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CALITĂȚII PRODUSELOR AGROALIMENTARE

Genetic diversity analysis of several pepper (*Capsicum annuum* L.) varieties-cultivated in Romania using ISSR and RAPD markers

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Research Center for Studies of Food Quality and Agricultural Products

Buzău region - an important area of biodiversity conservation in Romania

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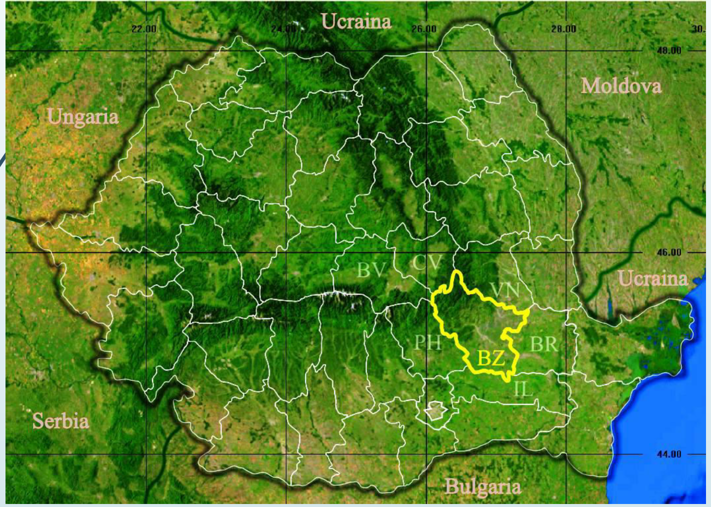
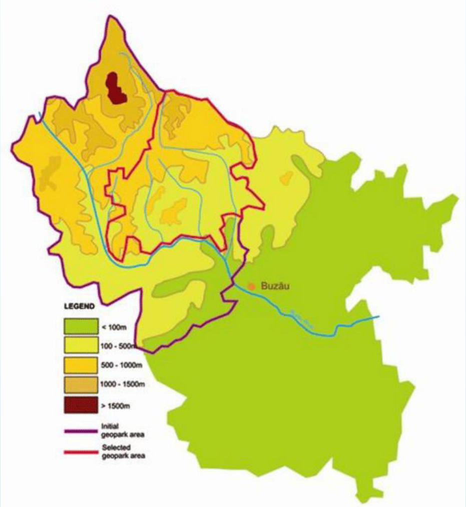
- Numerous varieties of *Capsicum annuum* L. with multiple valuable traits, such as adaptation to biotic and abiotic stress factors, can be found in south-east Romania, the Buzău region, well known for vegetable cultivation



ro.wikipedia.org



Seedling emergence and structure analysis of autochthonous pepper varieties

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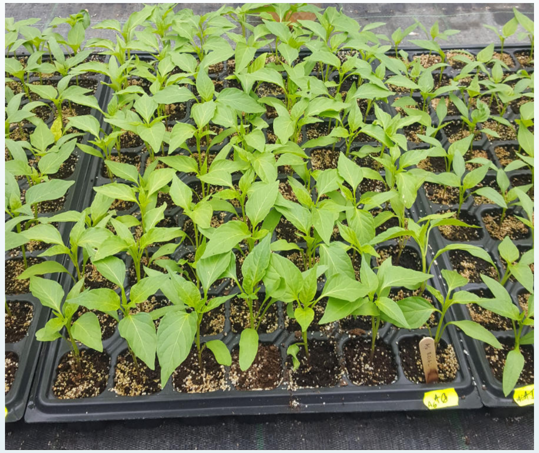
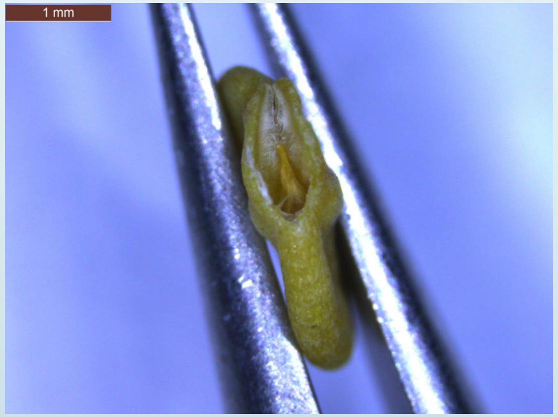


	L (mm)	l (mm)
A1	4.993	3.724
A2	3.621	2.69
A3	3.944	3.477
A4	4.55	3.59
A5	4.242	3.608
A6	4.492	3.172
A7	4.164	3.615

