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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

ASSESSMENT AND CLASSIFICATION OF BREEDING ACCESSIONS OF VEGETABLE PLANTS WITH THE USE OF DNA MARKERS

Document prepared by experts from Russian Federation

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The Annex to this document contains a copy of a presentation “Assessment and classification of breeding accessions of vegetable plants with the use of DNA markers” made at its fifteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

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[Annex follows]

Assessment and classification of breeding accessions of vegetable plants with the use of DNA markers

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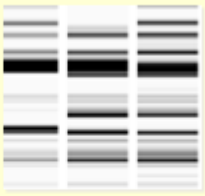
The common plant breeding accessions

Species – groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups. Species are the largest and most inclusive reproductive communities of sexual and cross – fertilizing individuals that share in a common gene pool (Mayr 1940; Dobzhansky 1950)
Variety – in classical taxonomy, heterogeneous grouping including non genetic variation of the phenotype, morph, domestic breeds, and geographic races (Mayr 1963).
Cultivar - grouping of plants selected for desirable characteristics that can be maintained by propagation.
Cultivar groups – the most closely related cultivars used for a long time for specific environment condition
Biotechnologically produced plants – DH, cloned accessions, genetically modified etc.

Variation matching in DNA sequence

Lack of sufficient locus (200-600 b.p.) in one genome and its presence in genome of other genotype
Differences in PCR product migration revealed by methods RAPD, AFLP, ISSR.....



Difference in 5-10 nucleotides at loci of genotypes studied by methods SSR, STS....

Variation of one nucleotide at locus studied by methods SSR, SNP, CAPS....

Phylogenetic relationships among Brassica species «U-triangle» (U, 1935; Karpechenko, 1922)

Naturally occurred hybridization of these species produced

- B.rapa (leafy Asian genotypes turnip, bird rape, nappa cabbage – genome A, n=10),
- B.nigra (black mustard - genome B, n=8),
- B.oleracea (head cabbage, Broccoli, Brussels sprout, Kohlrabi, Savoy, cauliflower , ornamental – genome C, n=9),

allotetraploid forms

- B. juncea (green mustard - AB, n=18),
- B.napus (rapeseed, rutabagas - AC, n=19),
- B.carinata (Abyssinian mustard, Ethiopian mustard) - BC, n=17).

«U-triangle»
(U, 1935; Karpechenko, 1922)

B.rapa
(геном A, n=10)

B.juncea
(AB, n=18)

B.napus
(AC, n=19)

B.nigra
(геном B, n=8)

B.carinata
(BC, n=17)

B.oleracea
(геном C, n=9)

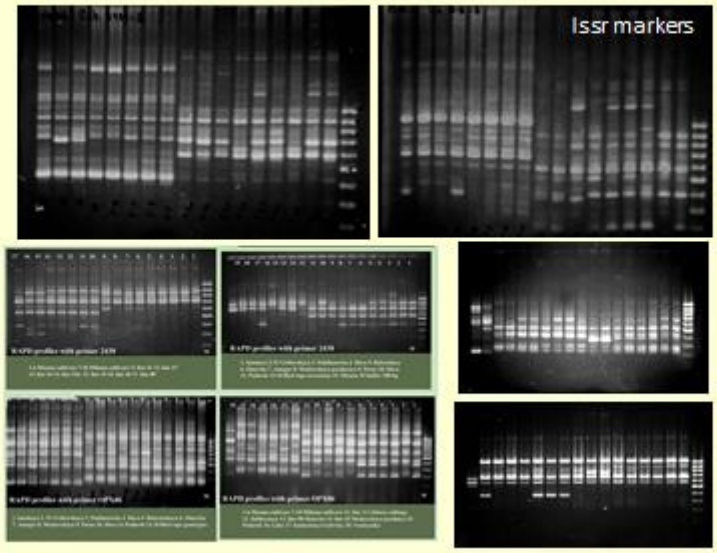
.

