E

BMT/15/15
ORIGINAL: English
DATE: April 19, 2016

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Fifteenth Session

Moscow, Russian Federation, May 24 to 27, 2016

NEW DEVELOPMENTS CONCERNING BIOCHEMICAL AND MOLECULAR TECHNIQUES IN BELARUS

Document prepared by an expert from Belarus

Disclaimer: this document does not represent UPOV policies or guidance

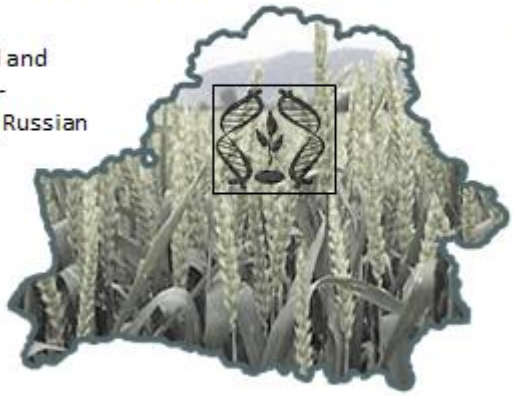
The Annex to this document contains a copy of a presentation “New developments concerning biochemical and molecular techniques in Belarus” to be made at its fifteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

Siamashka Tatiana, Deputy Director for DUS testing, Republican Central Laboratory for the Assessment of the Quality of New Varieties of Plants, State Inspection for Testing and Inspection of Plant Varieties, Belarus

[Annex follows]

New developments concerning biochemical and molecular techniques in Belarus

Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular, Moscow, Russian Federation, May 24-27, 2016



Deputy Director for DUS testing
Siamashka Tatiana

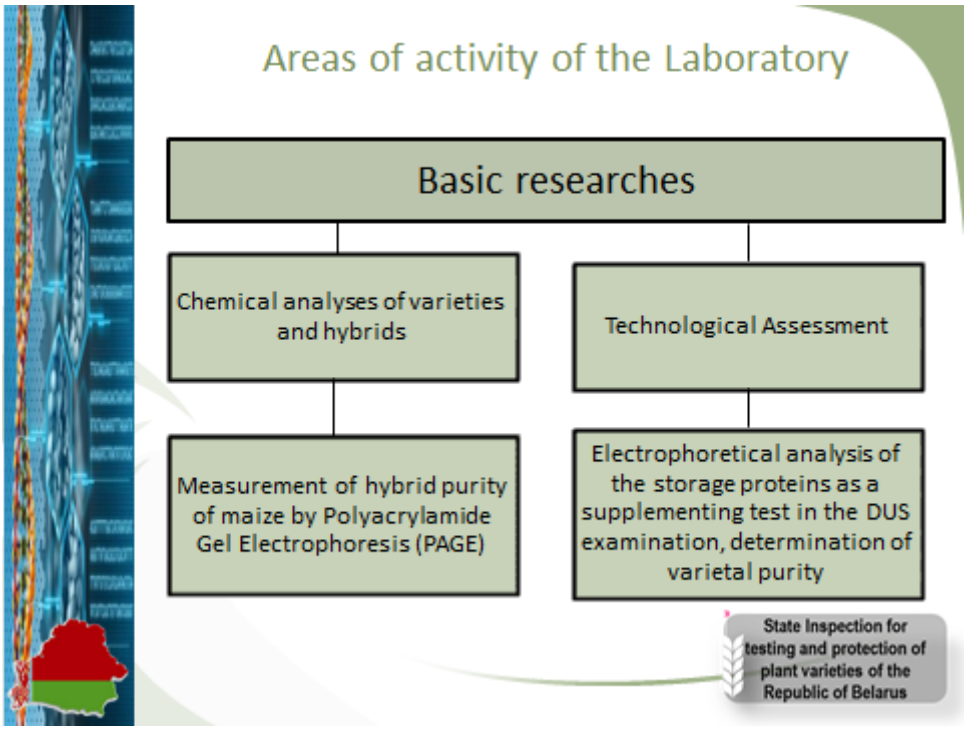
State Inspection for testing and protection of plant varieties of the Republic of Belarus