	L open. (mm)	l open. (mm)
A1	1.88	0.599
A2	1.496	0.266
A3	1.66	0.412
A4	2.349	0.521
A5	2.53	0.433
A6	3.06	0.323
A7	1.883	0.467



gDNA quantification prior PCR analysis

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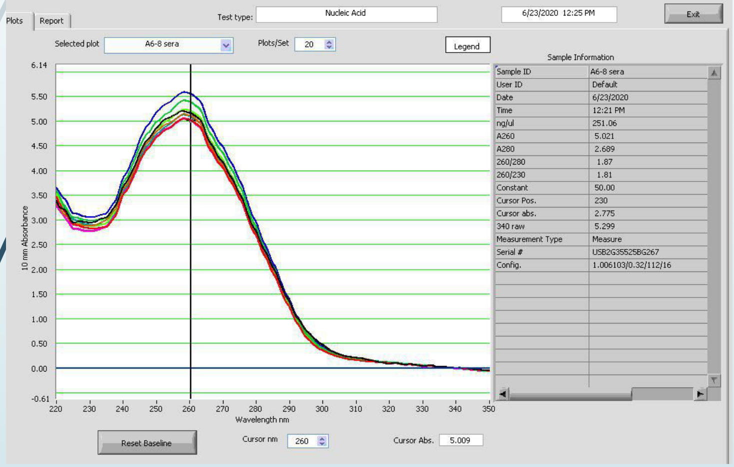
Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
A6-1 sera	Default	6/23/2020	12:13 PM	258.17	5.163	2.772	1.86	1.76	50.00	230	2.937	7.054
A6-2 sera	Default	6/23/2020	12:14 PM	251.38	5.028	2.680	1.88	1.78	50.00	230	2.825	3.285
A6-3 sera	Default	6/23/2020	12:15 PM	269.60	5.392	2.859	1.89	1.81	50.00	230	2.983	3.603
A6-4 sera	Default	6/23/2020	12:16 PM	255.59	5.112	2.751	1.86	1.78	50.00	230	2.880	6.020
A6-5 sera	Default	6/23/2020	12:17 PM	260.62	5.212	2.786	1.87	1.82	50.00	230	2.871	3.224
A6-6 sera	Default	6/23/2020	12:18 PM	254.93	5.099	2.736	1.86	1.76	50.00	230	2.899	2.390
A6-7 sera	Default	6/23/2020	12:19 PM	277.99	5.560	2.958	1.88	1.82	50.00	230	3.049	5.019



Test type: Nucleic Acid

6/23/2020 12:25 PM

Selected plot: A6-8 sera

Plots/Set: 20

Sample Information

Sample ID	A6-8 sera
User ID	Default
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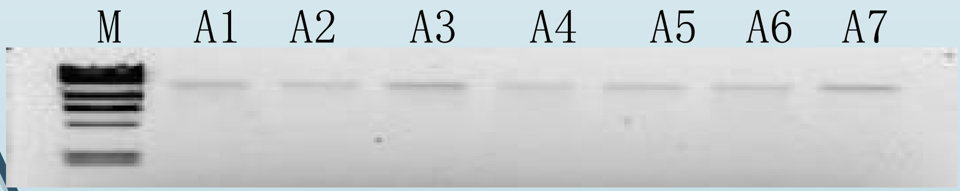
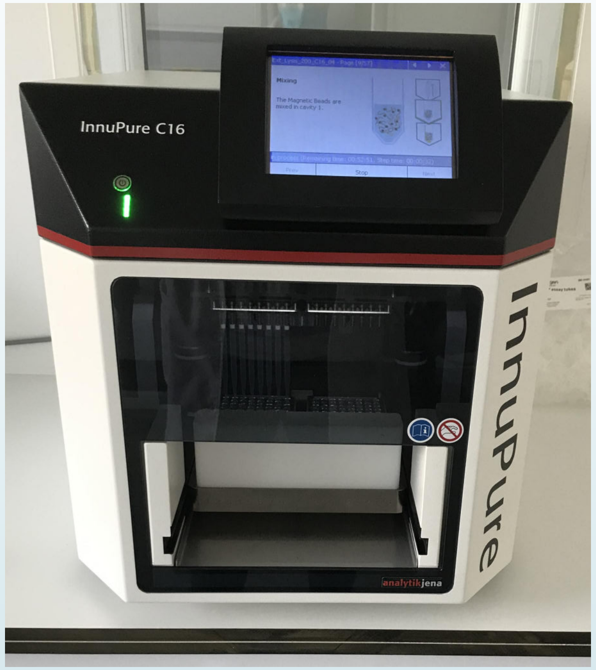
Time	12:21 PM
ng/ul	251.06
A260	5.021
A280	2.689
260/280	1.87
260/230	1.81
Constant	50.00
Cursor Pos.	230
Cursor abs.	2.775
340 raw	5.299
Measurement Type	Measure
Serial #	USB2G35525BG267
Config.	1.006103/0.32/112/16

Cursor nm: 260

Cursor Abs.: 5.009



-150 mg fresh young leaves
-SLS lysis solution
-InnuPure® C16 / innuPREP Plant DNA I Kit-IPC16
-NanoDrop 1000
-25-50 ng gDNA/ PCR reaction



