PROTEIN MARKERS AND METABOLOMIC APPROACH TO VARIETY IDENTIFICATION, SEED CONTROL, MEDICINAL, FOOD AND FEED UTILIZATION OF OATS

A. Konarev, I. Gavrilyuk, N. Gubareva, T. Shelenga, I.Perchuk, A.Shavarda and I.Loskutov

DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

N.I.VAVILOV INSTITUTE (VIR)

a.konarev@vir.nw.ru

Kurenok2006@yandex.ru

190000 SPb, B. Morskaya str., 42.

www.vir.nw.ru

- Discussed herein are facts and arguments “in favor of oats”;
- protein markers in oat gene pool registration;
- conservation and maintenance of oat genetic diversity and authenticity of its collection;
- oat seed control (fatuoids, etc.);
- oats in healthy and medicinal nutrition;
- metabolomic approach to oat gene pool analysis

Facts: "Oat is widespread over all continents embracing the whole grain belt of the Globe"

- It is a popular feed for horses.
- The widest popularity in the world it received as morning oatmeal porridge.

Arguments:

- most of the citizens of the United Kingdom and the Commonwealth have oatmeal porridge for breakfast;
- J.A. Hunter, the famous Scottish hunter who killed thousands of elephants, rhinos and lions, concludes his life-story with the information that all his years in Africa he had a huge supply of oatmeal;
- the second version of the breakfast menu for the Queen of the United Kingdom includes oatmeal porridge;
- the main character of A.I. Solzhenitsyn's *One Day in the Life of Ivan Denisovich*, who helped his author to become a Nobel Prize winner, longs in the labor camp canteen for the oat porridge cooked in the same way as for the Queen, with water;
- A.P. Chekhov depicts the degree of a widow's love for her late husband as the amount of oats that she gave to her husband's old horse (his story «*The Bear»*); oat also appears in his another story «*A Horsey Name»*;

A bear, lacking even minimal knowledge of biochemistry*, prefers an oat field (a bear hunt “on the oats”).

* - "The bear terribly dislikes the light of science" (from a Russian Scientific Journal of the middle of the 19th century).

The customer market offers a rich diversity of “oat” products, including oatmeal. However, “oat” is in quotes because, unlike the case with bears, there is no guarantee that it is oat we buy and not, for example, barley.

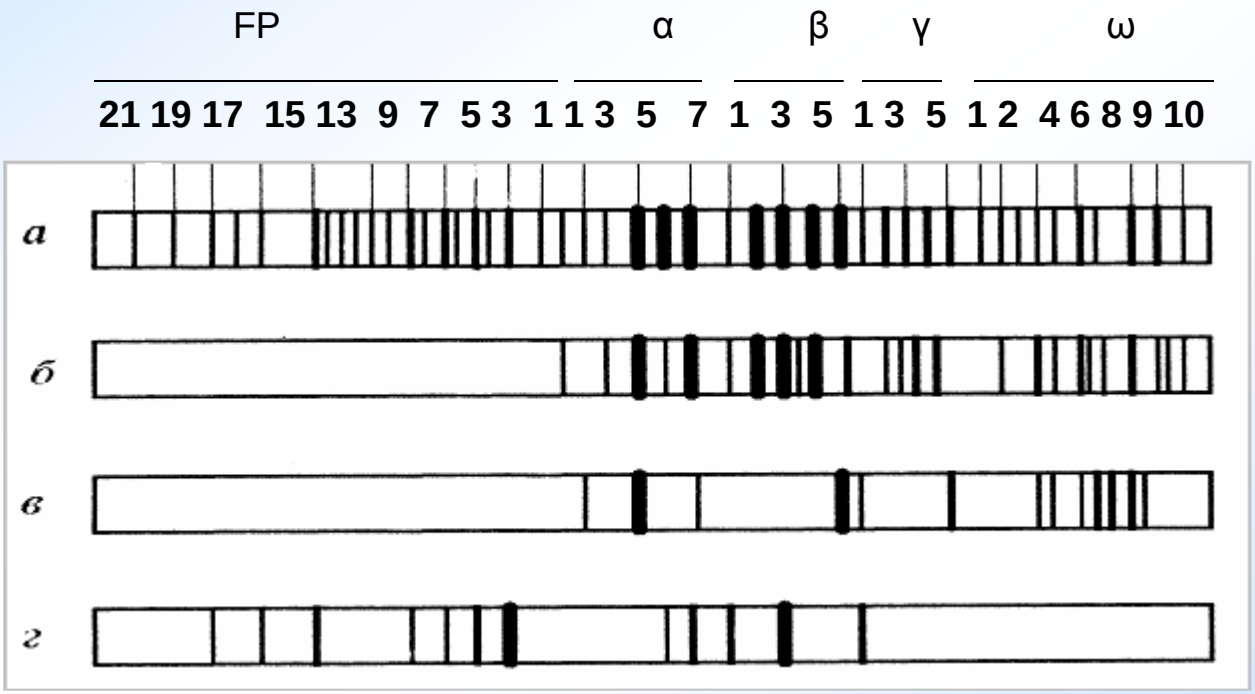
According to our data, the popular rolled oats for 40% or more consist of barley.

