E

BMT/15/18
ORIGINAL: English
DATE: May 10, 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

USING OF DNA – MARKER BASED TECHNIQUES FOR VARIETAL IDENTIFICATION AND FINGERPRINTING OF FRUIT CROPS AND GRAPE GENETIC RESOURCES

Document prepared by experts from Russian Federation

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation “Using of DNA – marker based techniques for varietal identification and fingerprinting of fruit crops and grape genetic resources” to be made at its fifteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

Ivan Suprun, head of the laboratory of genetics and microbiology, PhD in biology, North Caucasian Regional Research Institute of Horticulture and Viticulture, Krasnodar, Russian Federation

[Annex follows]

Using of DNA – marker based techniques for varietal identification and fingerprinting of fruit crops and grape genetic resources

Ivan Suprun,
head of the laboratory of genetics and microbiology, PhD in biology,
North Caucasian Regional Research Institute of Horticulture and Viticulture, Krasnodar

Marker – the identificator

Classical genetics: gene with known position and phenotypic characteristics
(phenotypic estimation only)
Development of molecular biology

Molecular markers

↓

Proteins

↓

DNA-markers: polymorphism of DNA

Molecular markers for accessions identification

- storage protein
- isozymes
- DNA-markers

Multi locus
RAPD
ISSR
AFLP
RBIP
REMAP

Locus specific
SSR
SNP

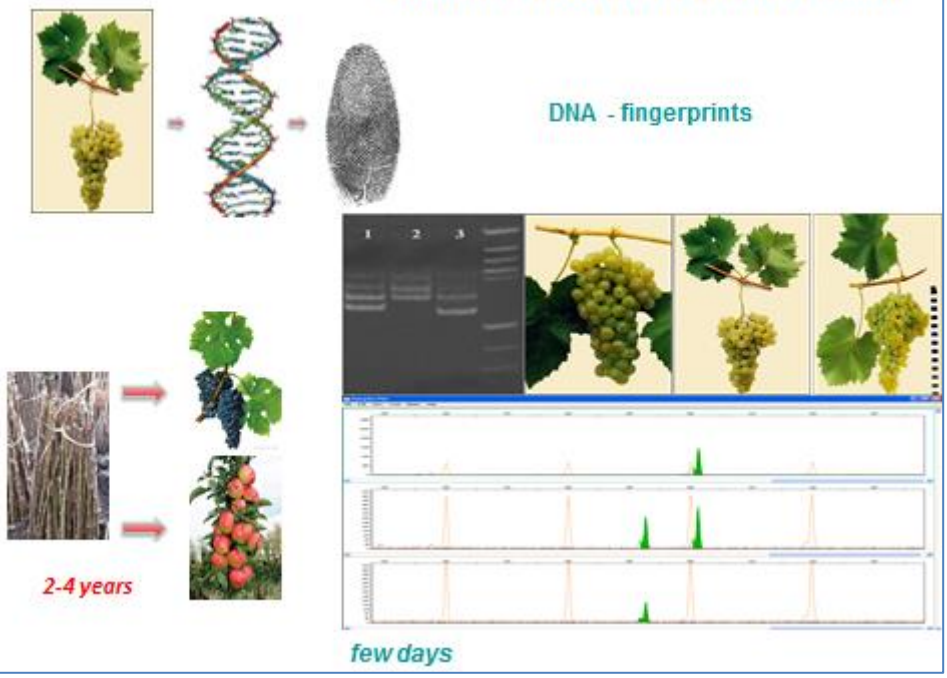
Key characteristics of DNA-markers

- High level of polymorphism
- Results easy to score
- Possibility for automatization
- High level of reproducibility
- Cost/quality

DNA – markers estimate accessions at the genome level

- ✓ Undependable from environment conditions
- ✓ No tissue specificity
- ✓ Can be estimate at the most level of vegetation from the young plantlets