RAPD profile analysis of seven local *C. annuum* varieties produced with primers P31/P32/P34/P37/P38

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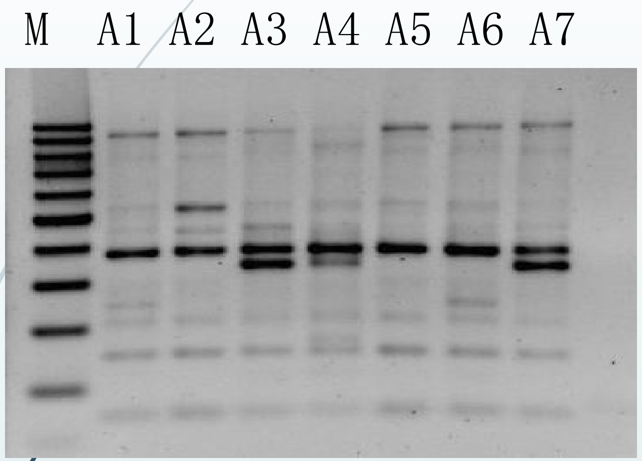
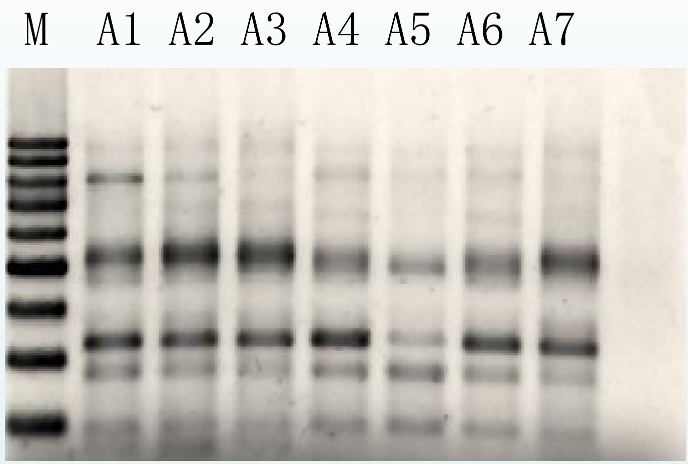
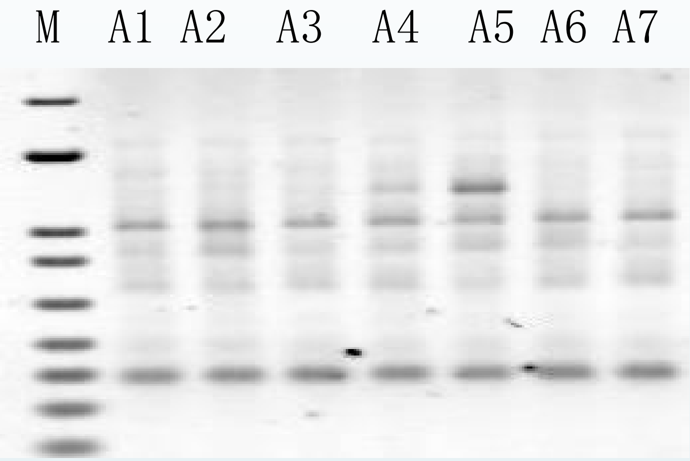
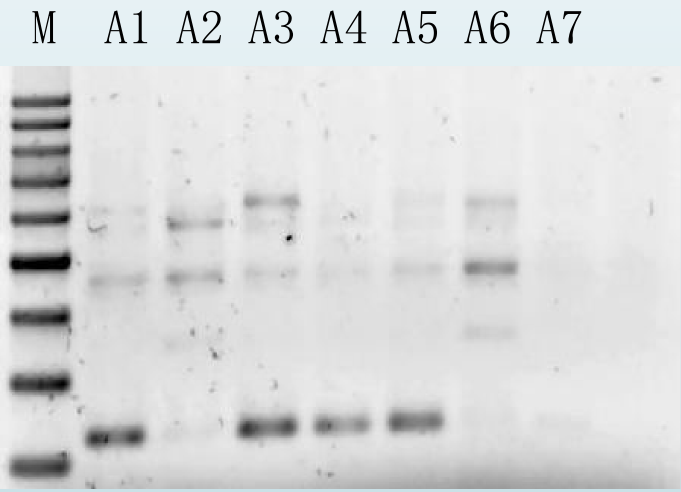
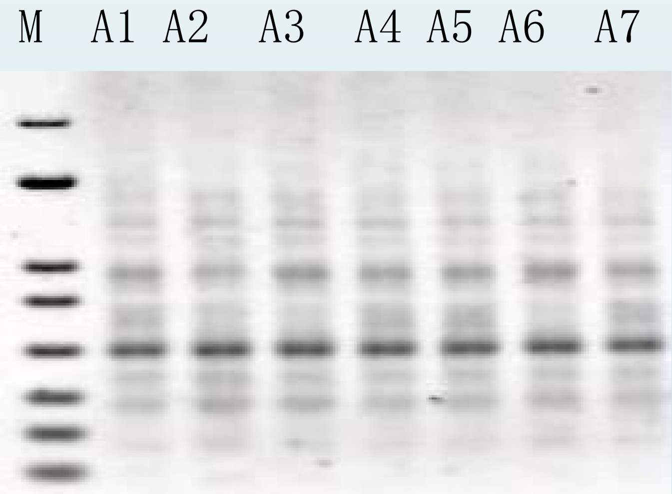