A paramount condition for implementing useful properties of a crop is the compliance of the contents of a sack, package, car, etc. with what is written on them.

It is equally valid for the “rolled oats” sold to the population, for seed collections held by genebanks, for breeding centers, for bulk grain (seed) batches, and so on.

Methods of crop variety identification according to the spectra of storage proteins developed with VIR’s participation and accepted as international standard techniques make it possible to determine with high accuracy the specific and varietal attribution of plant accessions (V.G. Konarev, I.P. Gavrilyuk, N.K. Gubareva, T.I. Peneva et al., 1972-1975).

Etalon electrophoretic pattern includes all possible protein component positions on the data of world collection study. Using etalon the varieties, biotypes of wheat, Barley, Oat are written down in form of protein formulae



а - **etalon electrophoretic pattern; protein formulae:**

б – **wheat (Var. Mir.808)** $\alpha 24\underline{5}6\underline{7}_1$ $\beta 1\underline{2}3_2\underline{4}5_2$ $\gamma 2_1 2_3\underline{3}\underline{4}$ $\omega 24_2 5\underline{6}_2 8_1 9_1 9_3 10_2$

в – **barley (Var. Krinichny)** α 357 $\beta\underline{5}_1 5_2$ $\gamma 5$ $\omega 4_1 4_3\underline{6}_3 7_2\underline{8}_{1,2}$

г – **oat (var. Astor) Fast Prolamines (FP) БП 17 15 7 5 3**$\underline{1}$ α 6 7_1 $\beta 1\underline{3}_2 5_2$

VIR (Vavilov Institute) has many years of experience in the use of protein markers to monitor the genetic integrity of its collection.

