



Report for the genetic diversity at inter- and intra-population level

DELIVERABLE 1.6

PulpIng

Developing of **Pumpkin Pulp** Formulation using a Sustainable **Integrated** Strategy





Index

Document Information	3
1. Scope of the document	4
2. Methodology	4
APPENDIX	21

Document Information

Deliverable Number	1.6
Deliverable name	Report for the genetic diversity at inter- and intra-population level
Contributing WP	WP1: Defining agronomic conditions for pumpkin production
Contractual delivery date	M36, August 2023
Actual delivery date	M36, August 2023
Dissemination level	Public
Responsible partner	UTH
Reviewers	UTH, CBBC/ISACM, BU
Version	1

1. Scope of the document

The scope of this document is to capture the genetic diversity at inter- and intra-population level among the study pumpkin landrace. The studied material included the landraces cultivated in Greece. Following genotypic evaluation in terms of agronomic performance and determination of morpho-agronomic diversity, the most promising genotypes were molecularly characterized in order to determine the existing genetic diversity within and among genotypes under study. Molecular analysis was conducted using RAPD markers.

2. Methodology

Genetic material

The genetic material consisted of two commercial varieties and eight local landraces. Genotypes under study are listed in Table 1.

Seeds were sown in pots containing soil:perlite mixture (3:1) under controlled conditions (temperature: 25°C, photoperiod: 16h light / 8h dark). At the stage of 4 true leaves, young and healthy leaves were harvested and stored at -80 °C deep freezer until use in molecular marker analyses. For each genotype under study, four replications were used, each consisting of two true leaves.

Table 1. Pumpkin germplasm employed in molecular marker analysis to determine the existing genetic diversity within and among populations.

N.	Type*	Description	Designation
1	Commercial variety 1	FYTRO FS 243	P1
2	Commercial variety 1	BIG MAX	P2
3	Local landrace 1	"Pumpkin for pie"	P3
4	Local landrace 2	"Nyxaki"	P4
5	Local landrace 3	"Melitis-BI / White"	P5
6	Local landrace 4	"Round / Deep orange"	P6
7	Local landrace 5	"Neapoli / Oval, small"	P7
8	Local landrace 6	"Lakonia / Bottle shape"	P8
9	Local landrace 7	"Makedonika / Green"	P9
10	Local landrace 8	"K-7"	P10

*The cultivars under study are thereof referred to as populations (P1 – P10).

DNA extraction

DNA was extracted using the CTAB method. Briefly, 200 mg of leaf tissue were placed in eppendorf tubes and grounded with 200 µl CTAB buffer, that was previously placed in water bath (60° C) for 15 min. Subsequently, 25 mg PVP were added and samples were thoroughly mixed. Following the addition of 200 µl CTAB, samples were thoroughly mixed and placed in a 25 °C water bath for 25 minutes. After thorough mixing (vortex), an equal volume of phenol/chloroform (200 µl each) was added and samples were mixed to homogenization. Samples were subsequently centrifuged at 13000 rpm for 5 min, to

separate the phases, and the aqueous upper phase was transferred to a new eppendorf tube. Following the addition of 200 μ l NaCl 5M, the samples were mixed and 800 μ l of ethanol 95 % were added. Samples were mixed and placed at -20 °C overnight to precipitate the DNA. Samples were centrifuged at 13000 rpm for 10 min and the supernatant was discarded without disturbing the pellet. A second purification step was performed, using 1 ml of ice cold 70 % ethanol, and samples were centrifuged at 13000 rpm for 5 min. Ethanol was decanted and the residual ethanol was removed by drying at room temperature for 15 min. Finally, the DNA pellet was dissolved in 50 μ l mQ H₂O.

DNA concentration, in ng/ μ L, was measured using a Nanodrop spectrophotometer at 260 nm. Sample purity was estimated using the ratio 260/280, which corresponds to the absorbance at the wavelengths 260 and 280 nm (Table 2). The 260/280 ratio provides an indication of how pure the sample is from contaminating protein, with an optimal 260/280 ratio for DNA ranging to 1.80.

Table 2. Nanodrop readings for genomic DNA of pumpkin samples under study.

Sample	Genotype	Absorbance at 260 nm	260/280 Ratio
1		96,7	2,16
2	Commercial variety FYTRO FS 243	176,7	2,1
3		83,4	2,07
4		190,1	2,02
5	Commercial variety BIG MAX	2.173,2	2,05
6		1.717,2	2,02
7		1.379,1	2,08
8		2.923,2	2,07
9	Local landrace 1 "Pumpkin for pie"	10,9	1,18
10		33,8	2,06
11		11	2,17
12		10,6	2,14
13	Local landrace 2 "Nyxaki"	2.943,2	2,09
14		2.005	2,07
15		2.171,2	2,09
16		1.266,4	2,12
17	Local landrace 3 "Melitis-BI / White"	409	2,08
18		217,4	2,14
19		331,2	2,03
20		184,2	1,84
21	Local landrace 4 "Round / Deep orange"	317,4	2,04
22		98,4	1,99
23		773,7	1,88
24		243,8	2,14
25	Local landrace 5 "Neapoli / Oval, small"	66	2,16
26		231,4	2,11
27		290,3	2,11
28		241,7	2,01
29	Local landrace 6	99,5	2,07
30		87,6	2,13

31	"Lakonia / Bottle	86,5	2,02
32	shape"	96,4	2,17
33		92,4	2,16
34	Local landrace 7	112,8	1,83
35	"Makedonika / Green"	105,6	2,07
36		142,1	2,01
37		3.972,2	2,03
38	Local landrace 8	3.553,9	2,05
39	"K-7"	3.016,9	2,08
40		3.642,2	2,06

RAPD analysis

For the RAPD analysis, random 10-mer primers were used for amplification, using the DNA from four individual plants of each genotype. PCR reaction and amplification conditions were accordingly standardized. Primers used for RAPD analyses are listed in Table 3, while the PCR reaction conditions are described in Table 4.

Following RAPD-PCR reactions, the amplification products were separated in 1.5-2 % agarose gels and detected by staining with MIDORI Green Advance, which is a non-carcinogenic and less mutagenic dye routinely employed as an alternative to the traditional ethidium bromide-mediated staining of nucleic acids. PCR products were visualized under UV light.

Table 3. Nucleotide sequences of primers used for molecular marker analysis.

N.	Type	Primer Name	Sequence 5' → 3'	Citation
1	RAPD	OPA-04	AATCGGGCTG	Radwan, 2014. Molecular discrimination and genetic relationships between some cultivars of <i>C. pepo</i> using RAPD analysis. African Journal of Biotechnology, 11(3): 1202-1209.
2	RAPD	OPB-01	GTTTCGCTCC	
3	RAPD	OPB-02	TGATCCCTGG	
4	RAPD	OPB-04	GGACTGGAGT	
5	RAPD	OPG-02	GGCACTGAGG	
6	RAPD	OPZ-03	CAGCACCGCA	Ntuli et al., 2015. Genetic diversity in <i>Cucurbita pepo</i> landraces revealed by RAPD and SSR markers. Scientia Horticulturae, 189: 192-200.
7	RAPD	CB9	GGTGACGCAG	
8	RAPD	CB12	AGTCGACGCC	
9	RAPD	CB13	ACGCATCGGA	
10	RAPD	CB15	GGCTGGTTCC	
11	RAPD	CB17	GTAACCAGCC	

Table 4. PCR reaction conditions used in RAPD marker analyses.

PCR Reaction Mix			
RAPD Primers	Reagent	Final concentration	Volume
OPA-04	Buffer	1x	5 µL

OPB-01	MgCl ₂	1.5 mM	1.5 µL
OPB-02	RAPD primer	10 pmole	1 µL
OPB-04	dNTPs	200 µM each	0.5 µL
OPG-02	Taq polymerase (KAPA Taq DNA Polymerase)	0.5 u	0.2 µL
OPZ-03	DNA	50 ng	2.5 µL
	ddH ₂ O		14.3 µL
	Total volume		25 µL

PCR Program

95 °C	3 min	
95 °C	2 min	
37 °C	1 min	45 cycles
72 °C	2 min	
72 °C	10 min	
4 °C	overnight	

Gel Electrophoresis

Agarose gel: 1.5 -2 % TBE	Ladder: 100 bp DNA Ladder (NEB)
---------------------------	---------------------------------

PCR Reaction Mix

RAPD Primers	Reagent	Final concentration	Volume
CB9	Buffer	1x	5µl
CB12	MgCl ₂	1.5 mM	1.5 µL
CB13	RAPD primer	0.4 µM	1 µL
CB15	dNTPs	200 µM each	0.5 µL
CB17	Taq polymerase (GoTaq Master Mix)	0.5 u	0.2 µL
	DNA	50 ng	2.5 µL
	H ₂ O		14.3 µL
	Total volume		25 µL

PCR Program

95 °C	5 min	
95 °C	30 sec	
36 °C	30 sec	40 cycles
72 °C	1 min	
72 °C	4 min	
4 °C	overnight	

Gel Electrophoresis

Agarose gel: 1.5 – 2 % TBA	Ladder: 100 bp DNA Ladder (NEB)
----------------------------	---------------------------------

Data analysis

Each PCR reaction was run in duplicate and only discrete and reproducible bands were analyzed. RAPD gel images were analyzed using GelAnalyzer 23.1 software (<http://www.gelanalyzer.com/>). The band size was determined using a 100 bp DNA Ladder (NEB).

The RAPD profiles for each primer were scored for presence or absence, as a binary character, and transferred to a binary data matrix using MS Excel. The presence coded

“1” and absence coded “0” of each band in a data matrix. Monomorphic bands were excluded from the analysis. An example data matrix using RAPD marker A04 is presented in the Appendix (Table 1). The total number of bands, number of polymorphic bands, and percentage of polymorphic bands were counted.