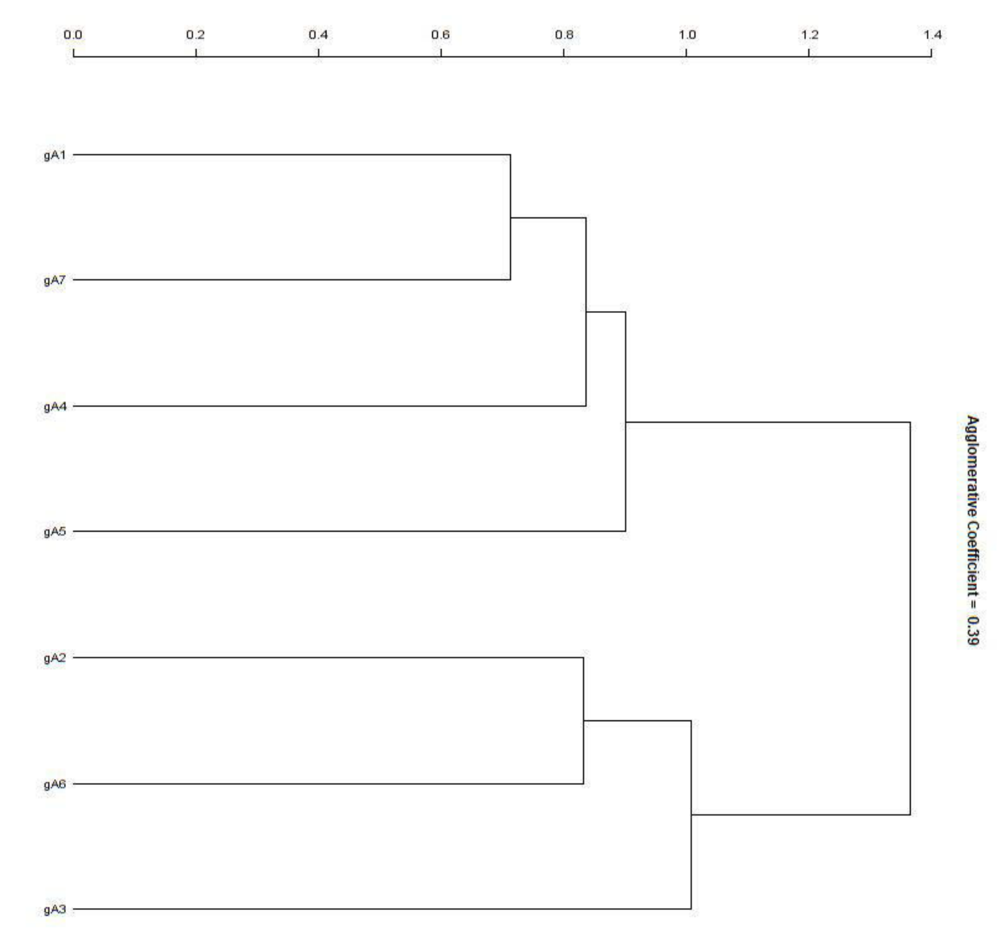


-RAPD-PCR profile analysis with 5 selected primers generated 200 reproducible bands from 7 samples.
-Each primer amplified 1-10 bands; the band size ranged between 190-2000 bp
-Platinum II Hot-Start PCR Master Mix 2X/ Invitrogen





Diversity in grups-RAPD cluster analysis /calculus per genotype



Genotype	He	Ho	Ae	rareness
gA1	0.489795918	0	1.96	1.8141898
gA2	0.471655329	0	1.892703863	3.110877742
gA3	0.471655329	0	1.892703863	2.499312555
gA4	0.498866213	0	1.995475113	2.372317028
gA5	0.471655329	0	1.892703863	2.792475072
gA6	0.498866213	0	1.995475113	2.380730412
gA7	0.471655329	0	1.892703863	1.743925072

-Dendrogram: cluster analysis with agglomerative coefficient 0.39
-Expected heterozygosity: 0.47-0.49
-Allelic frequency: 1.89-1.99
-Rareness range: 1.74-3.11
-BIO-R (Biodiversity analysis with R) program