Plant material

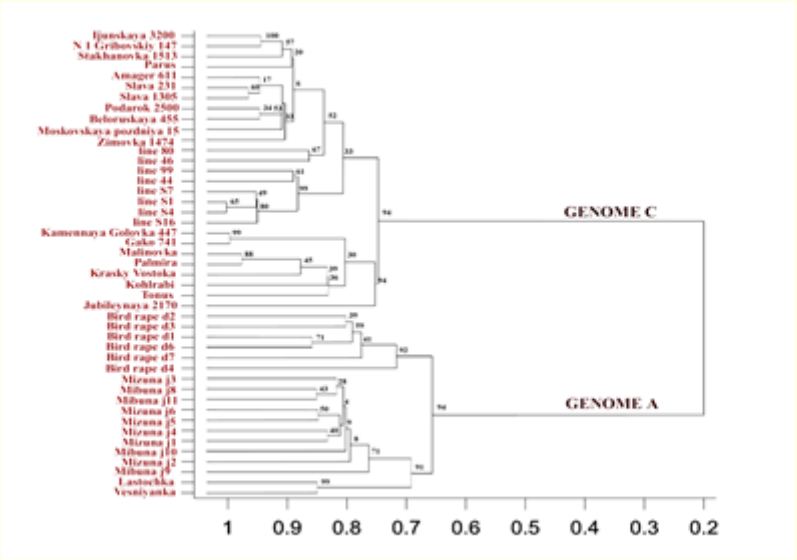
genome C was represented by 12 commercial cultivars and 8 inbred lines of head cabbage *Brassica oleracea* convar. *capitata(L.) Alef.* var. *capitata L. f. alba DC.*,
2 cultivars of red cabbage (convar. *Capitata (L.) Alef.* var. *capitata L. f. rubra(L.)* Thell.),
3 cultivars of ornamental cabbage (convar. *acefala D.C.)*,
1 cultivar of broccoli (var. *cymosa Duch)*,
1 cultivar of Savoy cabbage (convar. *capitata(L.) Alef.* var. *sabauda L.)*,
1 accession of kohlrabi (convar. *acefala(DC.) Alef.* var.*gongylodes L.)*

genome A,
2 cultivars of Chinese cabbage *Brassica rapa* ssp. *chinensis* (L.) Hanelt.,
6 accessions of bird rape (ssp. *oleifera* (DC.) Metzger f. *biennis*),
10 accessions of Mizuna and Mibuna salad green (ssp. *nipposinica* (Bailey) Hanelt)
from All-Russian Research Institute of Vegetable Breeding and Seed Production and N.I. Vavilov Research Institute of Plant Industry.