The binary matrix that included 40 individuals and 215 generated RAPD loci was used for further data analysis. The genetic diversity indices were calculated using the GenAlEx 6.5 package. The analysis involved determination of the observed allele number (N), the number of different alleles (Na), the effective allele number (Ne), Shannon’s information index (I), Nei’s genetic distance (D) and genetic identity (IN), gene diversity (h), and unbiased gene diversity (uh). The formulas employed for the determination of genetic parameters under study are presented in the Appendix (Table2).

Distribution of the genetic variation within populations and among populations based on the RAPD marker profiles was determined by analysis of molecular variance (AMOVA). Further, the pairwise population differentiation (PhiPT) was calculated with random permutation number $N = 999$.

Results

RAPD marker polymorphism

In the framework of characterizing the set of local landraces collected from various cultivation areas in Greece, RAPD marker analysis was employed. Eleven (11) primers were used to estimate the genetic diversity between two commercial varieties and eight local landraces by amplifying the extracted DNA using RAPD-PCR analysis.

The RAPD profiles generated by the eleven RAPD primers A-04, B-01, B-02, B-04, G-02, Z-03, CB-09, CB-12, CB-13, CB-15 and CB-19 are illustrated in Figures 1-11, respectively. The RAPD primer B-02 did not yield discrete and reproducible bands and, as such, was excluded from the analysis.

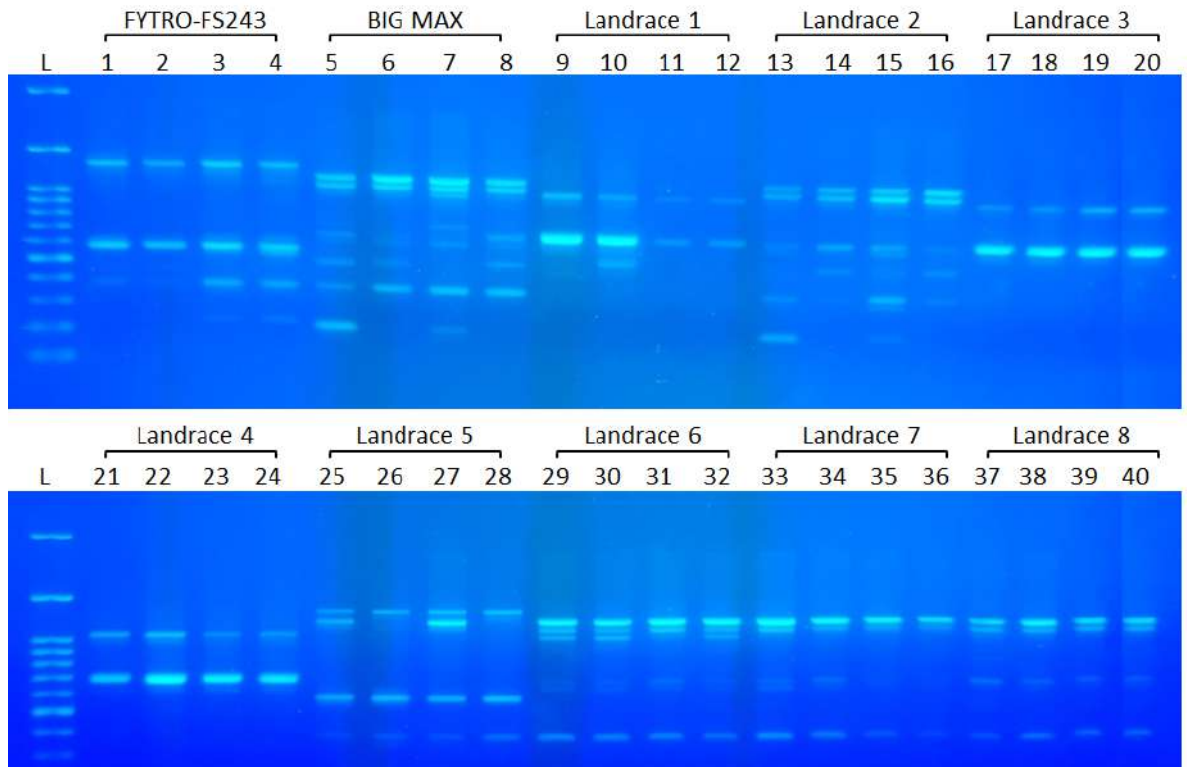


Figure 1. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker OPA-04. Ladder: 100 bp DNA Ladder (NEB).

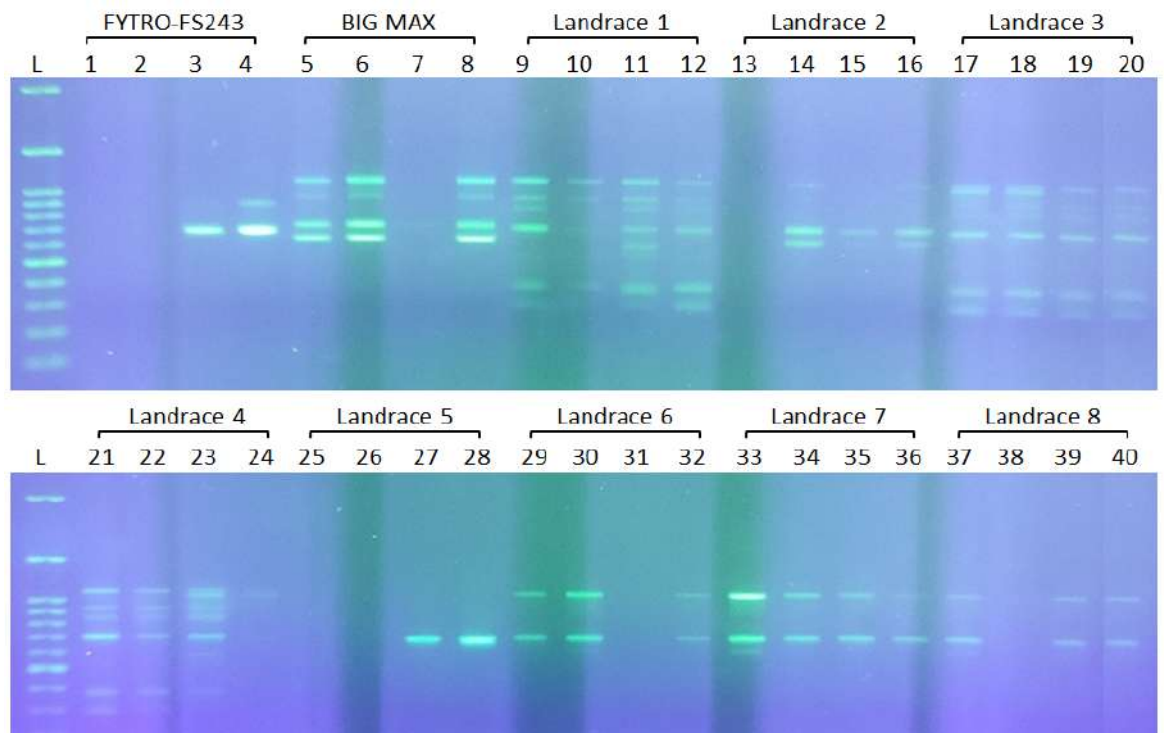


Figure 2. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker OPB-01. Ladder: 100 bp DNA Ladder (NEB).

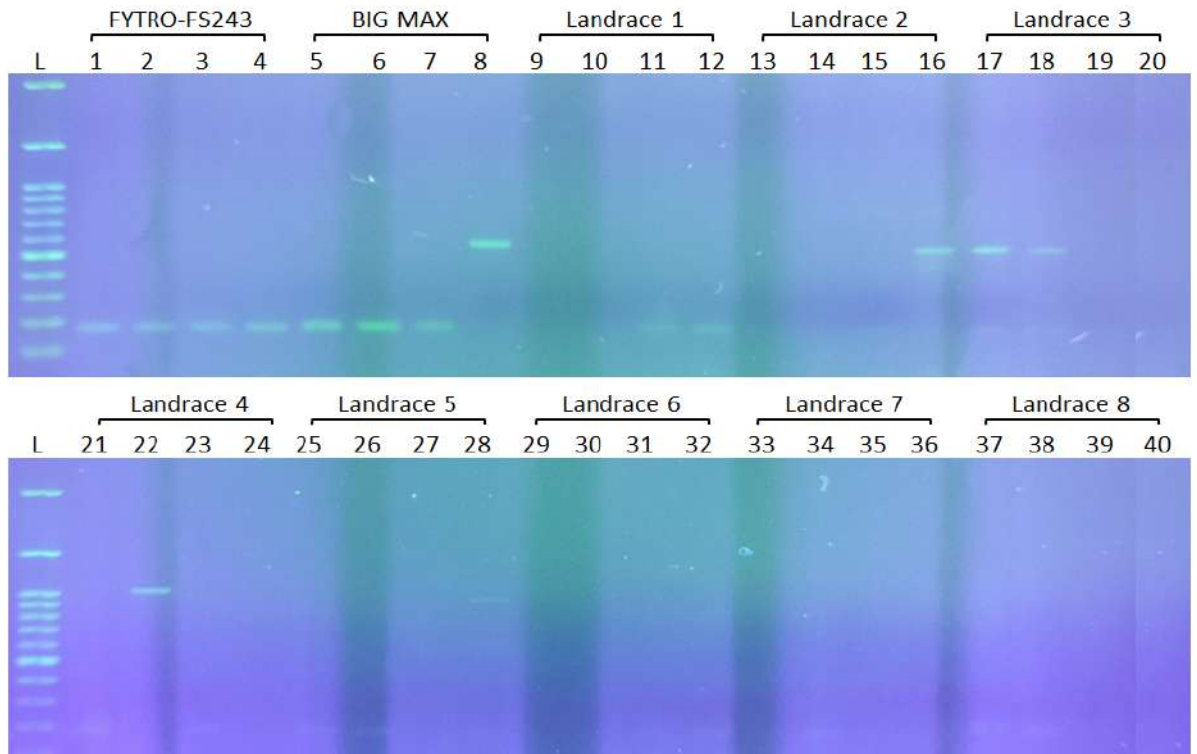


Figure 3. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker OPB-02. Ladder: 100 bp DNA Ladder (NEB).