RAPD profile for genetic distances calculated by Roger's distance and biodiversity analysis by distance matrix in GAP

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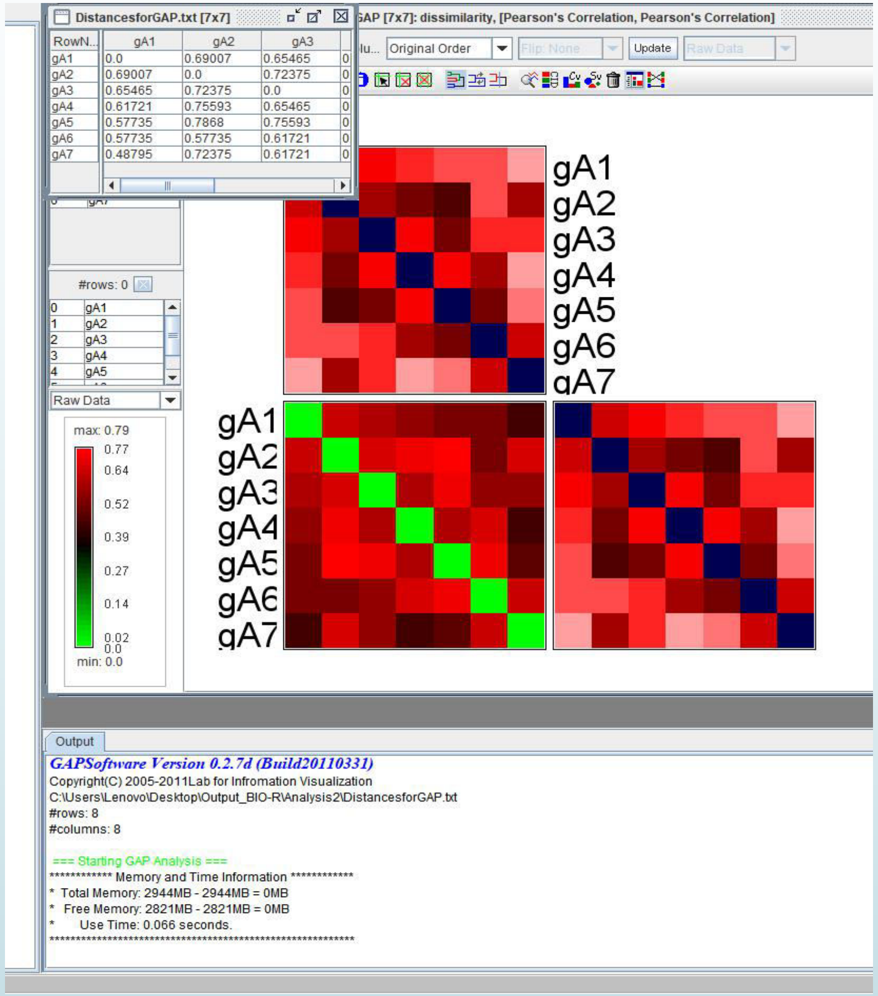
ID	NAME	1	2	3	4	5	6	7
1	gA1	0	0.69007	0.65465	0.61721	0.57735	0.57735	0.48795
2	gA2	0.69007	0	0.72375	0.75593	0.7868	0.57735	0.72375
3	gA3	0.65465	0.72375	0	0.65465	0.75593	0.61721	0.61721
4	gA4	0.61721	0.75593	0.65465	0	0.65465	0.72375	0.48795
5	gA5	0.57735	0.7868	0.75593	0.65465	0	0.75593	0.53452
6	gA6	0.57735	0.57735	0.61721	0.72375	0.75593	0	0.69007
7	gA7	0.48795	0.72375	0.61721	0.48795	0.53452	0.69007	0

- Genetic distance presented a similarity coefficient (SC) index ranging from 0.48 to 0.78.
- SC index was the lowest (0.48) between sample 1 and 7 and sample 4 and 7.
- SC index was the highest (0.78) between sample 2 and 5.