DNA-marker based control of cultivars identity



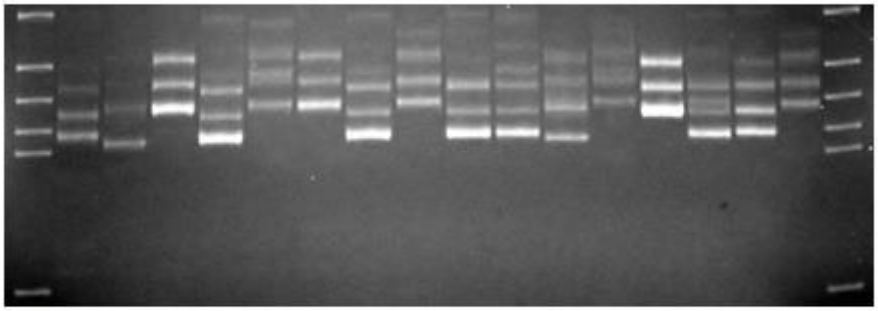
Microsatellite DNA – markers

- high level of polymorphism
- identification of heterozygosity
- uniform distribution on the genome
- locus specificity;
- high level of reproducibility
- possibility for automatization (if use genetic analyzers)
- possibility to use multiplex PCR *(up to 4-6 markers per one reaction)*

Main stages

- Analysis of international scientific publications as well as genetic databases for selection of polymorphic markers;
- Approbation and optimization of experimental parameters;
- Formation SSR multiplex sets;
- Optimization of experimental parameters for fragment; analysis on the genetic analyzer ABIprism 3130;
- Genotyping
- Data processing - GeneMapper 4.1;
- Development of SSR-fingerprints databases

Approbation of SSR-markers with electrophoresis in PAAG (SSR-marker CH03d07)



M – marker of DNA molecular weight; 1-16 apple cultivars samples

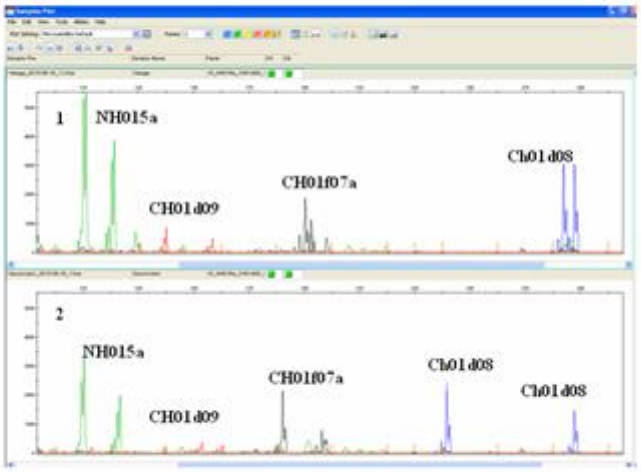
Multiplex SSR-analysis

SSR-маркер	Size range of PCR fragments (base pairs)	Fluorescent label	Wave length
Hi16d02	90-130	FAM	**520** nm
CN445290	230-242	ROX	**605** nm
CH04e03	170-220	R6G	**557** nm

Developed multiplex sets of SSR-markers

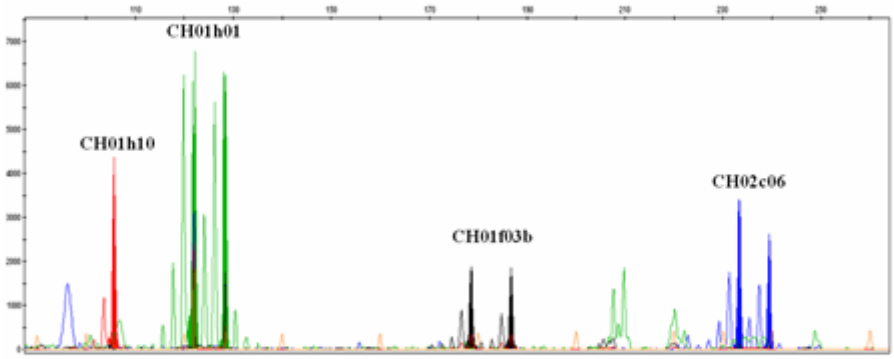
	Apple	Pear	Peach	Plum	Sweet and sour cherry	Grape	Walnut
Amount of SSR-markers	30	22	20	16	18	14	12
Amount of multiplex sets	9	6	7	4	5	4	3

Fragment analysis by SSR multiplex set with markers NH015a, CH01d09, CH01f07a, Ch01d08