Examples of agarose gel electrophoregams

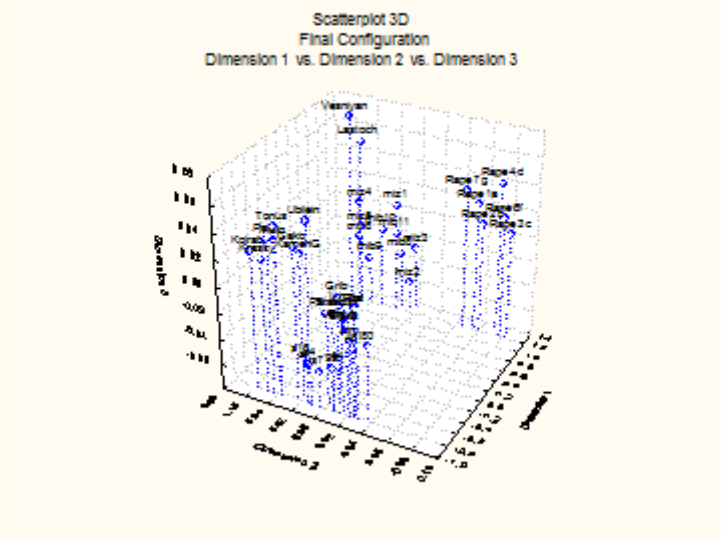


DNA classification of Brassica breeding accessions

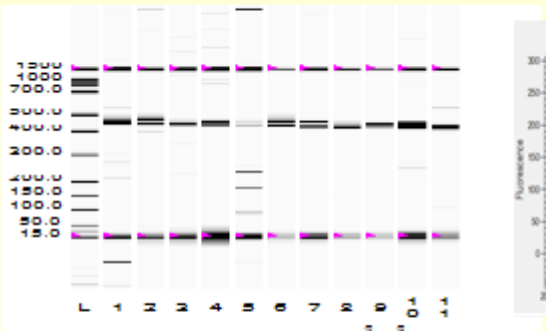


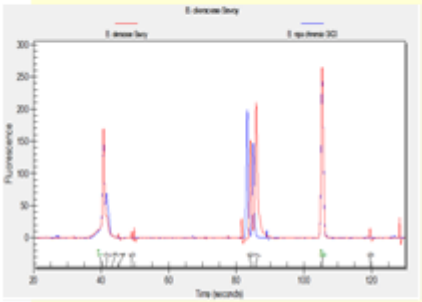
On the basisof RAPD, ISSR, SSR analysis

Three-dimensional plot of Brassica accessions



Results of gene locus amplification



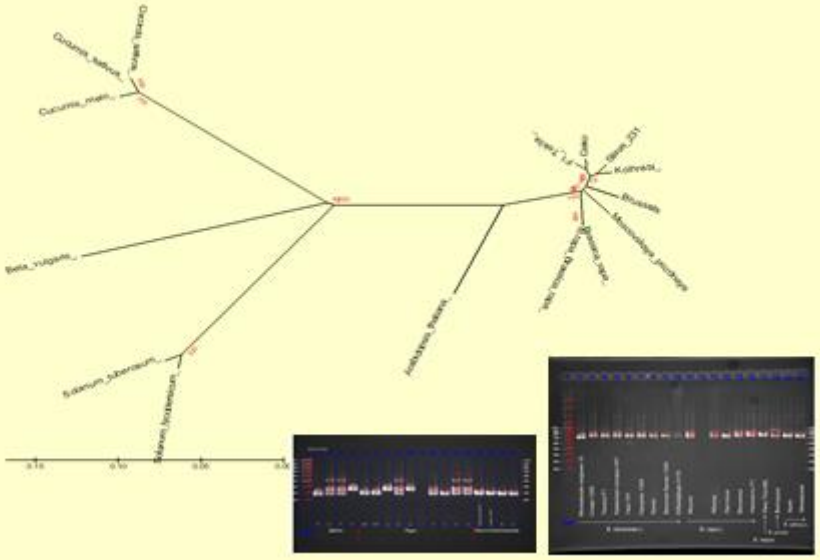


B. oleraceae L. (genome C)

B. rapa L. (genome A)

B. juncea (L.) Czern. (genome AB)

Variation among STS markers in Brassica accessions



Polymorphism of ISSR and SSR markers in accessions of *Allium* genus

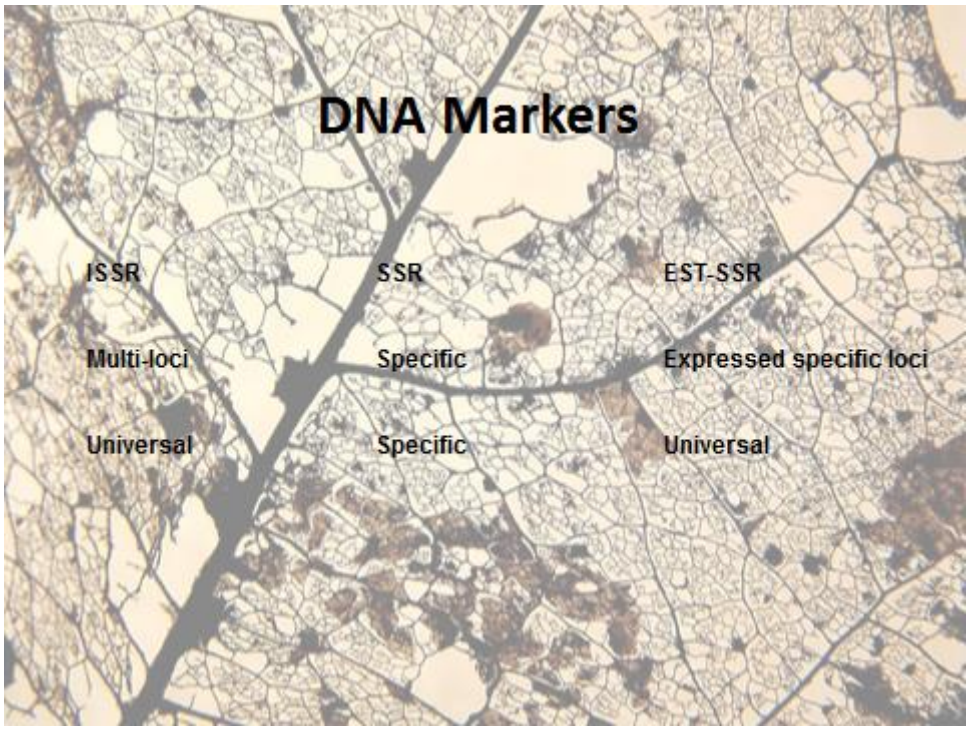
Allium species

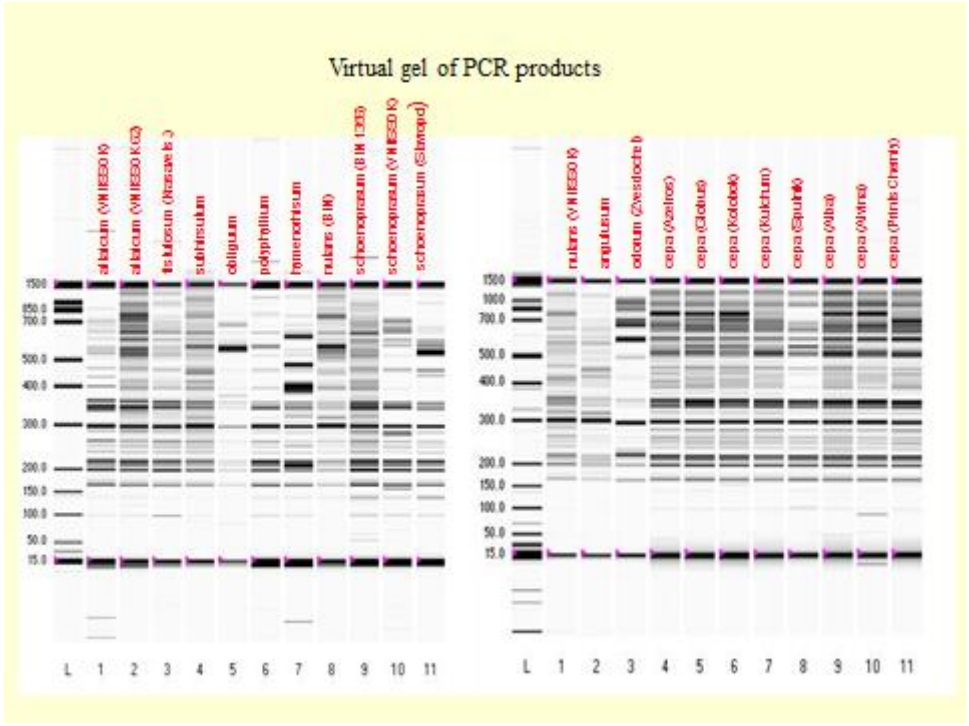
Allium species taken for the study

		New (by Friesen et al, 2006)	traditional (by Hanelt, 1992)
A.fistulosum L.	16	Cepa/Cepa	Rhizirideum/Cepa
A.altaicum Pall	16	Cepa/Cepa	Rhizirideum/Cepa
A.nutans L.	32	Rhizirideum/Rhizirideum	Rhizirideum/Rhizirideum
A.schoenoprasum L.	16, 32	Cepa/Schoenoprasum	Rhizirideum/Schoenoprasum
A.ramosum L.	16	Butomissa/Butomissa	Rhizirideum/Butomissa
A.obliguum L.	16	Polyprason/Oreiprason	Rhizirideum/Petroprason
A.anguiosum L.	16	Rhizirideum/Rhizirideum	Rhizirideum/Rhizirideum
A.hymenorrhizum Ledeb.	16	Polyprason/Falcatifolia	Rhizirideum/Oreiprason
A.subhirsutum L.	16	Amerallium/Mollium	Amerallium/Mollium
A.polyphyllum Kar&Kir	32	Polyprason/Falcatifolia	Rhizirideum/Oreiprason
A.cepa L.	16	Cepa/Cepa	Rhizirideum/Cepa