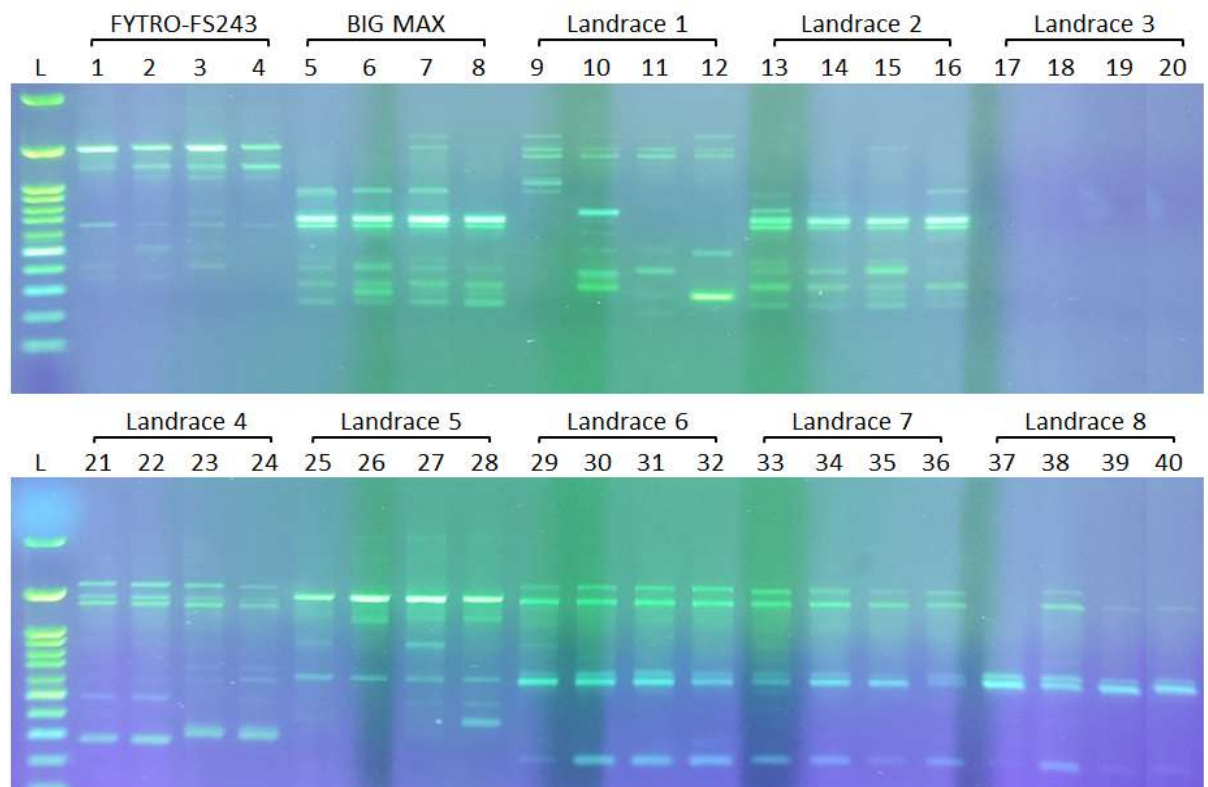


Figure 4. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker OPB-04. Ladder: 100 bp DNA Ladder (NEB).

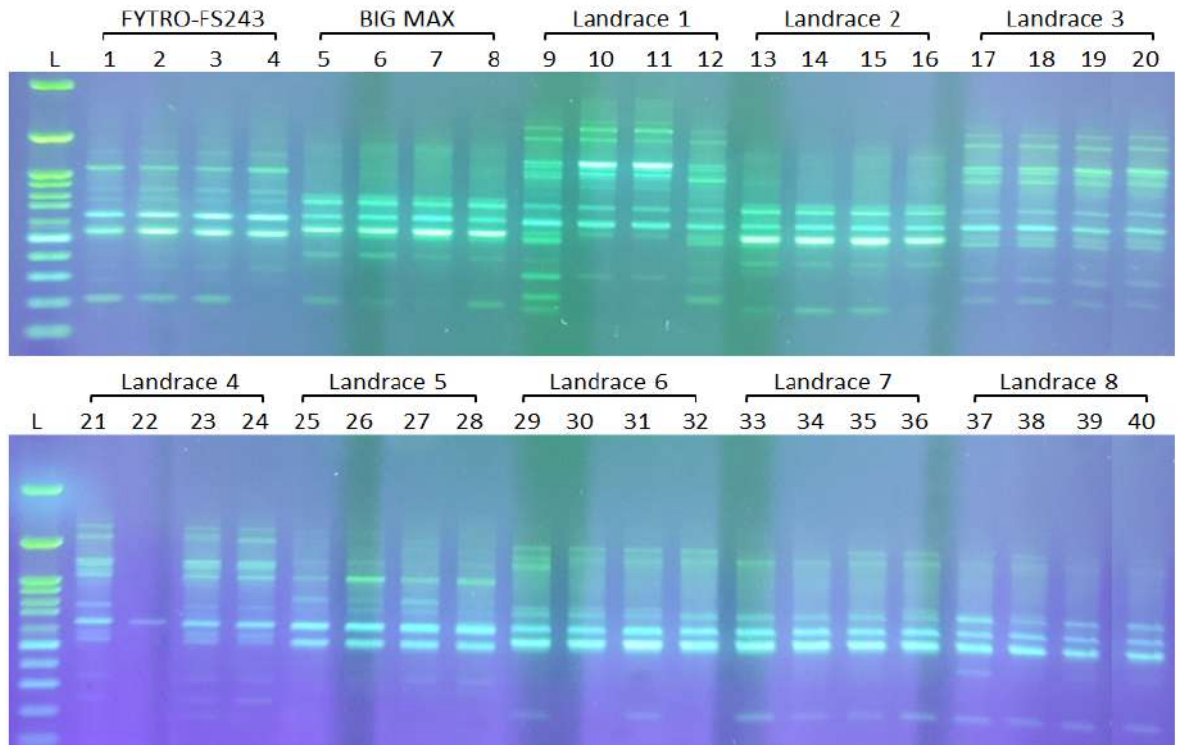


Figure 5. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker OPG-02. Ladder: 100 bp DNA Ladder (NEB).

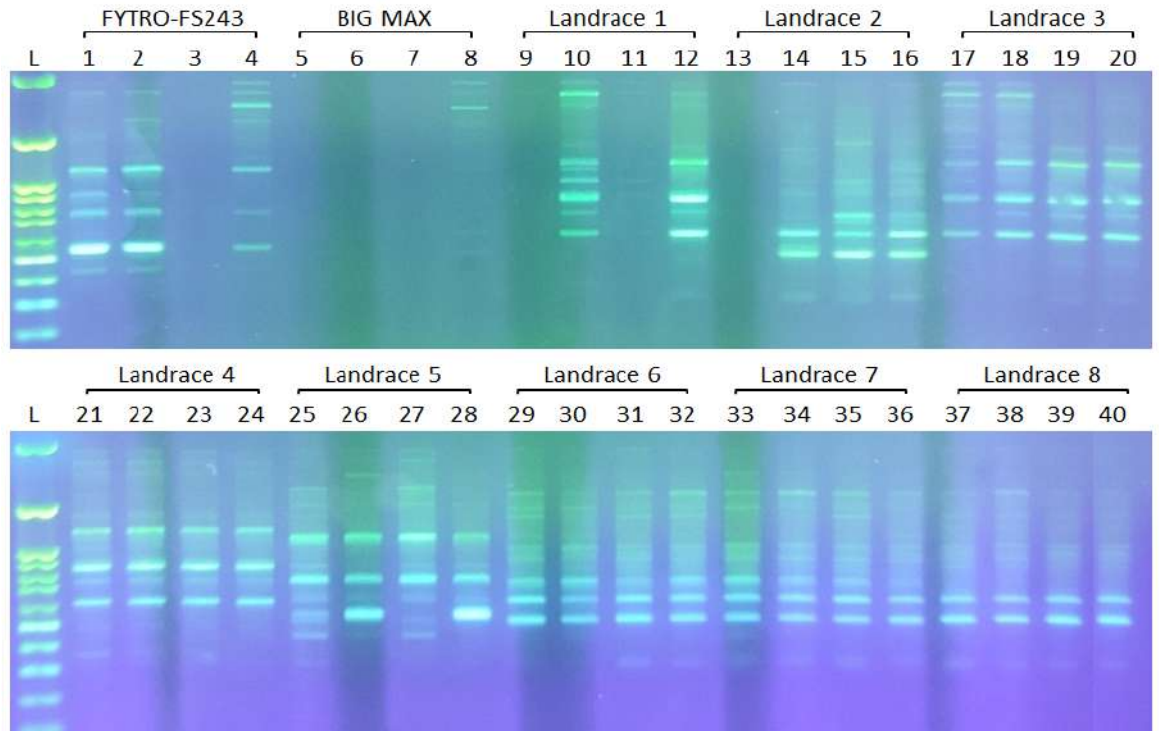


Figure 6. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker OPZ-03. Ladder: 100 bp DNA Ladder (NEB).