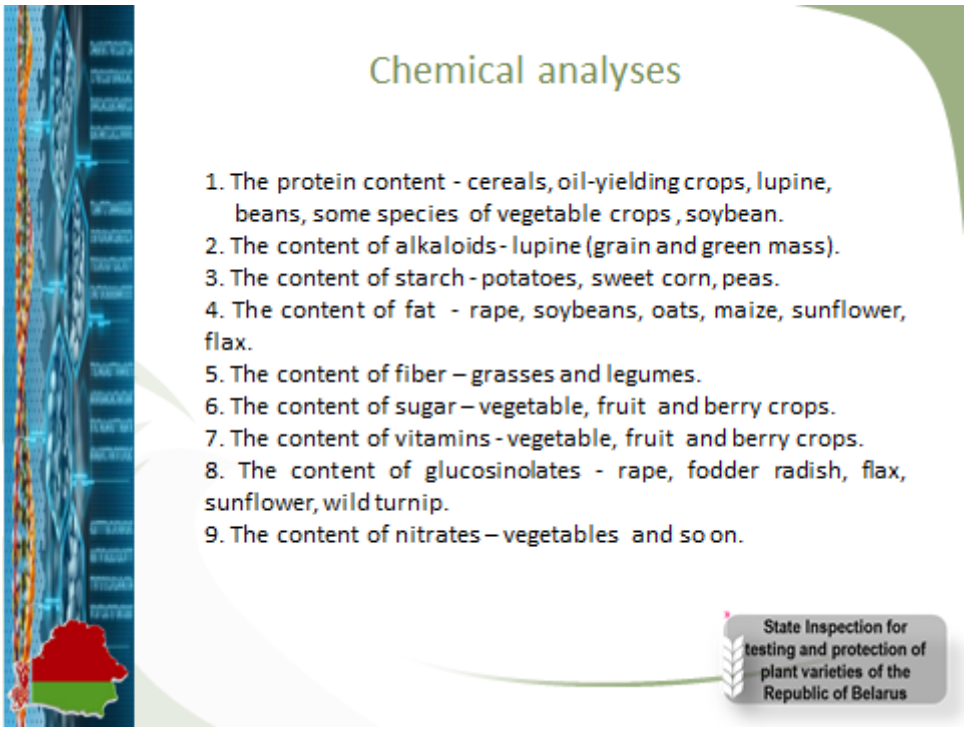
SE «State Inspection for Testing and Protection of Plant Varieties»

Republican Central Laboratory for the Assessment of the Quality of New Varieties of Plants

Minsk
The Republic of Belarus

State Inspection for testing and protection of plant varieties of the Republic of Belarus


Areas of activity of the Laboratory
Basic researches
Chemical analyses of varieties and hybrids
Technological Assessment
Measurement of hybrid purity of maize by Polyacrylamide Gel Electrophoresis (PAGE)
Electrophoretical analysis of the storage proteins as a supplementing test in the DUS examination, determination of varietal purity
State Inspection for testing and protection of plant varieties of the Republic of Belarus


Chemical analyses
1. The protein content - cereals, oil-yielding crops, lupine, beans, some species of vegetable crops , soybean.
2. The content of alkaloids - lupine (grain and green mass).
3. The content of starch - potatoes, sweet corn, peas.
4. The content of fat - rape, soybeans, oats, maize, sunflower, flax.
5. The content of fiber – grasses and legumes.
6. The content of sugar – vegetable, fruit and berry crops.
7. The content of vitamins - vegetable, fruit and berry crops.
8. The content of glucosinolates - rape, fodder radish, flax, sunflower, wild turnip.
9. The content of nitrates – vegetables and so on.
State Inspection for testing and protection of plant varieties of the Republic of Belarus