15 subgenera, 56 sections, 73 sections, 780 species

6 subgenera, 50 section and subsections, 600-700 species


DNA Markers
ISSR
Multi-loci
Universal
SSR
Specific
Specific
EST-SSR
Expressed specific loci
Universal

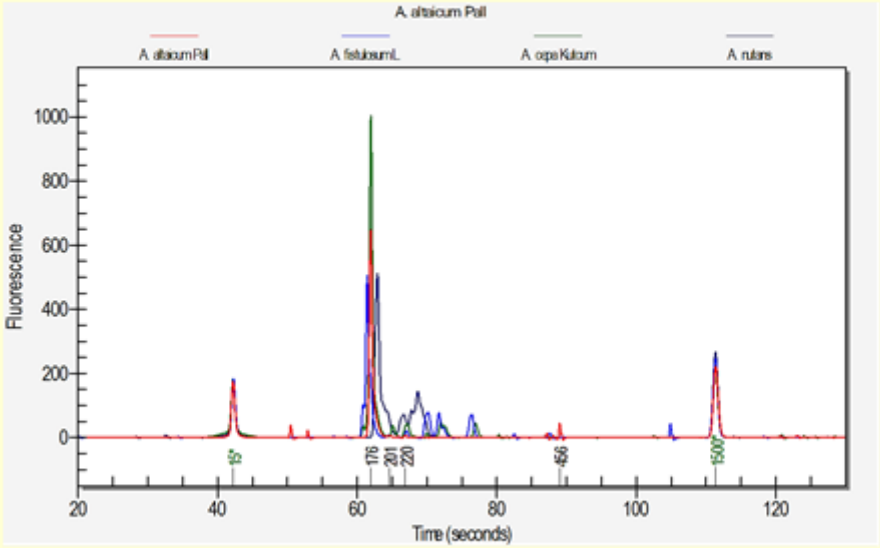

Virtual gel of PCR products
L 1 2 3 4 5 6 7 8 9 10 11
L 1 2 3 4 5 6 7 8 9 10 11

SSR-markers used for analysis

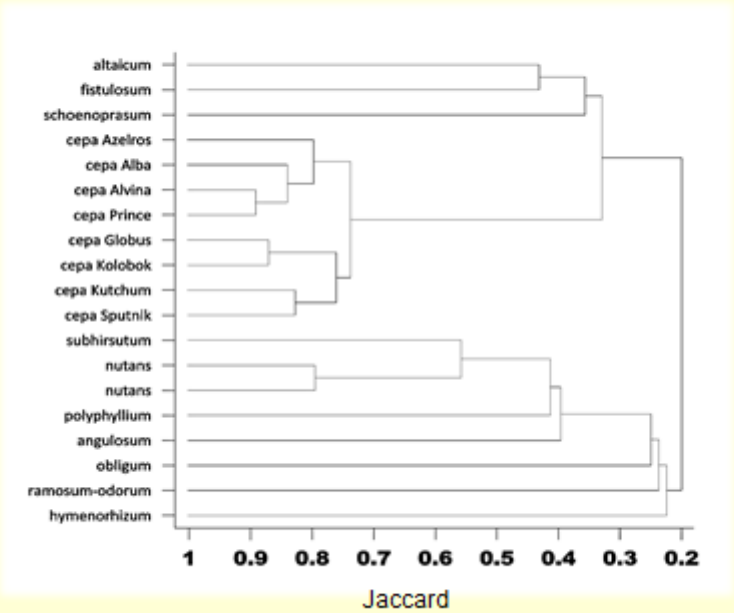
EST-SSR	T_a	Type of repeat	Fragment size (bp)	
ACM 004	55	(CCA)4	203-206-213	
ACM 018	53	(CCT)6	275-278	
ACM 024	57.5	(GCA)10-NNN-(GCA)4(ACA)4	150-190-200	
ACM 082	58	(TCT)13	190-200	
ACM 091	58	(TCT)10	190-200-220	
ACM 094	57	(TGG)5	120-150	