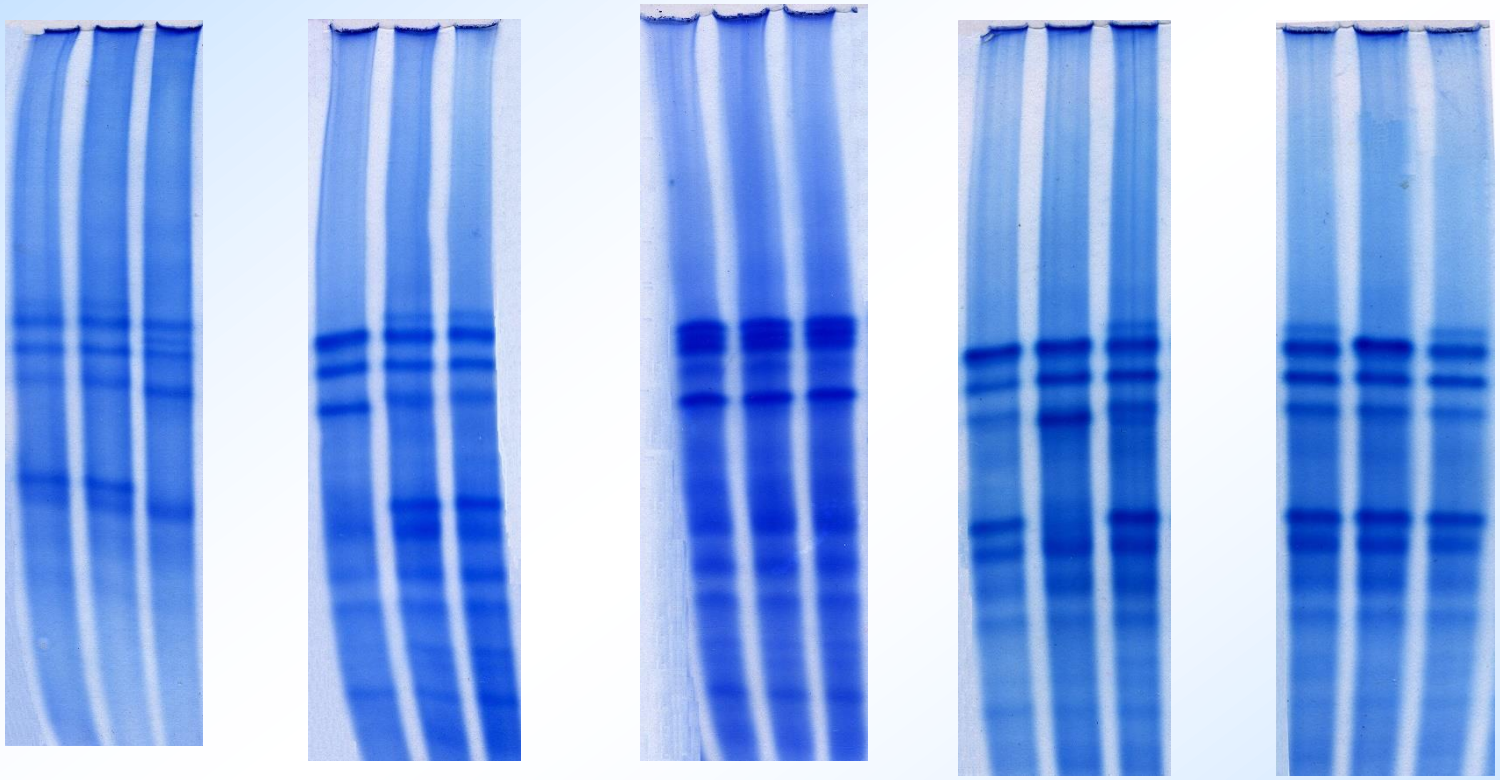
It is especially topical for the accessions of old and local varieties collected 80-100 ago (in N.I. Vavilov's times), as due to their age they were reproduced many times.

The marker system makes it possible to work with individual kernels and characterize the genotypic composition of accessions conserved for a long time: more than 100 years for cereal grasses.

Such works has been conducted in many genebanks of the world on different crops, also in cooperation with VIR (A.Konarev et al., 2005; I.N. Perchuk et al., 2016).

Using avenin patterns 175 original cultivated oat accessions (local old varieties of VIR collection from N.I.Vavilov times) and its reproductions 1989-2000 were compared.

Fig. 1. Avenin spectra of different reproductions of the sample k-8077 compared to the original. Visible differences.