RowN...	gA1	gA2	gA3
gA1	0.0	0.69007	0.65465
gA2	0.69007	0.0	0.72375
gA3	0.65465	0.72375	0.0
gA4	0.61721	0.75593	0.65465
gA5	0.57735	0.7868	0.75593
gA6	0.57735	0.57735	0.61721
gA7	0.48795	0.72375	0.61721

```
GAPSoftware Version 0.2.7d (Build20110331)
Copyright(C) 2005-2011Lab for Infromation Visualization
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ISSR profile analysis of seven local *C. annuum* varieties produced with primers P21/P22/P23/P24/P25/P26/P28

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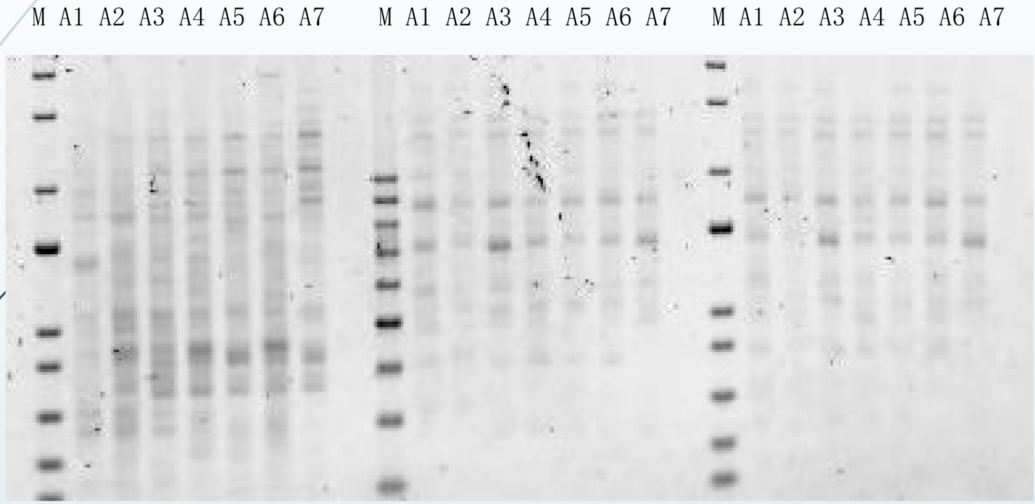

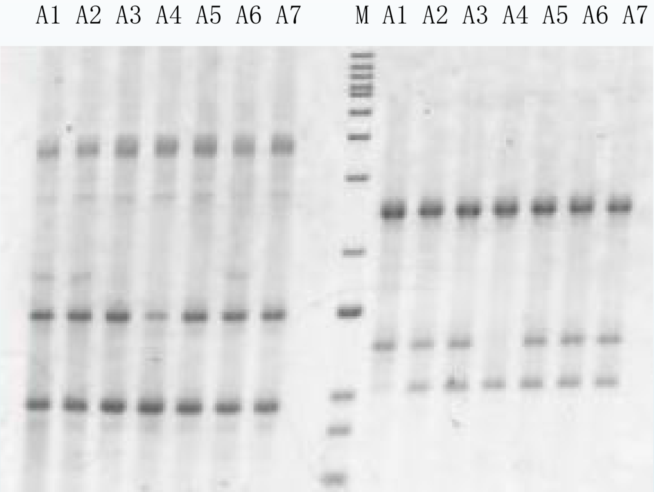

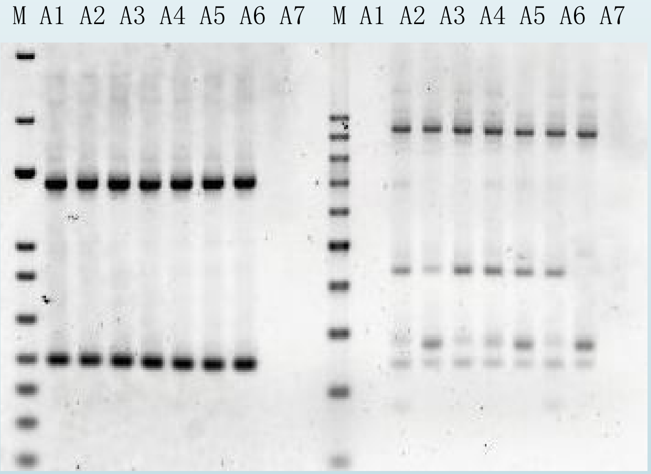

-ISSR-PCR profile analysis with 7 selected primers generated 295 reproducible bands from 7 samples.
-Each primer amplified 1-10 bands; the band size ranged between 350-4000 bp
-Platinum II Hot-Start PCR Master Mix 2X/ Invitrogen

Diversity in groups-ISSR cluster analysis /calculus per genotype

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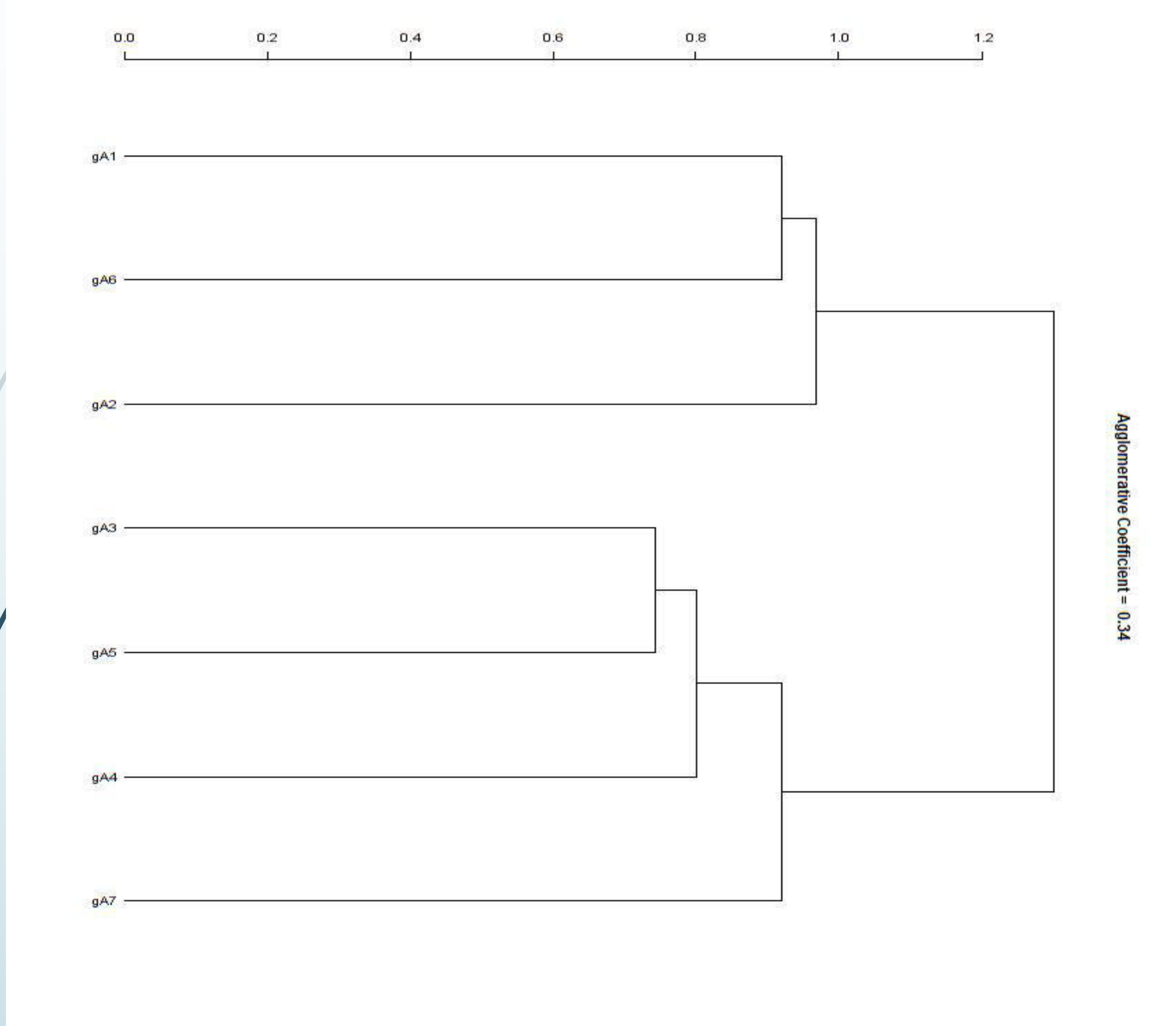
CALITĂȚII PRODUSELOR AGROALIMENTARE



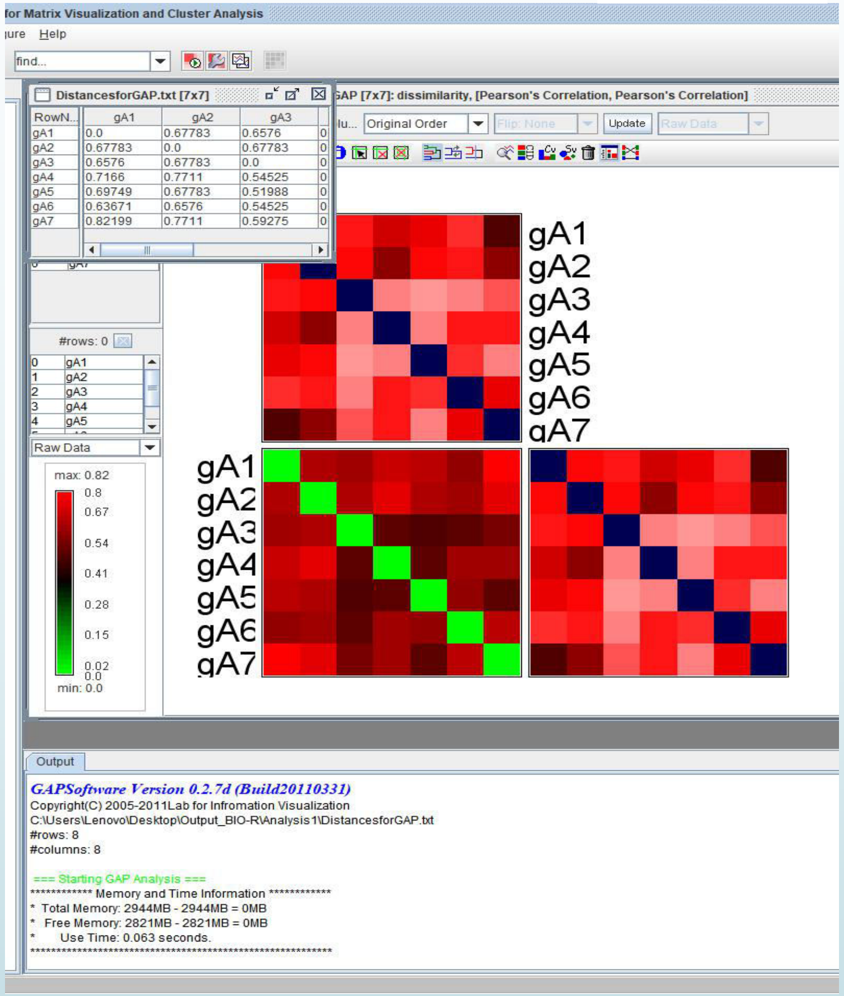
Genotype	He	Ho	Ae	rareness
gA1	0.482103725	0	1.930888575	5.239181849
gA2	0.496712929	0	1.986937591	5.247595233
gA3	0.417823229	0	1.717691343	2.95579525
gA4	0.470416362	0	1.888275862	4.204165777
gA5	0.482103725	0	1.930888575	3.214906848