Species	EST-SSR markers					
	ACM 004	ACM 018	ACM 024	ACM 082	ACM 091	ACM 094
Allium altaicum Pall	+	+	+	+	+	+
Allium fistulosum L.	+	+	+	+	+	+
Allium subhirsutum L.	-	+	-	-	+	-
Allium obliquum L.	-	+	+	+	+	-
Allium polyphyllum Kar. & Kir	-	+	-	-	+	-
Allium hymenorhizum Ledeb	-	+	+	+	+	-
Allium nutans L.	-	+	-	-	+	-
Allium angulosum L.	-	+	-	-	+	-
Allium schoenoprasum L.	+	+	+	+	-	-
Allium ramosum L.	-	+	+	-	-	-
Allium cepa L.	+	+	+	+	+	+

Capillary electrophoresis of amplified locus ACM091

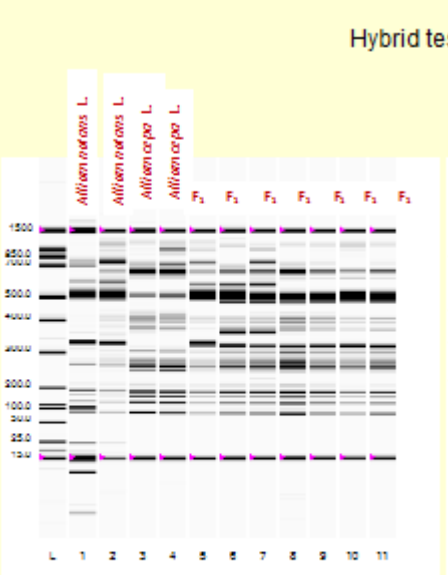


Relation among *Allium* accessions revealed by DNA markers

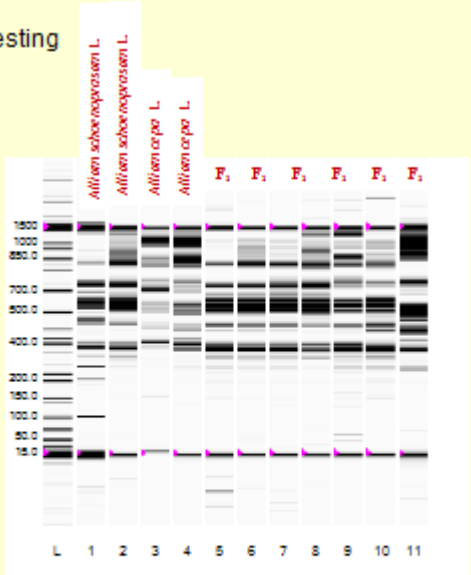


Interspesific hybrids

Hybrid testing

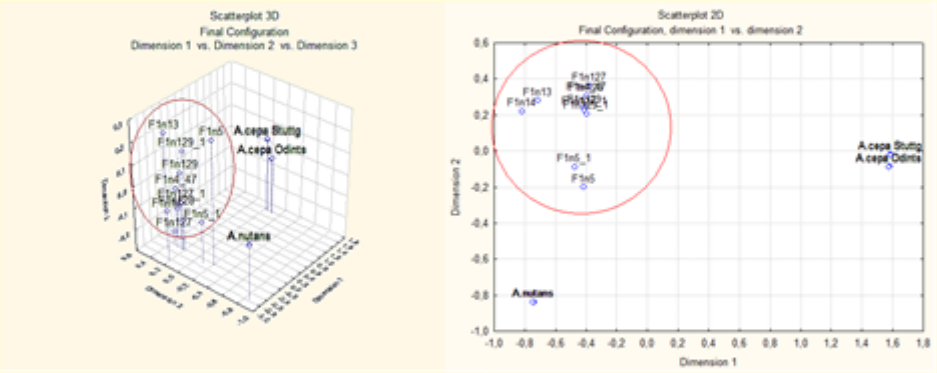


Virtual gel representing the PCR products observed after amplification ISSR loci of *Allium ostrowskianum* L., Stuttgarter Riesen (*Allium cepa* L.) and interspecific hybrid plants