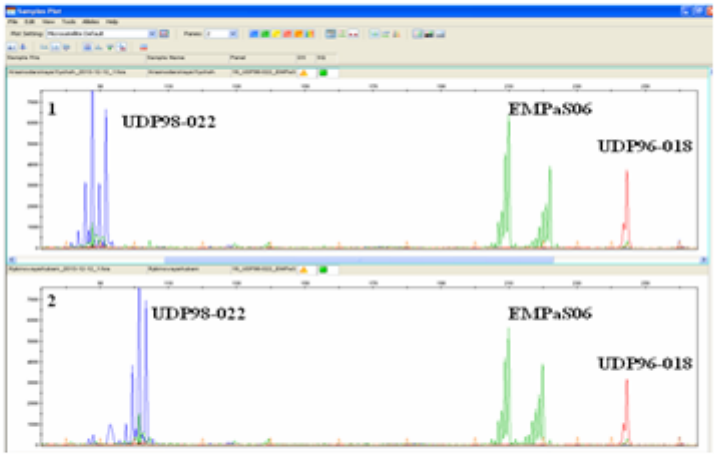
Pear cultivars: 1- 'Velesa', 2-'Samorodok'

Fragment analysis of apple cultivar 'Krasniy Jantar 'SSR multiplex set with markers CH01H10, CH01h01, CH01f03b u CH02c06



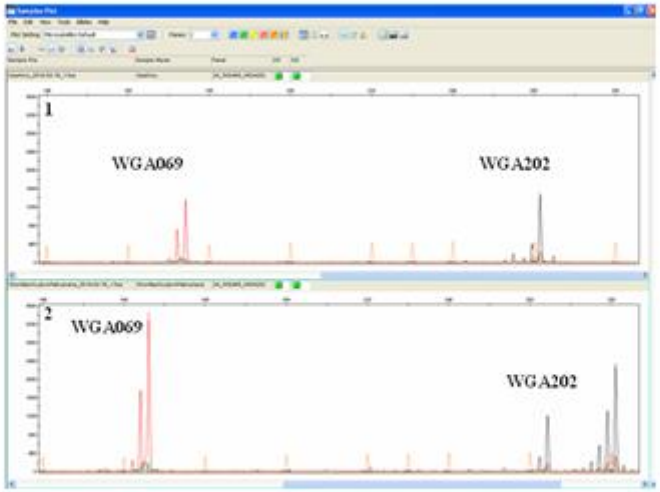
Fragment analysis by SSR multiplex set with markers

UDP98-022, EMPaS06 u UDP96-018



Sweet cherry cultivars 'Krasnodarskaya uluchshennaya'(1) and 'Rubinovaya Kubani'(2)

Fragment analysis by SSR multiplex set with markers WGA069 u WGA202

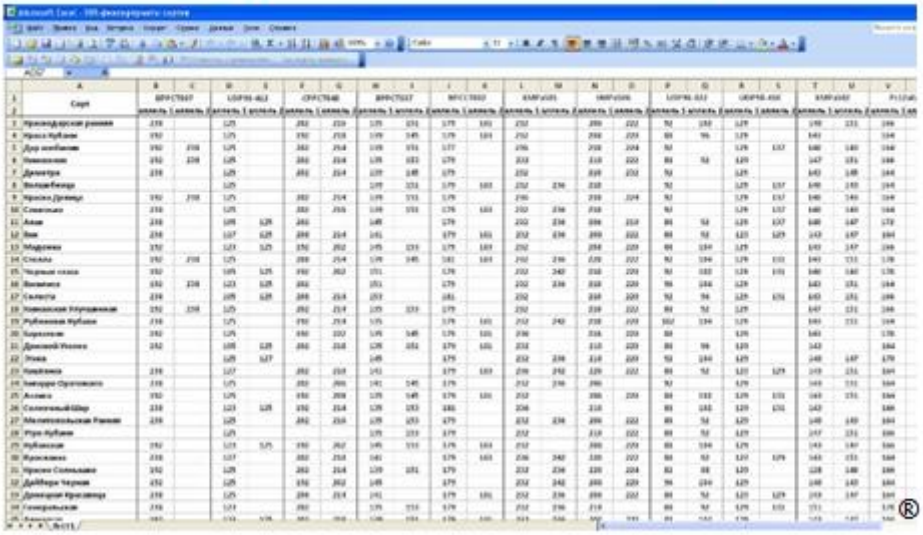


Walnut cultivars 'Izyashnyi' (1) and 'Lyubimets Petrosyana'(2)