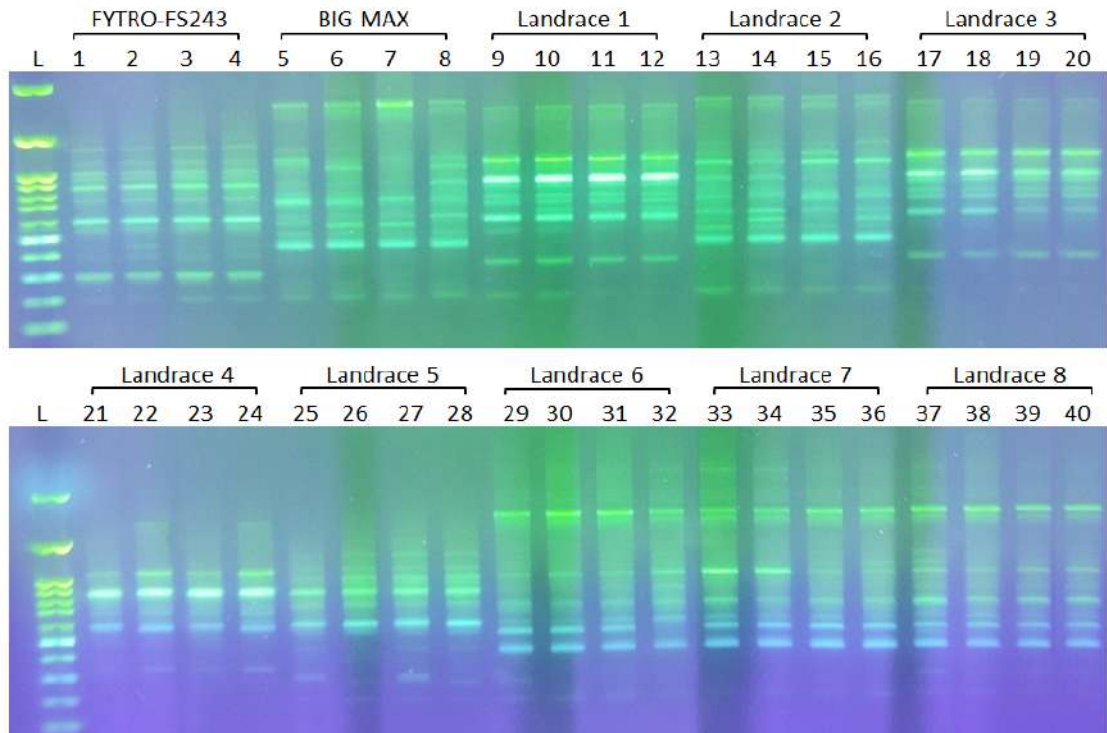


Figure 7. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker CB-9. Ladder: 100 bp DNA Ladder (NEB).

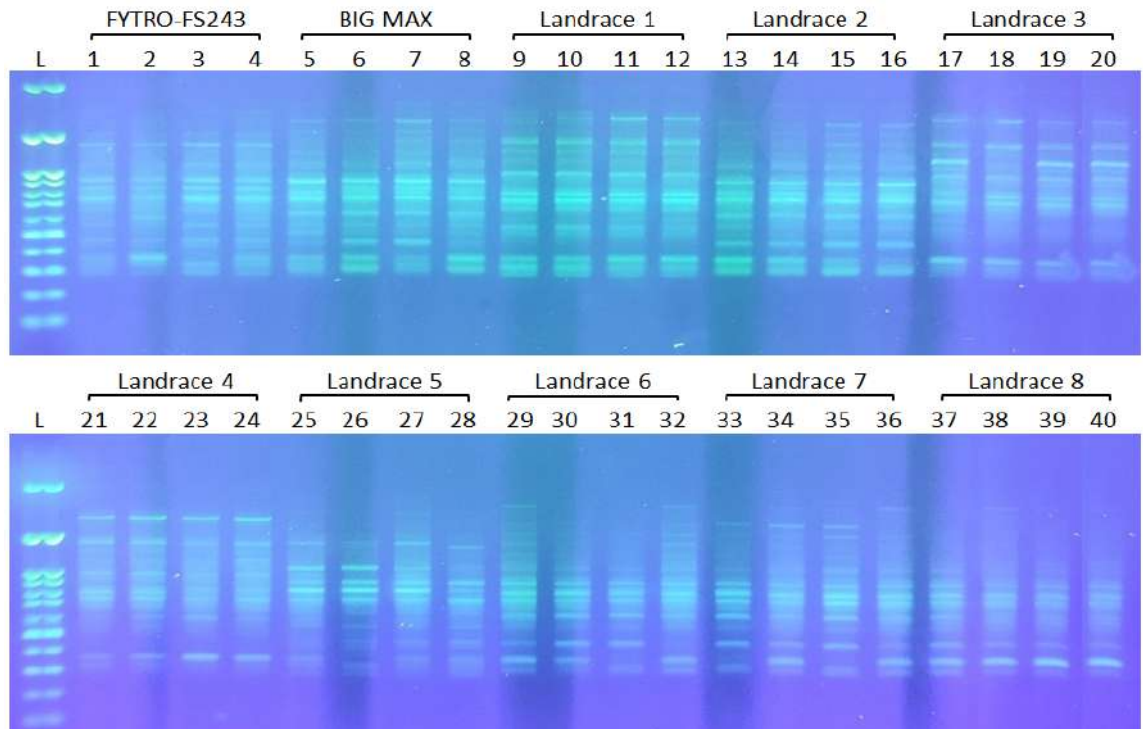


Figure 8. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker CB-12. Ladder: 100 bp DNA Ladder (NEB).

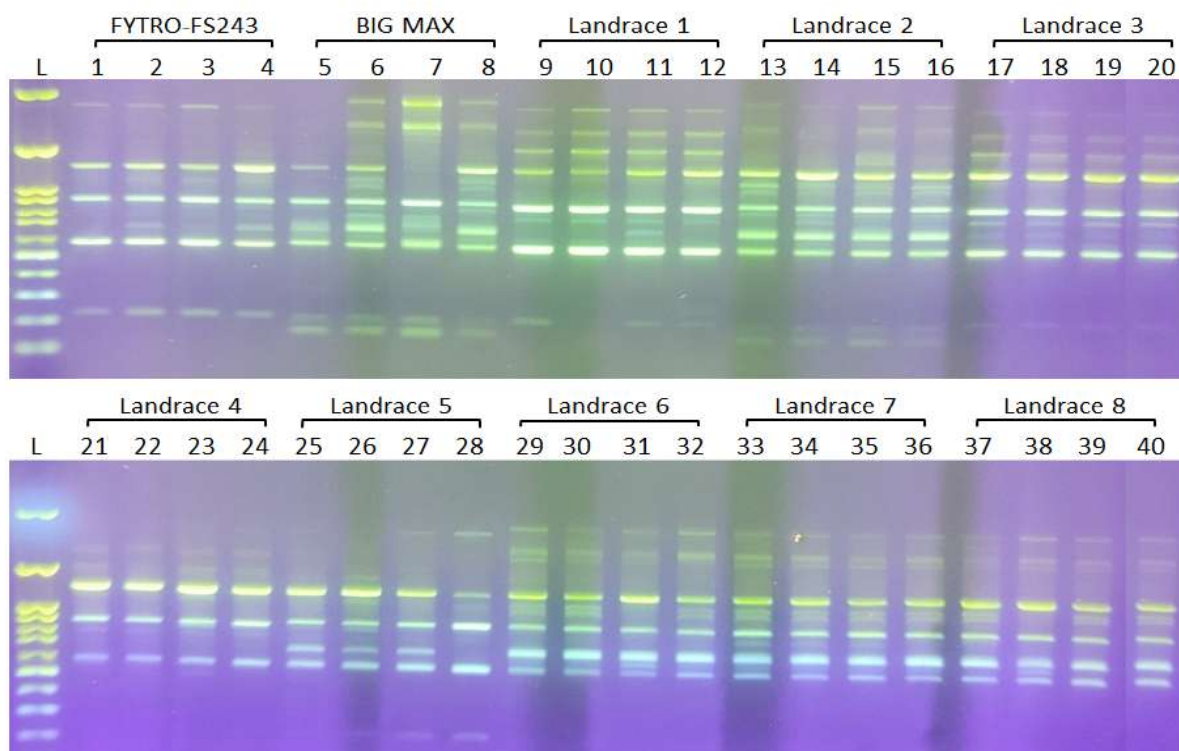


Figure 9. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker CB-13. Ladder: 100 bp DNA Ladder (NEB).