gA6	0.470416362	0	1.888275862	3.832325148
gA7	0.482103725	0	1.930888575	4.75717246

-Dendrogram: cluster analysis with agglomerative coefficient 0.34
-Expected heterozygosity: 0.47-0.49
-Allelic frequency: 1.71-1.98
-Rareness range: 2.95-5.24

ISSR profile for genetic distances calculated by Roger's distance and biodiversity analysis by distance matrix in GAP

ID	NAME	1	2	3	4	5	6	7
1	gA1	0	0.67783	0.6576	0.7166	0.69749	0.63671	0.82199
2	gA2	0.67783	0	0.67783	0.7711	0.67783	0.6576	0.7711
3	gA3	0.6576	0.67783	0	0.54525	0.51988	0.54525	0.59275
4	gA4	0.7166	0.7711	0.54525	0	0.54525	0.6576	0.6576
5	gA5	0.69749	0.67783	0.51988	0.54525	0	0.63671	0.54525
6	gA6	0.63671	0.6576	0.54525	0.6576	0.63671	0	0.69749
7	gA7	0.82199	0.7711	0.59275	0.6576	0.54525	0.69749	0

- Genetic distance presented a similarity coefficient (SC) index ranging from 0.51 to 0.82.
- SC index was the lowest (0.51) between sample 3 and 5.
- SC index was the highest (0.82) between sample 1 and 7.



for Matrix Visualization and Cluster Analysis

DistancesforGAP.txt [7x7]

RowN...	gA1	gA2	gA3
gA1	0.0	0.67783	0.6576
gA2	0.67783	0.0	0.67783
gA3	0.6576	0.67783	0.0
gA4	0.7166	0.7711	0.54525
gA5	0.69749	0.67783	0.51988
gA6	0.63671	0.6576	0.54525
gA7	0.82199	0.7711	0.59275

AP [7x7]: dissimilarity, [Pearson's Correlation, Pearson's Correlation]

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GAPSoftware Version 0.2.7d (Build20110331)