The protein content

Ignition block Gerhardt

Distillation unit Vapodest 45 with titrator TitroLine Easy

Infralum FT-10

Infratec

State Inspection for testing and protection of plant varieties of the Republic of Belarus

The content of starch

Automatic Polarimeter AP-300

Photocolorimeter

The content of alkaloids

State Inspection for testing and protection of plant varieties of the Republic of Belarus

The content of fat

SOXTHERM automatic rapid extraction system

Soxtec system HT 2 Extraction Unit

State Inspection for testing and protection of plant varieties of the Republic of Belarus

The content of Cellulose

Fibertec 2010
Hot Extraction

State Inspection for testing and protection of plant varieties of the Republic of Belarus

The content of glucosinolates in oil-yielding crops and assessment of fatty acids

Gas chromatograph GC-2010

Liquid chromatograph LC-2010 A

State Inspection for testing and protection of plant varieties of the Republic of Belarus

The content of Nitrates

Knife Mill Grindomix GM 200

Ionmeasurer EV-74

State Inspection for testing and protection of plant varieties of the Republic of Belarus


Technological Assessment: the baking properties.
State Inspection for testing and protection of plant varieties of the Republic of Belarus

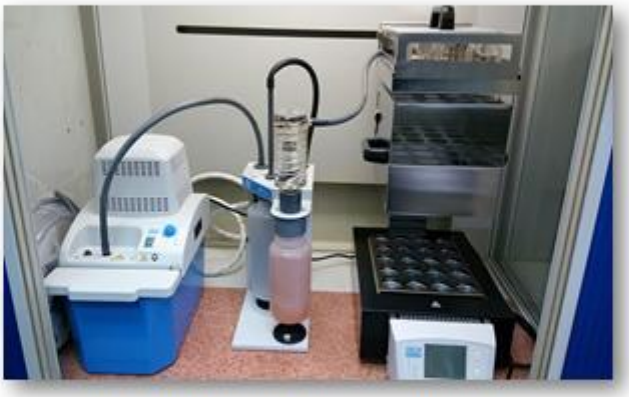

Technological Assessment: the malting value.
Micromalting machine M-3BX
Screening BUHLER
Mill BUHLER
FRIABILIMETER
Mashing Device LB 8 Electronic
State Inspection for testing and protection of plant varieties of the Republic of Belarus

Nitrogen analysis and protein determination

MIXOLAB 2

State Inspection for testing and protection of plant varieties of the Republic of Belarus

Nitrogen analysis and protein determination



Fully Automatic Kjeldahl Digestion Units

State Inspection for testing and protection of plant varieties of the Republic of Belarus

Automatic Distillation and Titration System under Kjeldahl Method (TKN)

The definition of moisture in solid and granular material

VELP Scientifica UDK-159

State Inspection for testing and protection of plant varieties of the Republic of Belarus

Automatic dual analysis system

Falling Number FN1700

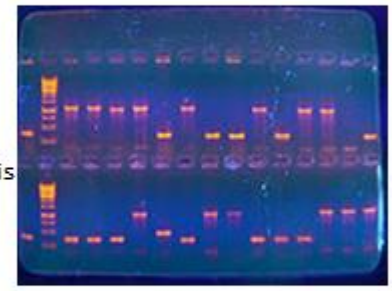
Diaphanoscope «Yantar»

The definition of glassiness in wheat grains

State Inspection for testing and protection of plant varieties of the Republic of Belarus

Measurement of hybrid purity of maize by Polyacrylamide Gel Electrophoresis (PAGE)

1. Measurement of hybrid purity of maize, identification of hybrids. Laboratory accreditation by the State Committee for Standardization of the Republic of Belarus (Gosstandart) on this analysis



State Inspection for testing and protection of plant varieties of the Republic of Belarus

Electrophoretical analyses of the storage proteins (PAGE) as a supplementing test in DUS examination, determination of varietal purity

Laboratory accreditation by The State Committee for Standardization of the Republic of Belarus (Gosstandart) on this analysis