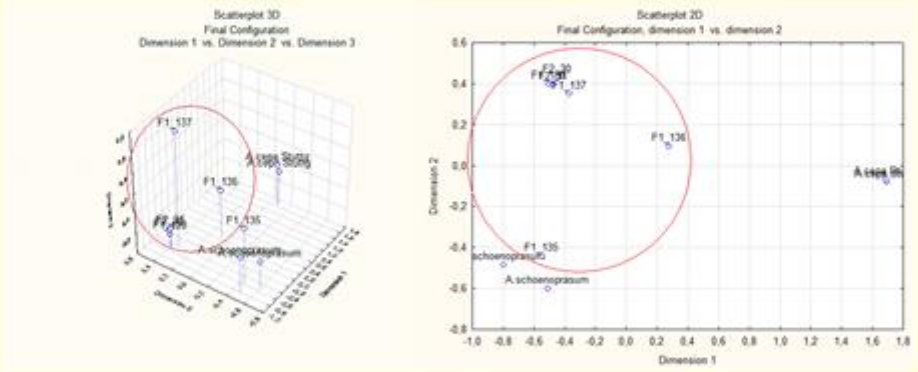
Virtual gel representing the PCR products observed after amplification ISSR loci of *Allium schoenoprasum* L., Stuttgarter Riesen (*Allium cepa* L.) and interspecific hybrid plants

Relationship among interspecific hybrid plants *A. nutans* L.× *A. cepa* L. shown with method of principal component analysis



*based on 77 ISSR loci and 25 SSR loci

Relationship among interspecific hybrid plants *A. schoenoprasum* L.× *A. cepa* L. shown with method of principal component analysis