Original, Kishinev, 1950

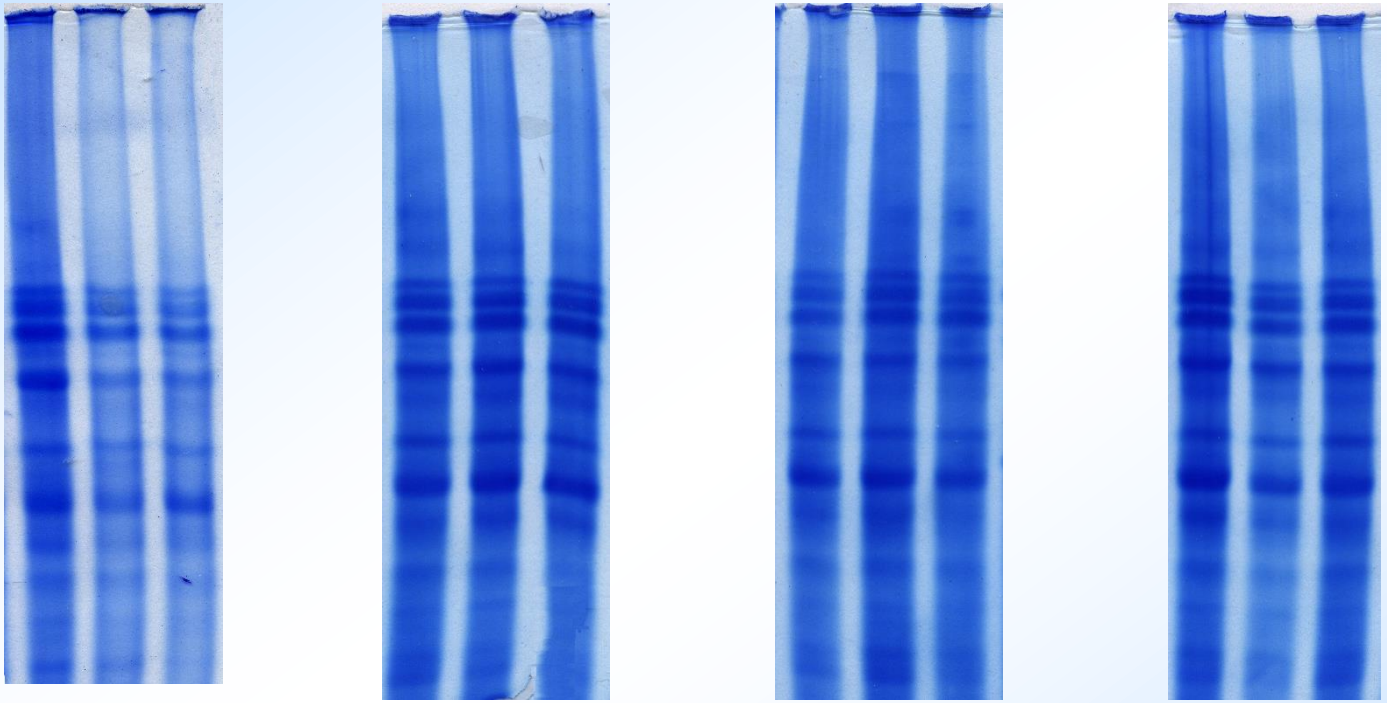
Repr., Mikhnevo, 1976

Repr. Ukraine, 1976

Repr. KExp.St, 1994

Repr. Kexp.St, 1995

Fig. 2. Avenin electrophoretic patterns of accessions к-2896.
Reproductions identical to the original



Original, 1922

Reproduction, 1977

Reproduction, 1992

Reproduction, 1999

Duplicate accession identification

Methods for identifying duplicate accessions in genebanks and the term “duplicate accession” are discussed. Field evaluation of crop accessions is interfaced with phenotypic variability of the majority of characters used in monitoring and also with its duration. Molecular methods are more objective and reproducible in different laboratories, which is important for coordination the work of different genebanks.

Our last work presents the results of comparative analysis performed on 112 pairs (potential duplicates) of cultivated oat (*Avena sativa* L.) from VIR and NordGen according to morphological and breeding-oriented characters as well as component composition (spectra) of avenin. The revealed conformity between the results of the field and laboratory tests shows that it is possible to use protein markers (avenin spectra) for identification of duplicate accessions in oat collections even before field trials.