1. Identification of wheat, barley, triticale and oats varieties, determination of varietal purity.
2. Identification of varieties of leguminous crops and determination of varietal purity.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

Electrophoretical analyses of the storage proteins (PAGE) as a supplementing test in DUS examination, determination of varietal purity

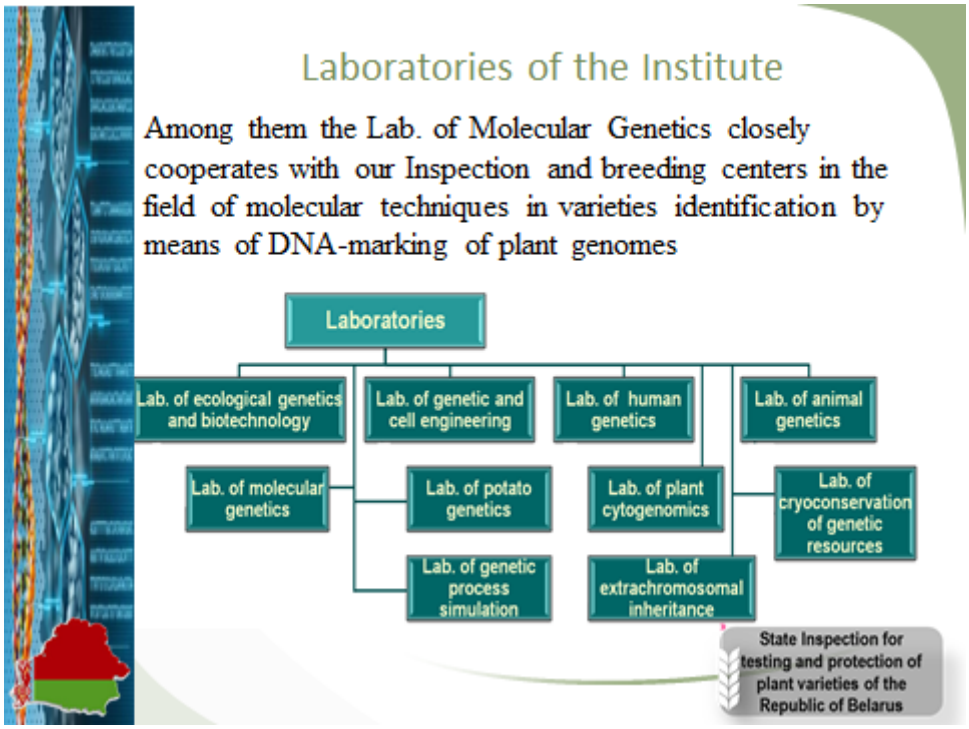
State Inspection for testing and protection of plant varieties of the Republic of Belarus