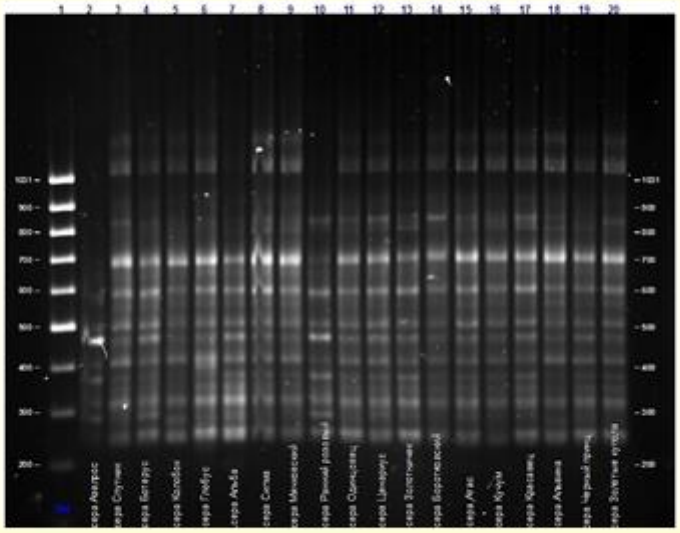


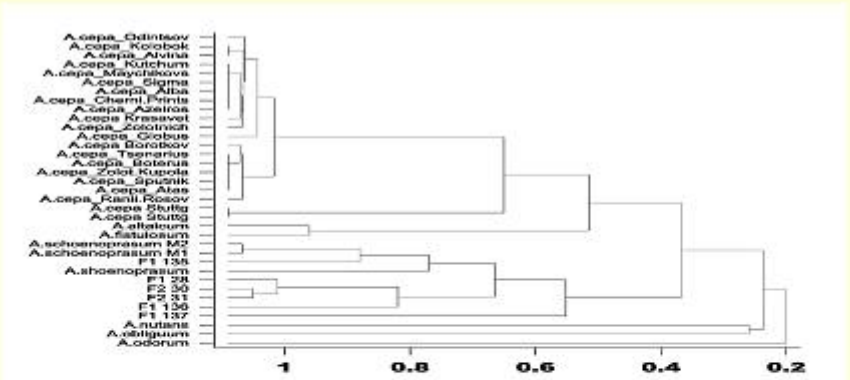
*Estimated 147 ISSR markers

Onion cultivars analyzed

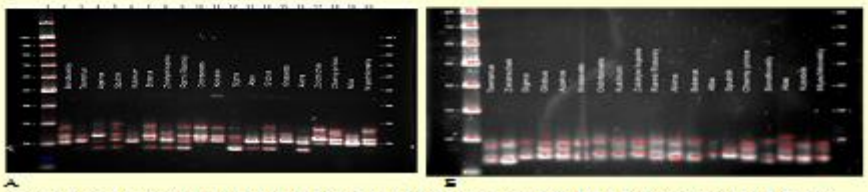
Cultivars	Bulb skin color	Shape of bulb
A.cepa Azelros	Yellow	Globe
A.cepa Globus	Yellow	Globe
A.cepa Kolobok	Yellow	Globe
A.cepa Kutchum	Yellow	Globe
A.cepa Sorodkovskiy	Yellow	Flat globe
A.cepa Sotanus	Yellow	Flat globe
A.cepa Ódintsovets	Yellow	Flat globe
A.cepa Zolotiye Kupola	Yellow	Flat globe
A.cepa Sigma	Yellow	Flat globe
A.cepa Tsenarius	Yellow	Flat globe
A.cepa Zolotnitchek	Yellow	Globe
A.cepa Atas	Yellow golden	Broad elliptic
A.cepa Alba	White	Globe
A.cepa Chamiy Printa	Dark violet	Globe
A.cepa Alvina	Violet	Flat
A.cepa Miyachikovskiy	Yellow	Flat
A.cepa Sputnik	Yellow	Flat
A.cepa Raniyi Rosoviy	Pink	Globe
A.cepa Krasnets	Dark red	Globe

ISSR analysis of onion cultivars



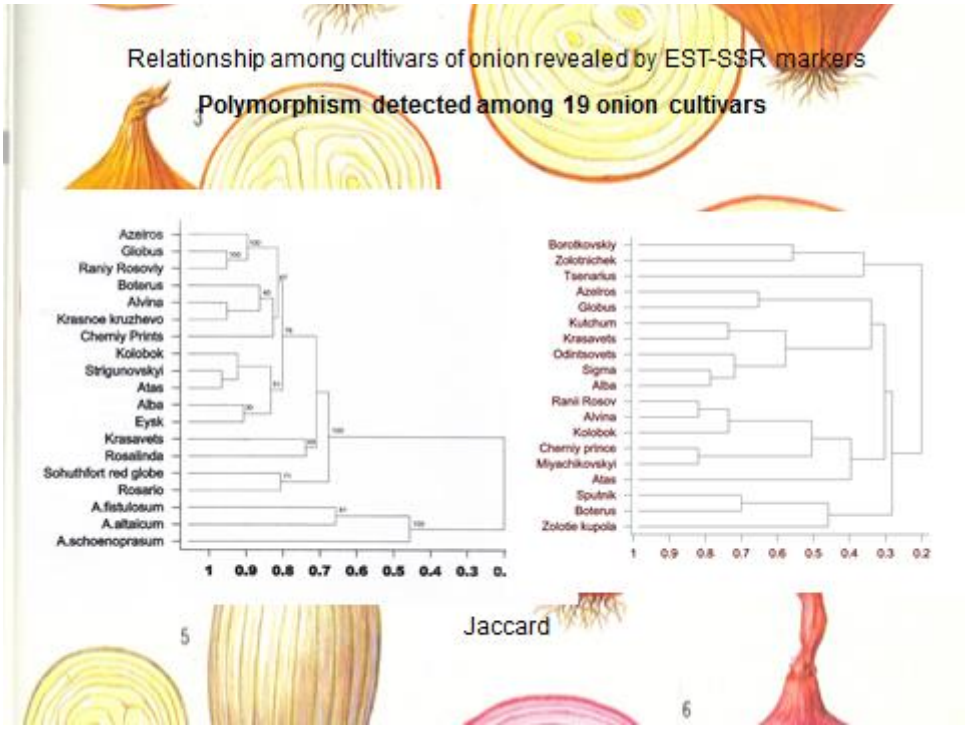


Дендрограмма генетических расстояний, построенная на основе полиморфизма ISSR маркеров и SSR



А Б

Электрофореграммы полученные в результате амплификации геномной ДНК сортов лука с микросателлитными локусами А – ACM132 и Б – ACM024



Thank you for your attention

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