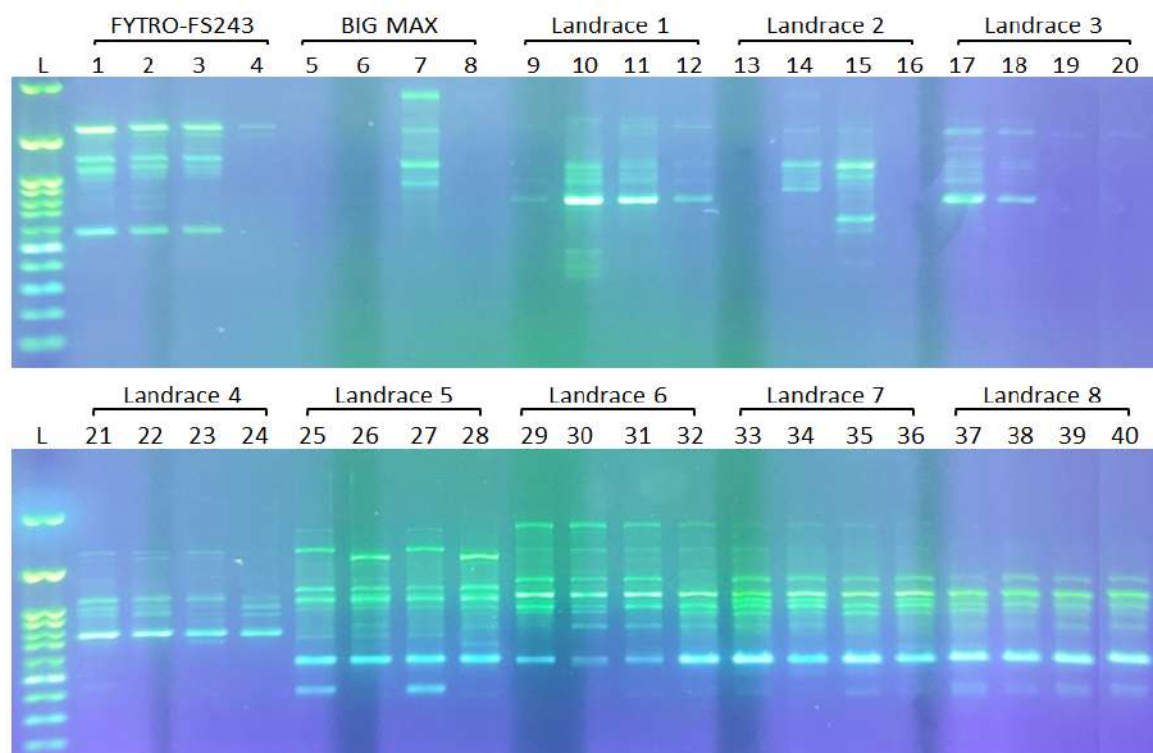


Figure 10. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker CB-15. Ladder: 100 bp DNA Ladder (NEB).

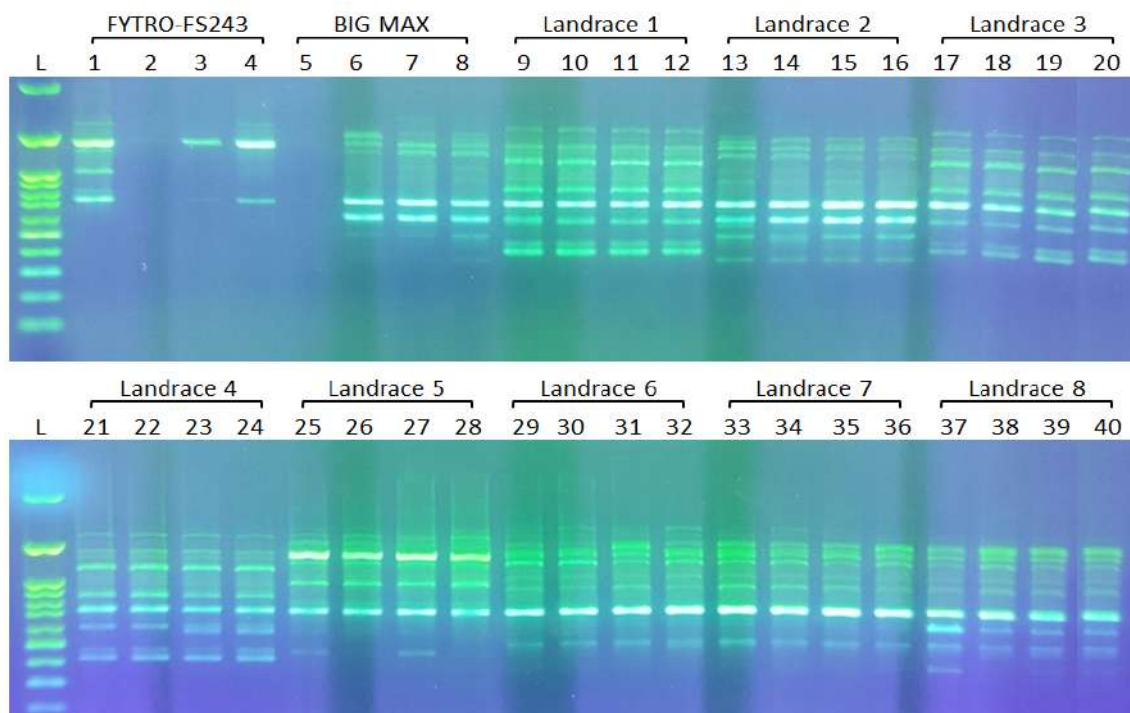


Figure 11. RAPD profiles illustrating bands amplification in the set of populations of *C. pepo* using the RAPD marker CB-17. Ladder: 100 bp DNA Ladder (NEB).

The set of 10-decamer arbitrary RAPD primers employed yielded highly polymorphic patterns and generated a total number of 215 amplification products. The degree of polymorphism and the size of amplification products are presented in Table 5.

Table 5. Analysis of the RAPD profiles of *C. pepo* populations under study. The genetic material consisted of two commercial varieties and eight local landraces.

RAPD primer	Sequence (5' – 3')	NTB	NPB	Band size range (bp)	PPB (%)
OPA-04	AATCGGGCTG	11	11	240 - 1400	100
OPB-01	GTTTCGCTCC	10	10	166 - 1190	100
OPB-04	GGACTGGAGT	21	21	209 - 1946	100
OPG-02	GGCACTGAGG	27	27	227 - 2618	100
OPZ-03	CAGCACCGCA	21	21	256 - 2975	100
CB-09	GGTGACGCAG	23	23	211 - 2399	100
CB-12	AGTCGACGCC	28	28	318 - 2406	100
CB-13	ACGCATCGGA	48	47	176 - 2879	97,91
CB-15	GGCTGGTTCC	26	26	351 - 2925	100

NTB: number of total bands, NPB: number of polymorphic bands, bp: base pair, PPB: percentage of polymorphic bands.

Amplification products ranged in size from 166 - 2975 bp. The highest number of bands was obtained using the RAPD marker CB-13 (48 bands), followed by markers CB-12,

OPG-02 and CB-15 (28, 27 and 26 bands, respectively). In contrast, the lowest number of bands was recorded in marker OPB-01 and OPA-04 (10 and 11 bands, respectively). The number of polymorphic bands ranged from 10 to 47 (OPB-01 and CB-13, respectively), with a mean value of 23,78 polymorphic bands. Overall findings are indicative of the fact that the RAPD markers employed in this study are suitable for assessing the genetic diversity existing within and among populations of *C. pepo*.

Table 6. Polymorphism of different RAPD primers and genetic diversity indices within ten *C. pepo* populations under study.

RAPD primer	Sequence (5' – 3')	N	Na	Ne	I	h	uh
OPA-04	AATCGGGCTG	4	0.491	1.089	0.072	0.050	0.067
OPB-01	GTTTCGCTCC	4	0.660	1.186	0.160	0.109	0.148
OPB-04	GGACTGGAGT	4	0.590	1.160	0.136	0.093	0.125
OPG-02	GGCACTGAGG	4	0.541	1.120	0.102	0.070	0.094
OPZ-03	CAGCACCGCA	4	0.833	1.234	0.199	0.136	0.185
CB-09	GGTGACGCAG	4	0.774	1.195	0.160	0.110	0.150
CB-12	AGTCGACGCC	4	0.864	1.250	0.203	0.140	0.192
CB-13	ACGCATCGGA	4	0.604	1.203	0.167	0.115	0.157
CB-15	GGCTGGTTCC	4	0.704	1.210	0.177	0.121	0.163
Total*		40	0.678	1.191	0.159	0.109	0.148

*Grand mean over genetic loci and populations

N: number of samples per population, Na: number of different alleles, Ne: number of effective alleles, I: Shannon's Information Index, h: gene diversity, uh: unbiased gene diversity.

Genetic diversity analysis

The genetic diversity existing within and among populations of *C. pepo* was determined based on the polymorphic band percentage (PBP %), the number of different alleles (Na), the number of effective alleles (Ne), Shannon's Information Index (I), gene diversity, Nei's genetic distance (D) and Nei's genetic identity (IN). The results for all parameters of genetic diversity are presented in Table 7. The percentage of polymorphic loci for pumpkin populations based on data from individual marker analysis is provided in the Appendix (Table 3).

The RAPD marker analysis revealed an average polymorphic band percentage (PBP) that ranged from 18.14 to 32.09 among *C. pepo* populations under study. The highest PBP was recorded in P6 (32.09 %), followed by P7 (30.23 %), whereas the lowest PBR was found in P5 and P10 (18.14 %). The average number of different alleles (Na) ranged from 0.530 to 0.781 for P10 and P3, respectively (Table 7).

In relation to the number of effective alleles (Ne), the values ranged from 1.145 to 1.228. The highest Ne value was recorded in P6 (1.228), while P5 and P10 showed the lowest Ne (1.145). In accordance with findings related to PBP and Ne, P6 showed also the highest Shannon index (I = 0.192) and the highest gene diversity (h = 0.131).

Accordingly, the lowest respective values were recorded in P5 and P10 ($I = 0.115$, $h = 0.081$) (Table 7).

Table 7. Genetic diversity indices of *C. pepo* populations based on RAPD marker analysis.