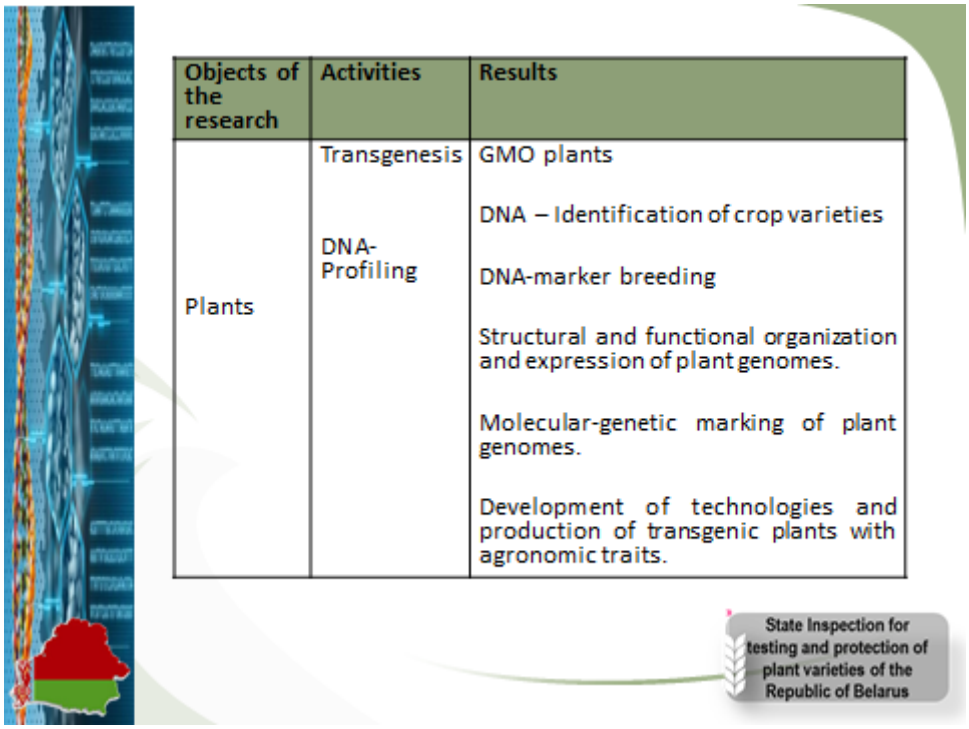
DNA-profiling in variety identification are not implemented in our Laboratory. We have in this field a long-term scientific cooperation with the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus

Grounded in 1965
More than 100 researchers
Doctors of Biological Sciences- 9
Candidates of Biological Sciences- 39

http://gens.by
Director – Lemesh Valentsina Alexandrovna
Tel: +375 (17) 294-93-03

State Inspection for testing and protection of plant varieties of the Republic of Belarus


Laboratories of the Institute
Among them the Lab. of Molecular Genetics closely cooperates with our Inspection and breeding centers in the field of molecular techniques in varieties identification by means of DNA-marking of plant genomes
Laboratories
Lab. of ecological genetics and biotechnology
Lab. of genetic and cell engineering
Lab. of human genetics
Lab. of animal genetics
Lab. of molecular genetics
Lab. of potato genetics
Lab. of plant cytogenomics
Lab. of cryoconservation of genetic resources
Lab. of genetic process simulation
Lab. of extrachromosomal inheritance
State Inspection for testing and protection of plant varieties of the Republic of Belarus


Objects of the research
Activities
Results
Plants
Transgenesis
DNA-Profiling
GMO plants
DNA – Identification of crop varieties
DNA-marker breeding
Structural and functional organization and expression of plant genomes.
Molecular-genetic marking of plant genomes.
Development of technologies and production of transgenic plants with agronomic traits.
State Inspection for testing and protection of plant varieties of the Republic of Belarus

Scientific and methodological support

Our Inspection provides scientific and methodological support on contractual basis for the correct development of these technologies in conformity with the UPOV Convention.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

Jointly conducted analytical work

Verification of the reliability of the link between the marker and the morphological characteristic

- Gene that determines the growth and development of the main shoot - Self-pruning (Sp)

State Inspection for testing and protection of plant varieties of the Republic of Belarus

DNA-marker breeding of plants (SSR markers)

Marker-related crop breeding technologies are designed, which can effectively identify genes of resistance and quality at any stage of plant development;

Research work is carried out on 70 genes associated with disease resistance and quality of products

Crop	Economic-valued characteristic
Potato	Resistance to diseases (phytophthora X, Y, L- viruses) and pests (nematode)
Tomato	carotenoid content, duration of storage, resistance to Cladosporium fulvum Cooke.
Wheat	Determination of varieties of durum wheat, the baking properties, resistance to leaf rust, short-stemmed
Apple	fruit ripening and storage; resistance to scab, powdery mildew, apple aphid
Rape	fatty acid content in the seeds
Soybean	photoperiodic response
Barley	the malting value
Flax	acid content

State Inspection for testing and protection of plant varieties of the Republic of Belarus

DNA-marker breeding of tomato

Developers: SSI "Institute of Genetics and Cytology of the National Academy of Sciences"
EE "BSAA" RUE "Institute of Horticulture»»

