlera | 0.001 |
| 4 | Hlukhivs'ki 33 | 0.000 |
| 5 | Zolotonis'ki 15 | 0.000 |

Table 2 – Mean THC content in French hemp varieties tested at the Federation Nationale des Producteurs de Chanvre, France, 2004-2007 (maximum allowed level: 0.2% dry weight)

| № | Variety | Mean THC content, % d.w. |
|---|-------------|--------------------------|
| 1 | Fedora 17 | 0.047 |
| 2 | Santhica 27 | 0.000 |
| 3 | Felina 32 | 0.060 |
| 4 | Epsilon 68 | 0.065 |
| 5 | Santhica 70 | 0.000 |
| 6 | Futura 75 | 0.055 |

Ukrainian and French hemp varieties differ in the duration of their vegetative periods, and offer a good complement of traits for user purposes, including the ability to grow under a wide range of environmental conditions in Europe, Canada, China, and elsewhere.

In the northern hemisphere, fast-ripening varieties are suitable for northern conditions, and are used to produce stem, fiber and seeds. Later-ripening varieties are suitable for these same uses in southern conditions. Because the process of fiber formation finishes a month before biological ripening, late varieties are often grown in northern regions to improve stem and fiber yield. Thus, producers harvest hemp at different phases of ripening depending on use. That is why two types of harvesting are used in breeding practice: at the phase of technical ripening (for fiber), and at the phase of biological ripening (for fiber and seeds).

A result of breeding work in Ukraine was the creation of different lines of preliminary breeding material, among which are samples lacking cannabinoids and the typical hemp aroma (e.g., variety USO-45). The absence of cannabinoids and aroma is apparently due to an absence of glandular trichomes; there are only cystolithic hairs on leaves and perianths. As a result of purposeful selection directed toward decreasing cannabinoid content, it appears that we have fixed this natural mutation which results in the elimination of hemp drug activity.

Agronomics results of the different varieties are given in tables 3 and 4.

Table 3 – Characteristics of Ukrainian hemp varieties, mean values for 2004-2006, tested at the Institute of Bast Crops UAAS.

| № | Variety | Yield, dT per ha | | | Fiber output, % d.w. | Vegetation period, days | |
|---|------------------|------------------|-------|-------|----------------------|--------------------------|---------------------------|
| | | straw | seeds | fiber | | until technical ripening | until biological ripening |
| 1 | USO-31, standard | 77.9 | 12.8 | 25.7 | 33.0 | 87 | 110 |
| 2 | Hlera | 90.3 | 16.1 | 28.5 | 32.7 | 95 | 120 |
| 3 | Hliana | 76.4* | 13.9* | 26.0* | 34.0* | 85* | 109* |
| 4 | Hlukhivs'ki 33 | 86.0 | 11.8 | 27.2 | 31.3 | 99 | 125 |
| 5 | Zolotonis'ki 15 | 92.5 | 8.1 | 25.9 | 29.0 | 113 | 134 |

* data for two years

Table 4 – Characteristics of French hemp varieties mean values for 2006-2007, tested at the Federation Nationale des Producteurs de Chanvre, France. Data for USO-31 grown in France are added.

| № | Variety | Yield, dT per ha | | | Fiber output, % d.w. | Full flowering date |
|---|-------------|------------------|-------|-------|----------------------|---------------------|
| | | straw | seeds | fiber | | |
| | USO-31, (*) | 83.8 | 11.2 | 31.8 | 37.9 | July 20 |
| 1 | Fedora 17 | 95.2 | 15.4 | 31.8 | 33.4 | August 1 |
| 2 | Santhica 27 | 92.6 | 10.8 | 34.2 | 36.9 | August 4 |
| 3 | Felina 32 | 95.4 | 13.8 | 32.4 | 34.0 | August 6 |

| | | | | | | |
|---|-------------|-------|------|------|------|-----------|
| 4 | Epsilon 68 | 100.7 | 11.5 | 33.6 | 33.4 | August 9 |
| 5 | Santhica 70 | 106.8 | 10.6 | 38.6 | 36.1 | August 11 |
| 6 | Futura 75 | 111.3 | 10.9 | 37.2 | 33.4 | August 15 |

* USO-31 was introduced in France and European countries by FNPC and CCPCSC.

Ukrainian and French hemp varieties can produce high straw yields, depending on the duration of the vegetative period. High seed yields can also be obtained, contingent on the availability of water in periods of rapid growth, bud-formation and flowering. High fiber yields are possible with the help of high fiber content in stems, which exceeds 30% d.w. in most varieties. Intensive multi-year work by breeders in both countries geared toward decreasing THC or total cannabinoid content made it impossible to grow these newly developed varieties for psychotropic use.

In the new Ukrainian hemp varieties, the combination of such traits as fast-ripening, low THC content (less than 0.05%), and high seed productivity and fiber yield enables them to be grown profitably in Europe, Russia, China and Canada.