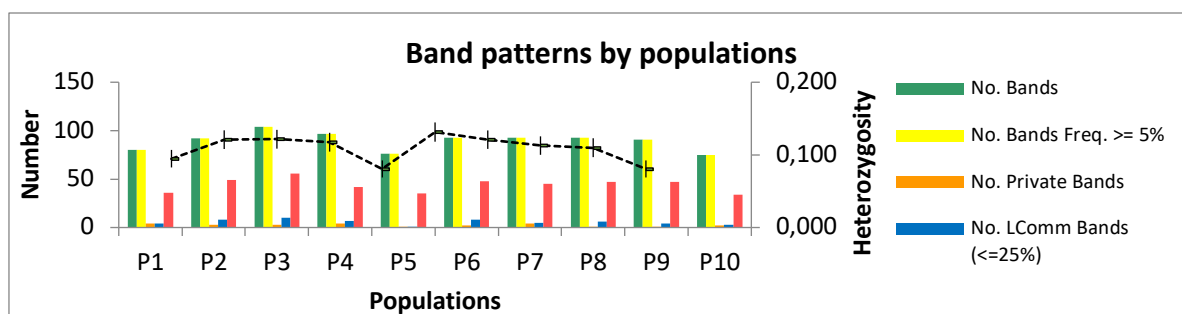
Population	Cultivar Name	PBP (%)	N	Na	Ne	I	h	uh
P1	FYTRO FS 243	22.79	4	0.600	1.167	0.138	0.095	0.126
P2	BIG MAX	29.77	4	0.726	1.208	0.177	0.121	0.161
P3	Pumpkin for pie	29.77	4	0.781	1.210	0.178	0.122	0.162
P4	Nyxaki	28.37	4	0.735	1.206	0.171	0.117	0.157
P5	Melitis-BI / White	18.14	4	0.535	1.145	0.115	0.081	0.121
P6	Round / Deep orange	32.09	4	0.753	1.228	0.192	0.131	0.175
P7	Neapoli / Oval, small	30.23	4	0.735	1.206	0.178	0.121	0.161
P8	Lakonia / Bottle shape	27.44	4	0.707	1.196	0.165	0.113	0.150
P9	Makedonika / Green	25.58	4	0.679	1.198	0.158	0.110	0.147
P10	K-7	18.14	4	0.530	1.145	0.115	0.081	0.121
Total*			40	0.678	1.191	0.159	0.109	0.148

*Grand mean over genetic loci and populations

PBP: percentage of polymorphic bands, N: number of samples, Na: number of different alleles, Ne: number of effective alleles, I: Shannon's Information Index, h: gene diversity (heterozygosity value), uh: unbiased gene diversity.

The band patterns for binary (haploid) data by populations are illustrated in Graph 1. The total band patterns for binary (haploid) data by populations for individual markers as well as for the entire set of markers are provided in the Appendix.

As shown in Graph 1, the highest number of locally common bands, present in 25 % or fewer populations, was found in P3 (10 bands) and P6 (8 bands). To the contrary, the lowest number was found in P5 (1 band). Furthermore, the analysis revealed that populations 1, 4 and 7 present the highest number of unique bands to a single populations (4 unique bands). In contrast, the lowest number of unique bands was found in populations 5, 8 and 9 (1 unique band).



Graph 1. Band patterns of RAPD markers by populations.

[No. Bands = No. of different bands, No. Bands Freq. $\geq 5\%$ = No. of Different Bands with a Frequency $\geq 5\%$,
 No. Private Bands = No. of Bands Unique to a Single Population, No. LComm Bands ($\leq 25\%$) = No. of Locally Common Bands (Freq. $\geq 5\%$) Found in 25% or Fewer Populations, No. LComm Bands ($\leq 50\%$) = No. of Locally Common Bands (Freq. $\geq 5\%$) Found in 50% or Fewer Populations]

Further, analysis of molecular variance (AMOVA) was conducted as a means to determine the distribution of genetic diversity both among and within populations under study. AMOVA revealed that 59 % of total genetic variation was attributed to differences among populations and 41 % of genetic variance was accordingly attributed to differences within populations (Table 8). The PhiPT value was 0.587.

Table 8. Analysis of molecular variance (AMOVA) of ten *C. pepo* populations using RAPD markers. The genetic material consisted of two commercial varieties and eight local landraces.

Source of variation	DF	SS	MS	Est. Var.	%	PhiPT
Among populations	9	928,171	103,130	22,922	59%	0.587**
Within populations	30	451,750	16,134	16,134	41%	
Total	39	1379,921		39,056	100%	

DF: degrees of freedom, SS: sum of squares, MS: mean square deviations, Est.Var.: estimated variance, percentage of total variance (%), PhiPT: significance of variance (p-value) [PhiPT = $AP / (WP + AP) = AP / TOT$], ** $p < 0.001$.

The pairwise population PhiPT values were estimated in order to estimate the genetic differentiation among populations under study. The PhiPT values ranged from 0.345 to 0.671. The analysis revealed a high differentiation (PhiPT = 0.671) of P3 and P9 populations, whereas the lowest differentiation was recorded between P8 and P9 populations (PhiPT = 0.345) (Table 9).

Table 9. Pairwise population PhiPT values among ten *C. pepo* populations using RAPD markers. The values are calculated with permutation number $N = 999$.

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
------------	----	----	----	----	----	----	----	----	----	-----

P1	-									
P2	0.547	-								
P3	0.624	0.555	-							
P4	0.616	0.494	0.587	-						
P5	0.665	0.631	0.454	0.610	-					
P6	0.584	0.541	0.457	0.578	0.464	-				
P7	0.505	0.585	0.576	0.593	0.588	0.524	-			
P8	0.642	0.620	0.631	0.529	0.655	0.570	0.543	-		
P9	0.651	0.642	0.671	0.590	0.659	0.609	0.581	0.345		
P10	0.652	0.636	0.666	0.591	0.670	0.606	0.593	0.541	0.424	-

Further, Nei's genetic distance and identity were determined in order to provide estimates for the genetic relationship among populations. Nei's genetic distance ranged from 0.139 to 0.510, while the genetic identity ranged accordingly from 0.600 to 0.870 (Table 10). In relation to genetic distance among populations, the highest values were recorded for P3 and P9 ($D = 0.510$), whereas the corresponding lowest values were found in P8 and P9 populations ($D = 0.139$). Accordingly, P8 and P9 showed the highest genetic identity ($IN = 0.870$), followed by P9 and P10 ($IN = 0.845$). In contrast, P3 and P9 were the least identical populations ($IN = 0.600$). The Nei's genetic distance and genetic identity based on the analysis of individual markers are provided in the Appendix.

Table 10. Nei's genetic distance and genetic identity among ten *C. pepo* populations using RAPD markers. The genetic material consisted of two commercial varieties and eight local landraces.

Pairwise Population Matrix of Nei Genetic Distance										
Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
P1	0.000									
P2	0.268	0.000								
P3	0.369	0.322	0.000							
P4	0.348	0.250	0.360	0.000						
P5	0.370	0.390	0.201	0.347	0.000					
P6	0.330	0.321	0.237	0.366	0.221	0.000				
P7	0.229	0.364	0.351	0.369	0.325	0.301	0.000			
P8	0.379	0.404	0.426	0.272	0.409	0.345	0.293	0.000		
P9	0.387	0.440	0.510	0.340	0.407	0.402	0.335	0.139	0.000	
P10	0.350	0.399	0.462	0.321	0.373	0.377	0.331	0.256	0.168	0.000

Pairwise Population Matrix of Nei Genetic Identity										
Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
P1	1.000									
P2	0.765	1.000								
P3	0.691	0.724	1.000							
P4	0.706	0.779	0.698	1.000						
P5	0.690	0.677	0.818	0.707	1.000					
P6	0.719	0.725	0.789	0.693	0.802	1.000				
P7	0.795	0.695	0.704	0.692	0.723	0.740	1.000			
P8	0.684	0.668	0.653	0.762	0.664	0.708	0.746	1.000		
P9	0.679	0.644	0.600	0.712	0.665	0.669	0.715	0.870	1.000	
P10	0.705	0.671	0.630	0.725	0.689	0.686	0.718	0.774	0.845	1.000

Principal coordinate analysis (PCoA) was used to generate a coordinate graph which provides visualization of the genetic relationships among populations of *C. pepo*. In the coordinate graph, the points represent the individuals from the ten populations under study (Figure 12). The results of PCoA are in good agreement with those obtained by the genetic distance and identity indices, supporting the separation of individuals into three main groups. As shown in Figure 12, populations P8, P9 and P10 are clustered together, while the analysis further revealed close genetic relationships among P3, P5 and P6. The third group, involving populations P1, P2, P4 and P7, shows relatively greater genetic divergence as compared to the others.

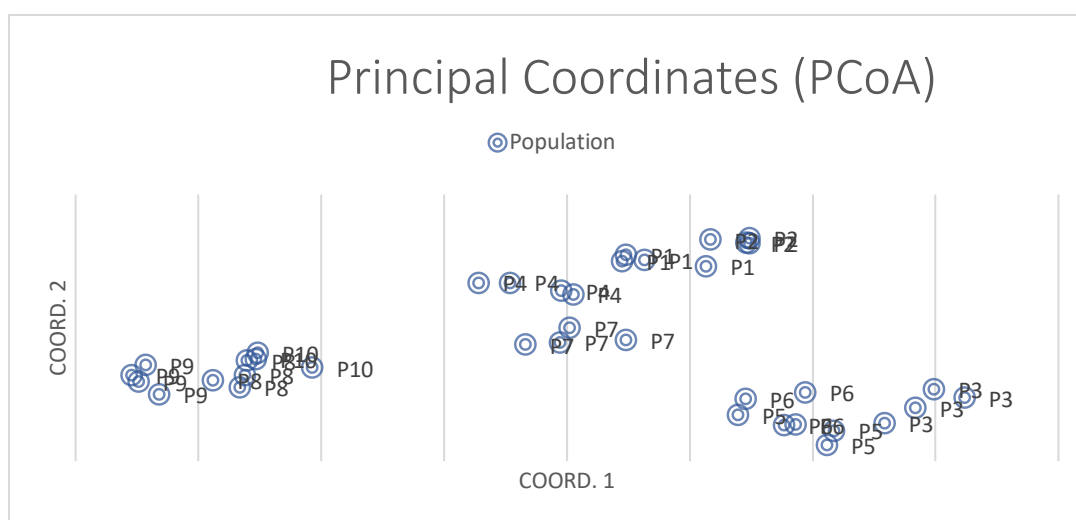


Figure 12. Principal coordinate analysis of ten populations of *C. pepo* based on RAPD molecular marker analysis.