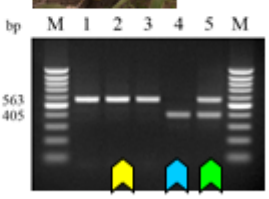
The following methods of DNA-typing were developed:

- Genes of high and altered carotenoid content- *Beta carotene (B), old-gold (og), old-gold crimson (ogc), tangerine (t), yellow-flesh (r), Delta (Del), high pigment 1 (hp-1), high pigment -2dg (hp-2dg), green flesh (gf), green flesh-3 (gf-3), green flesh-5 (gf-5), t (tangerine)* **and anthocyanin**(*Ant-1*)

- Genes of long storage of fruits- *ripening inhibitor (rin), non-ripening (nor) и alcobaça (norA)*

- Genes that determine the growth and development of the main and side shoots- *Self-pruning (Sp), Blind (bl-1, bl-2, to-1) u Lateral suppressor (ls1, ls2).*

- Genes of resistance to brown spot*Cf-6, Cf-2, Cf-5, Cf-4, Cf-9;* ***to fusarium:*** *I-2, I-3;* **to nematode** *Mi-1.2*



Was created the collection of constant highly-productive lines with long storage period and highly-carotinoid forms of tomato.

Were bred 14 varieties of tomato with complex disease resistance. Created molecular genetic passports.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

DNA-marker breeding of sweet pepper

Developers: SSI "Institute of Genetics and Cytology of the National Academy of Sciences, UU «BSCA», «VNIISOK»

The following methods of DNA-typing were developed:

-Genes of long storage of vegetables– *polygalacturonase (S), ripening inhibitor (rin), non-ripening (nor)* (3 гена);

Variety Маяк (*Sp+, Ccs+, cl–, Psy+, S–, nor–, rin+/ rin –*)

- Genes of high and altered carotenoid content - *chlorophyll retainer (Cl), capsanthin-capsorubin synthase (Ccs), Psy (phytoene synthase)*

- Gene that determines the growth and development of the main shoot - *Self-pruning (Sp)*

Variety Л-45-11 (*Sp⁻, Ccs⁻, cl⁺, Psy⁺, S⁻, nor⁺, rin⁺/ rin⁻*)

Variety Желтоплодный (*Sp⁻, Ccs⁻, cl⁻, Psy⁺, S⁻, nor⁺, rin⁺/ rin⁻*)

Variety Л-236/09 (*Sp⁻, Ccs⁺, cl⁺, Psy⁺, S⁻, nor⁻, rin⁺/ rin⁻*)

Variety Л-160-10 (*Sp⁻, Ccs⁺, cl⁻, Psy⁺, S⁻, nor⁺, rin⁺/ rin⁻*)

DNA- typing was carried out with populations of F2 and forms of sweet pepper, were created lines with the combination of 2 and 3 genes of quality. Under the basis of selected forms were created 12 hybrids F1 with a complex of vegetable quality genes. Created molecular genetic passports.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

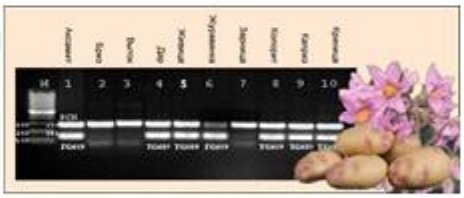
DNA-marker breeding of potato

Developers: SSI "Institute of Genetics and Cytology of the National Academy of Sciences", Institute of potato

Proven methods of DNA identification of 11 most important for the breeding of potato genes of resistance to diseases and pests.

L-virus (ВС/ЛК)

On the basis of a DNA-profiling were screened potato varieties of domestic and foreign selection for resistance to the X-, Y- and L-viruses and potato nematode. Were selected the forms, promising for breeding.