Oats as an example of a healthy (functional) food product

- Oats – culture unique to obtain a dietary and healing food. Proteins of oats is easily digestible.

The high content in oat grain polysaccharides – beta-glucans with daily consumption of oats significantly reduces the risk of cardio-vascular disease (Factor Liveliness of Horses).

Oat oil and starch has dietary properties. In the grain contains antioxidants.

Oats are officially recognized (FoodDrugAdm., USA) a functional product.

Oats are valuable as a source of forms is non-toxic in celiac disease – the disease of humans in the use of the flour of wheat, barley (and sometimes oats).

Search for non-toxic varieties and forms of oats are progressing in collaboration with nutritionists and physicians SPb (see the stand).

Hulless forms of oats have a higher dietary and medicinal properties.

In VIR, where a rich collection of oats, and is working to find and introduce valuable forms in breeding and practice.

OAT VARIETIES FOR THE PRODUCTION OF GLUTEN-FREE PRODUCTS

The aim of this work is to investigate the composition of proteins in grains of different oat varieties and the degree of their toxicity in celiac disease.

The composition of proteins was determined by electrophoresis. The intensity of reaction of proteins studied grains with IgA and IgG were estimated by the immuno-chemical analysis with sera of patients with celiac disease and healthy people.

All patients diagnosed with celiac disease at the DNA markers by PCR identified genes predisposing to celiac disease, control group, these genes do not exist.

- Installed: some patients react to oats proteins as intensely as wheat proteins. Others also weak, as healthy people.
  This testimony pronounced the individual response of patients to the proteins of oats.
  The individuality of reactions emerged with varieties of hulless oat that has a number of advantages in food and processing qualities. Varieties of Pushkin and Rhiannon proved to be nontoxic for 3 of the 4 patients.

Conclusion

- For 40% - almost all varieties are toxic. 10% - respond differently to the different varieties of oats.
- One of the main tests for the diagnosis of celiac disease is the reaction of immunoglobulins of the patient with the protein of wheat (gliadin).
- With the aim of individualization of the diet appropriate to include in the test system evaluation of the reaction with the protein of oats.
- The inclusion of oats in gluten-free products to diversify the diet and increase the range of food for patients diagnosed with celiac disease.

THE METABOLOMIC APPROACH TO THE COMPARATIVE ANALYSIS OF WILD AND CULTIVATED SPECIES OF OATS (*AVENA* L.)

Seed metabolomic profiles have been investigated in wild and cultivated forms (cultivars) of oat (*Avena* L.). Seed accessions from the VIR oat collection were used for the research. Metabolome analysis employed gas liquid chromatography-mass spectrometry (GLC-MS) using an Agilent 6850 chromatographer (USA).