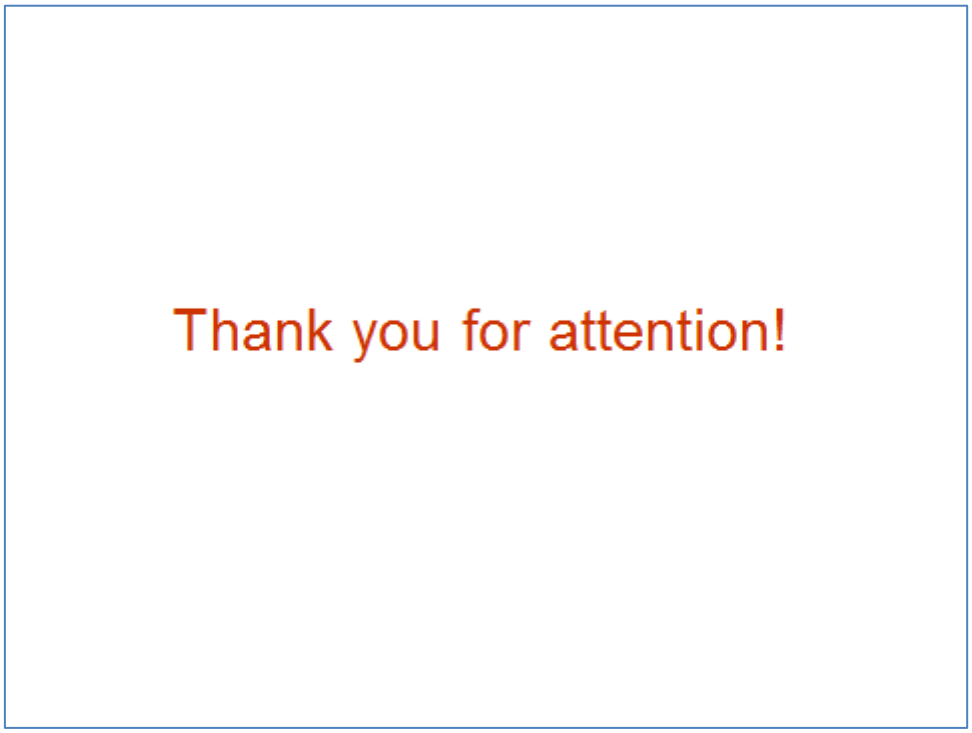
SSR-fingerprints of apple cultivars

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Сорт		CH01f03b			CH01h01			CH01h10			CH02c06		
2			аллель 1	аллель 2	аллель 3	аллель 1	аллель 2	аллель 3	аллель 1	аллель 2	аллель 3	аллель 1	аллель 2	аллель 3
26	Юнона		162	182		120	124		100			238	242	
27	Купава		141	182		120	137		94	100		238	242	
28	Фея		141	148	174	118	120		94	100	104	220	256	
29	Любава		141	162		124			99			242	254	
30	Фортуна		174			118	122		100			204	232	
31	Талида		162	182		120	124		94	100		238		
32	Кармен		162			122	124		100			238	242	
33	Золотое летнее		141	174		120	124		104	112		242	254	
34	Талисман		162	174		122			100			204	254	
35	Василиса		162			120	122		100			238		
36	Рассвет		174			118	120		100	104		204		
37	Родничок		148	174		120			94	100		238	256	
38	Ноктюрн		141	182		124			94	100		242	254	
39	Зефир		148	174		118	120		100	104		220	232	
40	Сокровое 3		148	162		118	120		104	108		246	254	
41	Валюта		148	174		120			94	100		242	256	
42	Благая весть		174			120	122		100	114		204	252	
43	Кубанское румяное		162	174		120	126		100	104		220	232	
44	Имант		174			120	122		94	100		220	256	
45	Осенняя красавица		148	174		120	128		100			232	266	
46	Зори Кубани		174	182		120	122		94	100		254	256	
47	Кубанское багряное		141			122			94	100		252	270	
48	Поспех		162	174		120			100	118		246	256	
49	Память Коваленко		153	162		118	120		100			232	254	

SSR-fingerprints of sweet cherry cultivars



SSR-fingerprints databases



[End of Annex and of document]