X-virus

Y- virus

potato nematode

The results of DNA-profiling of resistance to potato cyst nematode (1, 4-6, 8-10 – varieties with gene H1, determining the resistance to type Ro1 of nematode) Created molecular genetic passports.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

A set of DNA markers of agronomic characters of spring and winter wheat

Developers:
SSI «Institute of Genetics and Cytology of the National Academy of Sciences», SPC NASB for agriculture

PCR analysis on allelic content of:

genes of grain hardness.

genes controlling the synthesis of seed storage proteins – glutenin Glu-A1x, Glu-B1x и Glu-B1y, Glu-D1x и Glu-D1y in varieties and lines of winter wheat. Gene Vp1 that controls the characteristic "Pre-harvest germination"

Created molecular genetic passports.

Wheat material in the amount of 75 hybrid combinations was created.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

DNA-marker breeding of apple

- Developed and introduced in the selection process effective methods (based on DNA-markers) of identifying genes for apple resistance to diseases and pests.
- Created molecular genetic passports of apple varieties grown in Belarus.
- Developed systems of crossing and created hybrids of a new generation with complex resistance to scab, powdery mildew, apple aphids, blight, long-term storage of fruits by means of complying genes in one genome.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

DNA-marker breeding of soybean

Developer:
SSI «Institute of Genetics and Cytology of the National Academy of Sciences»

Development of DNA-technology

Creation of DNA-passports

A genotyping of soybean varieties of Belarusian and foreign breeding. It was established that some varieties (33%) have economically significant E7 allele.

Were developed guidelines describing the use of microsatellite markers for the identification of soybean breeding material.

State Inspection for testing and protection of plant varieties of the Republic of Belarus

DNA-marker breeding of barley under the brewing characteristic

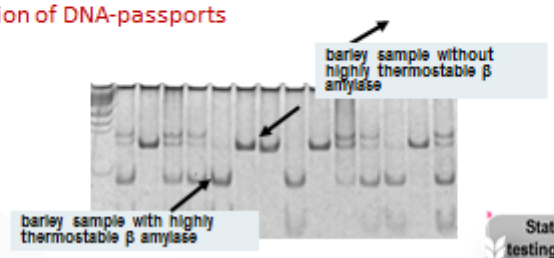
Developers:
SSI «Institute of Genetics and Cytology of the National Academy of Sciences»,
SPC NASB for agriculture

- Developed approach to the differentiation of samples of barley for malting / feed.

- Completed the screening of barley varieties and samples under 6 markers associated with the characteristic of brewing.

- Selected the forms, perspective for breeding.

-Creation of DNA-passports

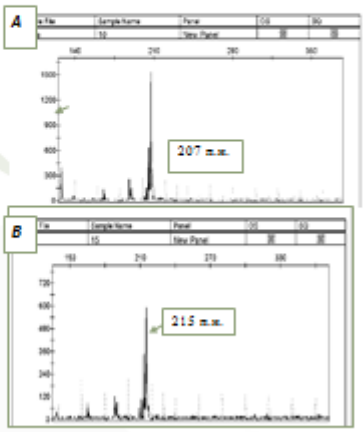


State Inspection for testing and protection of plant varieties of the Republic of Belarus

DNA-marker breeding of flax

Developer:
SSI «Institute of Genetics and Cytology of the National Academy of Sciences»

Carried out the evaluation of polymorphisms of 12 microsatellite loci of varieties of oil flax.



Obtained varieties of rare and unique alleles, as well as varieties with the highest values of heterozygosity SSR loci studied.

Revealed allele Lu8207, which can serve as a marker SSR- low linoli acid content forms of oil flax.

Creation of DNA-passports

State Inspection for testing and protection of plant varieties of the Republic of Belarus

Use of biochemical and molecular techniques

- a supplementing test in DUS examination
- DNA-marker breeding
- in the process of certification of crops as additional testing methods for varietal identity
- by titleholders as a rapid tool in enforcement situations
- creation of National DNA database

State Inspection for testing and protection of plant varieties of the Republic of Belarus

[End of Annex and of document]