The area of hemp in Ukraine is 1000-2000 ha. The main obstacle to hemp growing in Ukraine is an imperfect law that mandates all producers to obtain a license and guard their hemp crops, irrespective of THC content. In the EU, fields of registered hemp varieties with THC content less than 0.2% have to be grown under license, but do not have to be guarded. Moreover, in Russia it is unnecessary to be licensed to grow such varieties.

Breeders of the Institute of Bast Crops maintain that monoecious hemp varieties grown for industrial use should be well defined by law to distinguish them from wild hemp, which can be grown for drug use. Thus, it is necessary to make changes in Ukrainian legislation corresponding to the EU laws.

Hemp growing in countries of the EU (France, Poland, Hungary, United Kingdom, Germany and Italy) and elsewhere, without guarding, has become possible with the work of breeders.

Today, variety USO-31 is registered in France, and the Federation Nationale des Producteurs de Chanvre has introduced it in the EU. French varieties are investigated and used for the creation of new initial breeding material at the Institute of Bast Crops. Such cooperation benefits both countries, and the rest of the world besides.

REFERENCES

- Hramchenko H.I., 1935. Sistematically harakteristika konopli. Trudy Vsesoiuznogo NII konopli Biologii konopli 8: 9-21.
- Puzanov M.A., 1937. Konoplia i eia produkty. Sovet Imperatorskogo Moskovskogo Obschestva. Komitet zemledeliya: 224 – 279.
- Vavilov N.I., 1987. Teoreticheskie osnovy selektsii. Nauka, Moskva, Rossia, 512 pp.
- Virovets V.G., 1992. Sozdanie vysokoproduktivnyh sortov konopli ne obladaiuschih narkoticheskoy aktivnostiu. Avtoreferat doctorskoi dissertatsii: 42.

- Kisin M.V., Cemkin E.P., Zhuravleva N.M., 1974. Izuchenie zavisimosti sodержaniya kannabinoidov ot sorta konopli. Tez. dikl. nauch. konf. Problemy ekspertnogo issledovaniya narcoticheskikh veschestv:46-47
- Senchenko G.I. Sazhko M.M., Horshkova L.M., 1975. K metodike opredeleniia sodержaniia kannabinoidov v konople. Sb. nauch. tr. VNIILK Biologiya, vzdelyvanie i pervichnaia obrabotka konopli i kenafa: 39-44
- Sazhko M.M., Vyrovets V.G., Gorshkova L.M., 1985. Metodicheskie ukazaniya po kachestvennoy otsenke konopli na sodержanie kannabinoidov, polucheniuiu tetraploidnyh form i ispol'zovaniuiu estrela. Moskva, Rossia, 16 pp.
- Virovets V.G., Gorshkova L.M., Senchenko G.I., Sazhko M.M., 1985. Metodicheskie ukazaniya po selektsii konopli na snizhenie sodержaniia kannabinoidov. Moskva, Rossia, 14 pp.
- Bocsa I., Karus M., Lohmeyer, 2000. Der Hanfanbau Botanik, Sorten, Anbau und Ernte, Markte und der produktlinien. Landwirtschaftsverlag GmbH, Munster, Anflag, 195 pp.
- Fournier G., Paris M. Détermination de chimiotypes à partir des cannabinoïds chez le Chanvre à fibres monoïque (*Cannabis sativa* L.). Possibilités de sélection. *Physiol. Vég.* 1980; 18: 349-356.
- Fournier G. Les chimotypes du chanvre (*Cannabis sativa* L.). Intérêt pour un programme de sélection. *Agronomie.* 1981; 1: 679-688.
- Mathieu J.P., Fournier G. La sélection du Chanvre en France. *Psychotropes* 1996; 2: 53-60.
- Fournier G. L sélection du Chanvre à fibres (*Cannabis sativa* L.) en France. *Chanvre et THC.* C.R. Acad. Agric. Fr. 2000; 86: 209-217
- Fournier G., Beherec O., Bertucelli S., Mathieu J.P. A propos des conditions d'échantillonnage pour le dosage du delta-9-tétrahydrocannabinol dans les variétés de chanvre à usage industriel. *Ann. Toxicol. Anal.* 2001; 13: 275-281
- Fournier G., Beherec O., Bertucelli S. Intérêt du rapport Δ -9-THC/CBD dans le contrôle des cultures de chanvre industriel. *Ann. Toxicol. Anal.* 2003; 15: 250-259
- Fournier G., Beherec O., Bertucelli S. Santhica 23 et 27: deux variétés de chanvre (*Cannabis sativa* L.) sans Δ -9-THC. *Ann. Toxicol. Anal.* 2004; 16: 128-132
- Fournier G., Bausset J., Beherec O., Desvals M., Bertucelli S. Lettre à la rédaction: une nouvelle souche de chanvre à fibres sans cannabinoïds. *Ann. Toxicol. Anal.* 2005; 17: 109-111
- Fournier G., Bausset J., Beherec O., Desvals M., Bertucelli S. La simplification du contrôle des cultures de chanvre industriel est possible. *Ann. Toxicol. Anal.* 2007; 19: 201-209