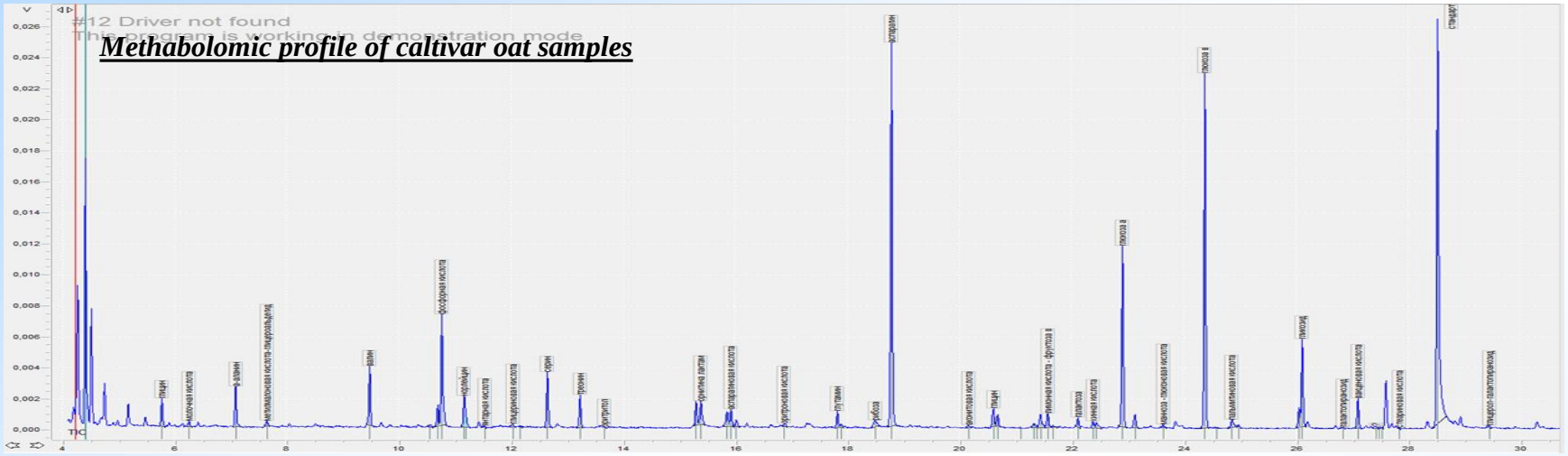
The analysis included composition and contents of organic and fatty acids, amino acids, polyatomic spirits and sugars. The content fluctuation range for the studied groups of compounds was found to be narrower (significantly in some cases) in cultivars than that in the wild species. Along with a sharp increase in the oleic acid content, cultivars demonstrated a decrease in that of the linoleic acid.

THE METABOLOMIC APPROACH TO THE COMPARATIVE ANALYSIS OF WILD AND CULTIVATED SPECIES OF OATS (*AVENA* L.)

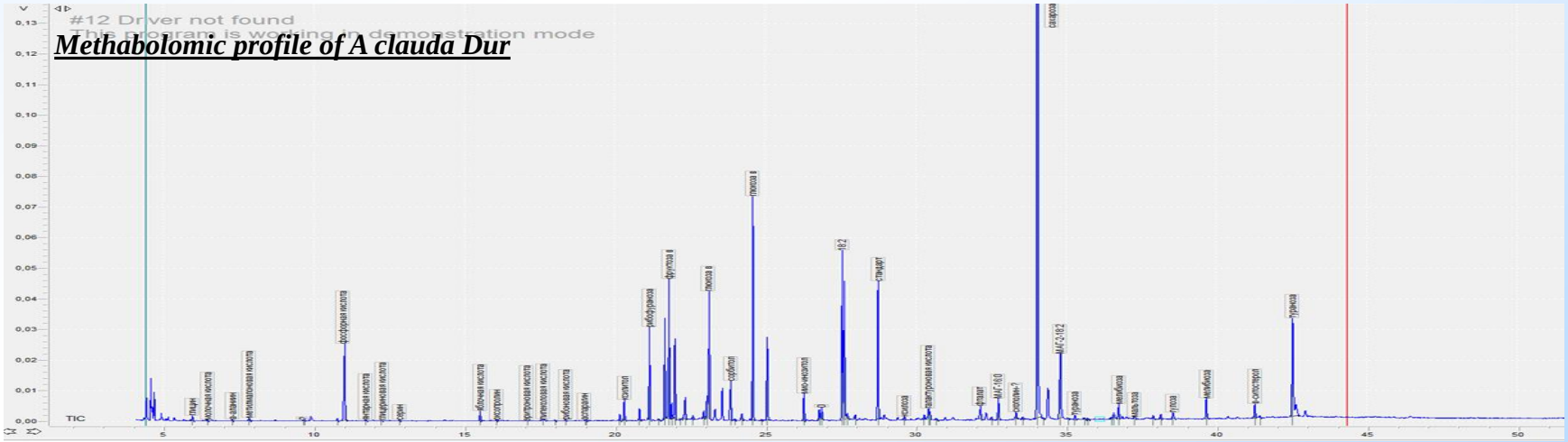
Application of a new methodological approach has proved that the global collection of oat conserved at VIR is a valuable source of different forms that possess economically important traits with a varying degree of their manifestation, and thus can serve as a reliable basis for creating cultivated forms (cultivars) that can meet modern requirements of the food and feed industries, as well as of dietotherapy, etc. A number of wild species can be recommended as a potential source of biochemical quality traits for breeding purposes.