Conclusively, the findings underline the suitability of RAPD markers for determining the genetic diversity both at the intra- and inter-population level. RAPD analysis of the germplasm under study revealed a significant level of genetic diversity both within and among populations, while it is interesting both from an agronomic and breeding



perspective that the Greek local landraces derived from different geographic regions under study exhibit a considerable genetic variation.

APPENDIX

Table 1. A data matrix for RAPD marker A04. Polymorphic bands are scored as a binary character, “1” stands for a presence of a band and “0” stands for its absence.

Lane #	1400	1300	1200	1100	970	690	630	460	380	300	240
2	1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	1	0	0	0	0
2	0	0	0	0	0	0	0	0	1	0	0
3	1	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	1	0	0	0	0
4	1	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	1	0	0	0	0
4	0	0	0	0	0	0	0	0	1	0	0
4	0	0	0	0	0	0	0	0	0	0	1
5	1	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	1	0	0	0	0
5	0	0	0	0	0	0	0	0	1	0	0
5	0	0	0	0	0	0	0	0	0	0	1
6	0	0	1	0	0	0	0	0	0	0	0
6	0	0	0	1	0	0	0	0	0	0	0
6	0	0	0	0	0	0	1	0	0	0	0
6	0	0	0	0	0	0	0	1	0	0	0
6	0	0	0	0	0	0	0	0	1	0	0
6	0	0	0	0	0	0	0	0	0	0	1
7	0	0	1	0	0	0	0	0	0	0	0
7	0	0	0	1	0	0	0	0	0	0	0
7	0	0	0	0	0	0	1	0	0	0	0
7	0	0	0	0	0	0	0	1	0	0	0
8	0	0	1	0	0	0	0	0	0	0	0
8	0	0	0	1	0	0	0	0	0	0	0
8	0	0	0	0	1	0	0	0	0	0	0
8	0	0	0	0	0	1	0	0	0	0	0
8	0	0	0	0	0	0	1	0	0	0	0
8	0	0	0	0	0	0	0	0	1	0	0
8	0	0	0	0	0	0	0	0	0	0	1
9	0	0	1	0	0	0	0	0	0	0	0
9	0	0	0	1	0	0	0	0	0	0	0
9	0	0	0	0	0	0	1	0	0	0	0
9	0	0	0	0	0	0	0	1	0	0	0
9	0	0	0	0	0	0	0	0	1	0	0
10	0	0	0	0	1	0	0	0	0	0	0
10	0	0	0	0	0	0	1	0	0	0	0
11	0	0	0	0	1	0	0	0	0	0	0
11	0	0	0	0	0	0	1	0	0	0	0
11	0	0	0	0	0	0	0	1	0	0	0
12	0	0	0	0	1	0	0	0	0	0	0
12	0	0	0	0	0	0	1	0	0	0	0
13	0	0	0	0	1	0	0	0	0	0	0
13	0	0	0	0	0	1	0	0	0	0	0
14	0	0	0	1	0	0	0	0	0	0	0
14	0	0	0	0	1	0	0	0	0	0	0
14	0	0	0	0	0	0	1	0	0	0	0
14	0	0	0	0	0	0	0	0	0	1	0
14	0	0	0	0	0	0	0	0	0	0	1
15	0	0	0	1	0	0	0	0	0	0	0
15	0	0	0	0	1	0	0	0	0	0	0
15	0	0	0	0	0	0	1	0	0	0	0
15	0	0	0	0	0	0	0	1	0	0	0
15	0	0	0	0	0	0	0	0	0	1	0
16	0	0	0	1	0	0	0	0	0	0	0
16	0	0	0	0	1	0	0	0	0	0	0

16	0	0	0	0	0	0	1	0	0	0	0
16	0	0	0	0	0	0	0	0	0	1	0
16	0	0	0	0	0	0	0	0	0	0	1
17	0	0	0	1	0	0	0	0	0	0	0
17	0	0	0	0	1	0	0	0	0	0	0
17	0	0	0	0	0	0	1	0	0	0	0
17	0	0	0	0	0	0	0	1	0	0	0
17	0	0	0	0	0	0	0	0	0	1	0
18	0	0	0	0	1	0	0	0	0	0	0
18	0	0	0	0	0	0	1	0	0	0	0
19	0	0	0	0	1	0	0	0	0	0	0
19	0	0	0	0	0	0	1	0	0	0	0
20	0	0	0	0	1	0	0	0	0	0	0
20	0	0	0	0	0	0	1	0	0	0	0
21	0	0	0	1	0	0	0	0	0	0	0
21	0	0	0	0	0	1	0	0	0	0	0
22	0	0	0	1	0	0	0	0	0	0	0
22	0	0	0	0	0	1	0	0	0	0	0
23	0	0	0	1	0	0	0	0	0	0	0
23	0	0	0	0	0	1	0	0	0	0	0
24	0	0	0	1	0	0	0	0	0	0	0
24	0	0	0	0	0	1	0	0	0	0	0
25	1	0	0	0	0	0	0	0	0	0	0
25	0	1	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	1	0	0	0	0
25	0	0	0	0	0	0	0	0	1	0	0
26	1	0	0	0	0	0	0	0	0	0	0
26	0	1	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	1	0	0	0	0
26	0	0	0	0	0	0	0	1	0	0	0
27	1	0	0	0	0	0	0	0	0	0	0
27	0	1	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	1	0	0	0	0
27	0	0	0	0	0	0	0	0	1	0	0
28	1	0	0	0	0	0	0	0	0	0	0
28	0	1	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	1	0	0	0	0
28	0	0	0	0	0	0	0	0	1	0	0
29	0	1	0	0	0	0	0	0	0	0	0
29	0	0	1	0	0	0	0	0	0	0	0
29	0	0	0	1	0	0	0	0	0	0	0
29	0	0	0	0	0	1	0	0	0	0	0
29	0	0	0	0	0	0	0	0	1	0	0
30	0	1	0	0	0	0	0	0	0	0	0
30	0	0	1	0	0	0	0	0	0	0	0
30	0	0	0	1	0	0	0	0	0	0	0
30	0	0	0	0	0	1	0	0	0	0	0
30	0	0	0	0	0	0	0	0	1	0	0
31	0	1	0	0	0	0	0	0	0	0	0
31	0	0	1	0	0	0	0	0	0	0	0
31	0	0	0	0	0	1	0	0	0	0	0
31	0	0	0	0	0	0	0	0	1	0	0
32	0	1	0	0	0	0	0	0	0	0	0
32	0	0	1	0	0	0	0	0	0	0	0
32	0	0	0	1	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	1	0	0
33	0	1	0	0	0	0	0	0	0	0	0
33	0	0	1	0	0	0	0	0	0	0	0
33	0	0	0	0	0	1	0	0	0	0	0
33	0	0	0	0	0	0	0	0	1	0	0
34	0	1	0	0	0	0	0	0	0	0	0
34	0	0	1	0	0	0	0	0	0	0	0
34	0	0	0	0	0	1	0	0	0	0	0

34	0	0	0	0	0	0	0	0	1	0	0
35	0	1	0	0	0	0	0	0	0	0	0
35	0	0	1	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	1	0	0
36	0	1	0	0	0	0	0	0	0	0	0
36	0	0	1	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	1	0	0
37	0	1	0	0	0	0	0	0	0	0	0
37	0	0	1	0	0	0	0	0	0	0	0
37	0	0	0	0	0	1	0	0	0	0	0
37	0	0	0	0	0	0	0	0	1	0	0
38	0	1	0	0	0	0	0	0	0	0	0
38	0	0	1	0	0	0	0	0	0	0	0
38	0	0	0	0	0	1	0	0	0	0	0
38	0	0	0	0	0	0	0	0	1	0	0
39	0	1	0	0	0	0	0	0	0	0	0
39	0	0	1	0	0	0	0	0	0	0	0
39	0	0	0	0	0	1	0	0	0	0	0
39	0	0	0	0	0	0	0	0	1	0	0

4.

Table 2. Formulas used for the estimation of genetic parameters.

Genetic parameter	Formula	Designations
Ne	$Ne = \text{No. of Effective Alleles} = 1 / (p^2 + q^2)$	
I	$I = \text{Shannon's Information Index} = -1 * (p * \ln(p) + q * \ln(q))$	
h	$h = \text{Diversity} = 1 - (p^2 + q^2)$	Where for Haploid Binary data, p = Band Freq. and q = 1 - p
uh	$uh = \text{Unbiased Diversity} = (N / (N-1)) * h$	Where for Haploid Binary data, p = Band Freq. and q = 1 - p
PhiPT	$\text{PhiPT} = AP / (WP + AP) = AP / \text{TOT}$	AP = Est. Var. Among Pops WP = Est. Var. Within Pops

Table 3. Percentage of polymorphic loci for pumpkin populations under study.