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Conclusions- Diversity analysis within each group

RAPD Profile

Diversity	
% of polymorphic loci	0.525
Expected Heterozygosity	0.3692906
Standar desviation for HE	0.0207887
Observed Heterozygosity	0
Standar desviation for HO	0
Number of effective allele	1.6193725
Standar desviation for Ae	0.0517061
Shannon diversity Index	0.7958966
Standar desviation for ShanIn	0.0338248

ISSR Profile

Diversity	
% of polymorphic loci	0.637931
Expected Heterozygosity	0.3706564
Standar desviation for HE	0.0180592
Observed Heterozygosity	0
Standar desviation for HO	0
Number of effective allele	1.6365341
Standar desviation for Ae	0.0462377
Shannon diversity Index	0.7959768
Standar desviation for ShanIn	0.0291559

Pacheco, Ángela; Alvarado, Gregorio; Rodríguez, Francisco;Crossa, Jose; Burgueño, Juan, 2020/08/17, BIO-R (Biodiversity analysis with R for Windows) Version 3.0, International Maize and Wheat Improvement Center

ADER 7.2.6.

Thank you for attention!

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Acknowledgements

This research work is supported by the Romanian Ministry of Agriculture and Rural Development (MADR-Bucharest), under the agricultural research and development program 2019-2022, ADER 7.2.6 project.

https://www.usamv.ro/index.php/ro/850-ader-7-2-6