A series of identified metabolites (compounds) was found either to change in quantity in the course of domestication, or differentiate wild oat species from cultivars of the crop. Along with such well-known healthy food chemical factors as oleic acid, glucose and fructose, etc., differences concerning monoacylglycerol compounds (MAG 16:0 and MAG-2-18:2, etc.) have been found. The latter have been supposed to be related to the formation of adaptive traits, in particular, to resistance to diseases and pests, and to environmental abiotic stresses.

Methabolomic profile of caltivar oat samples

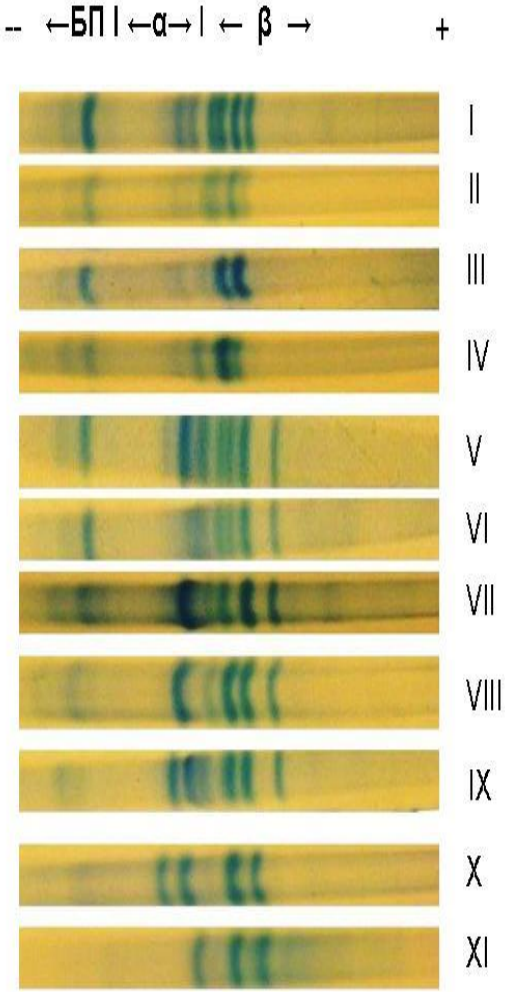


Methabolomic profile of A clauda Dur



- Avenin electrophoresis - in seed production and seed control

Electrophoresis of avenin in seed production and seed control of oats (identification fatwaid and the European part of the USSR)



•*Avenin types spectra var. Privet, separated from his fatwaid (I-IX) and wild oat (X—XI)*

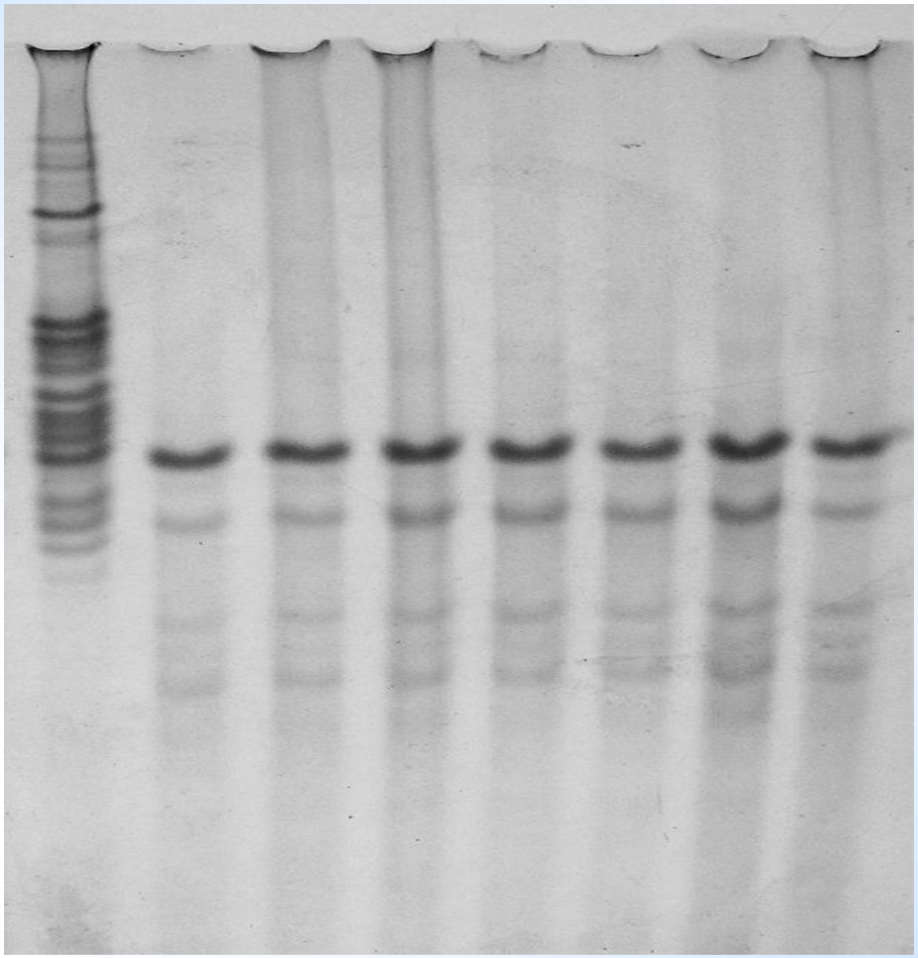
In crops of oats appear fatbody plants with morphological characteristics of wild oat – the wild oat. This ground for rejection of the party of oats.

The problem fatwaid escalated to varieties-populations

(var. Privet).

In the spectra of Avenida weevil varieties hi and fatbody identical and differ from the European part of the USSR. Detection fatwaid, with the spectra of oats should not be cause for rejection of seed lots of oat.

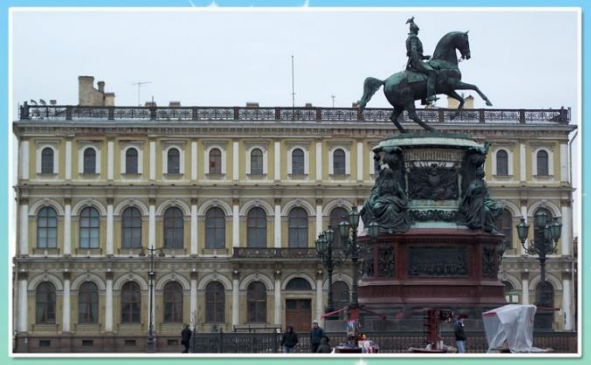
Oats S. Borrus. Seeds original from Germany (1), “superelite”(2) and the second reproduction (3-7) ZAO PZ Gomontovo homogeneous, and identical on avenin spectra



1 2 3 4 5 6 7

1915-2006

- Ideas N. Vavilov and N. N. Ivanov were on a modern level, implemented by VIR V. G. Konarev and his disciples. Received the development of molecular and others methods of exploration, including the identification of plant genetic resources and seed control (1967-2010.).



Коллектив авторов

OATS 2016

БЛАГОДАРЮ ЗА ВНИМАНИЕ!

THANK YOU FOR YOUR ATTENTION!