Population	Percentage of Polymorphic Loci									
	% Polymorphism									
	OPA-04	OPB-01	OPB-04	OPG-02	OPZ-03	CB-09	CB-12	CB-13	CB-15	Total
P1	18,18	20,00	28,57	11,11	42,86	8,70	25,00	16,67	38,46	22,79
P2	36,36	50,00	28,57	3,70	28,57	26,09	32,14	43,75	23,08	29,77
P3	18,18	40,00	47,62	44,44	42,86	13,04	7,14	25,00	38,46	29,77
P4	18,18	30,00	19,05	3,70	52,38	34,78	39,29	25,00	34,62	28,37
P5	0,00	20,00	0,00	3,70	33,33	4,35	50,00	18,75	19,23	18,14
P6	0,00	50,00	14,29	44,44	19,05	30,43	35,71	37,50	38,46	32,09
P7	0,00	10,00	28,57	22,22	42,86	34,78	39,29	29,17	38,46	30,23
P8	18,18	20,00	28,57	11,11	33,33	26,09	32,14	35,42	26,92	27,44
P9	9,09	10,00	14,29	14,81	23,81	43,48	53,57	14,58	34,62	25,58
P10	0,00	20,00	19,05	11,11	14,29	39,13	14,29	27,08	3,85	18,14
Mean	11,82	27,00	22,86	17,04	33,33	26,09	32,86	27,29	29,62	26,23
SE	3,85	4,73	4,00	4,91	3,82	4,20	4,57	2,99	3,63	1,58

Total Band Patterns for Binary (Haploid) Data by Populations

OPA-04

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	4	8	3	6	2	2	4	5	4	3
No. Bands Freq. \geq 5%	4	8	3	6	2	2	4	5	4	3
No. Private Bands	0	0	0	1	0	0	0	0	0	0
No. LComm Bands (\leq25%)	0	0	0	0	0	0	0	0	0	0
No. LComm Bands (\leq50%)	3	7	2	4	1	2	3	5	4	2
Mean h	0,080	0,148	0,068	0,091	0,000	0,000	0,000	0,068	0,045	0,000
SE of Mean h	0,054	0,063	0,046	0,061	0,000	0,000	0,000	0,046	0,045	0,000
Mean uh	0,106	0,197	0,091	0,121	0,000	0,000	0,000	0,091	0,061	0,000
SE of Mean uh	0,072	0,084	0,061	0,081	0,000	0,000	0,000	0,061	0,061	0,000

No. Bands = No. of Different Bands

No. Bands Freq. \geq 5% = No. of Different Bands with a Frequency \geq 5%

No. Private Bands = No. of Bands Unique to a Single Population

No. LComm Bands (\leq 25%) = No. of Locally Common Bands (Freq. \geq 5%) Found in 25% or Fewer Populations

No. LComm Bands (\leq 50%) = No. of Locally Common Bands (Freq. \geq 5%) Found in 50% or Fewer Populations

h = Diversity = $1 - (p^2 + q^2)$

uh = Unbiased Diversity = $(N / (N-1)) * h$

Where for Haploid Binary data, p = Band Freq. and $q = 1 - p$.

OPB-01

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	2	5	8	3	7	6	1	2	3	2
No. Bands Freq. \geq 5%	2	5	8	3	7	6	1	2	3	2
No. Private Bands	0	0	0	0	1	0	0	0	0	0
No. LComm Bands (\leq25%)	0	2	2	0	0	0	0	0	0	0
No. LComm Bands (\leq50%)	1	4	7	1	4	4	0	0	1	0
Mean h	0,088	0,188	0,150	0,138	0,089	0,188	0,050	0,075	0,038	0,089
SE of Mean h	0,059	0,063	0,061	0,071	0,059	0,063	0,050	0,050	0,038	0,059
Mean uh	0,117	0,250	0,200	0,183	0,133	0,250	0,067	0,100	0,050	0,133
SE of Mean uh	0,079	0,083	0,082	0,094	0,089	0,083	0,067	0,067	0,050	0,089

OPB-04

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	9	9	13	9	0	7	8	9	6	6
No. Bands Freq. \geq 5%	9	9	13	9	0	7	8	9	6	6

No. Private Bands	0	0	1	0	0	0	0	0	0	1
No. LComm Bands (<=25%)	0	1	1	0	0	1	0	1	0	0
No. LComm Bands (<=50%)	7	7	9	7	0	6	6	7	4	3
Mean h	0,125	0,107	0,190	0,071	0,000	0,065	0,113	0,107	0,065	0,085
SE of Mean h	0,045	0,038	0,045	0,033	0,000	0,036	0,040	0,038	0,036	0,039
Mean uh	0,167	0,143	0,254	0,095	0,000	0,087	0,151	0,143	0,087	0,127
SE of Mean uh	0,060	0,051	0,060	0,044	0,000	0,048	0,054	0,051	0,048	0,059

OPG-02

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	10	7	18	8	11	13	10	8	8	7
No. Bands Freq. >= 5%	10	7	18	8	11	13	10	8	8	7
No. Private Bands	0	0	1	0	0	1	0	0	0	0
No. LComm Bands (<=25%)	2	1	3	1	1	1	2	0	1	0
No. LComm Bands (<=50%)	8	6	13	4	8	9	7	4	5	4
Mean h	0,046	0,014	0,190	0,014	0,016	0,171	0,088	0,046	0,060	0,049
SE of Mean h	0,026	0,014	0,042	0,014	0,016	0,038	0,033	0,026	0,029	0,027
Mean uh	0,062	0,019	0,253	0,019	0,025	0,228	0,117	0,062	0,080	0,074
SE of Mean uh	0,035	0,019	0,057	0,019	0,025	0,050	0,043	0,035	0,038	0,041

OPZ-03

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	9	6	9	14	11	11	13	11	12	9
No. Bands Freq. >= 5%	9	6	9	14	11	11	13	11	12	9
No. Private Bands	0	0	0	1	0	1	2	0	0	0
No. LComm Bands (<=25%)	0	0	0	1	0	1	0	0	0	0
No. LComm Bands (<=50%)	4	1	0	2	2	2	4	3	4	2
Mean h	0,185	0,107	0,179	0,208	0,148	0,077	0,173	0,131	0,089	0,063
SE of Mean h	0,049	0,038	0,047	0,045	0,047	0,036	0,045	0,042	0,036	0,035
Mean uh	0,246	0,143	0,238	0,278	0,222	0,103	0,230	0,175	0,119	0,095
SE of Mean uh	0,065	0,051	0,063	0,060	0,070	0,048	0,060	0,056	0,048	0,052

CB-09

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	11	13	12	13	9	9	13	11	12	15
No. Bands Freq. >= 5%	11	13	12	13	9	9	13	11	12	15
No. Private Bands	1	0	0	0	0	0	0	0	0	0

No. LComm Bands (<=25%)	1	1	0	1	0	0	1	0	1	1
No. LComm Bands (<=50%)	2	3	2	4	2	1	5	3	5	6
Mean h	0,033	0,109	0,065	0,147	0,019	0,130	0,130	0,098	0,196	0,174
SE of Mean h	0,023	0,040	0,036	0,044	0,019	0,043	0,038	0,035	0,048	0,046
Mean uh	0,043	0,145	0,087	0,196	0,029	0,174	0,174	0,130	0,261	0,261
SE of Mean uh	0,030	0,053	0,048	0,058	0,029	0,057	0,051	0,047	0,064	0,069

CB-12

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	14	16	13	16	16	14	15	17	19	10
No. Bands Freq. >= 5%	14	16	13	16	16	14	15	17	19	10
No. Private Bands	1	0	0	0	0	0	1	0	0	0
No. LComm Bands (<=25%)	0	1	1	0	0	0	0	0	0	0
No. LComm Bands (<=50%)	2	5	5	3	5	4	3	6	7	2
Mean h	0,098	0,138	0,031	0,161	0,222	0,165	0,156	0,143	0,223	0,063
SE of Mean h	0,033	0,039	0,022	0,039	0,043	0,043	0,038	0,041	0,041	0,030
Mean uh	0,131	0,185	0,042	0,214	0,333	0,220	0,208	0,190	0,298	0,095
SE of Mean uh	0,044	0,052	0,029	0,052	0,064	0,058	0,050	0,054	0,054	0,045

CB-13

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	10	22	16	19	14	18	14	18	13	15
No. Bands Freq. >= 5%	10	22	16	19	14	18	14	18	13	15
No. Private Bands	1	3	0	2	0	0	0	0	0	1
No. LComm Bands (<=25%)	1	1	3	2	0	3	1	4	1	2
No. LComm Bands (<=50%)	7	15	12	14	12	13	10	14	11	12
Mean h	0,070	0,185	0,096	0,104	0,083	0,151	0,117	0,156	0,065	0,120
SE of Mean h	0,023	0,031	0,024	0,027	0,025	0,029	0,027	0,031	0,023	0,029
Mean uh	0,094	0,247	0,128	0,139	0,125	0,201	0,156	0,208	0,087	0,181
SE of Mean uh	0,031	0,042	0,033	0,036	0,038	0,038	0,036	0,042	0,031	0,043

CB-15

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	11	6	12	9	6	13	15	12	14	8
No. Bands Freq. >= 5%	11	6	12	9	6	13	15	12	14	8
No. Private Bands	1	0	1	0	0	0	1	1	1	0
No. LComm Bands (<=25%)	0	1	0	2	0	2	1	1	1	0

No. LComm Bands (<=50%)	2	1	6	3	1	7	7	5	6	3
Mean h	0,154	0,091	0,154	0,144	0,085	0,149	0,159	0,106	0,154	0,017
SE of Mean h	0,039	0,034	0,039	0,040	0,035	0,038	0,041	0,035	0,043	0,017
Mean uh	0,205	0,122	0,205	0,192	0,128	0,199	0,212	0,141	0,205	0,026
SE of Mean uh	0,053	0,045	0,053	0,054	0,053	0,051	0,054	0,047	0,057	0,026

ALL MARKERS

Population	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
No. Bands	80	92	104	97	76	93	93	93	91	75
No. Bands Freq. >= 5%	80	92	104	97	76	93	93	93	91	75
No. Private Bands	4	3	3	4	1	2	4	1	1	2
No. LComm Bands (<=25%)	4	8	10	7	1	8	5	6	4	3
No. LComm Bands (<=50%)	36	49	56	42	35	48	45	47	47	34
Mean h	0,095	0,121	0,122	0,117	0,081	0,131	0,121	0,113	0,110	0,081
SE of Mean h	0,012	0,013	0,013	0,013	0,012	0,013	0,013	0,013	0,013	0,012
Mean uh	0,126	0,161	0,162	0,157	0,121	0,175	0,161	0,150	0,147	0,121
SE of Mean uh	0,016	0,017	0,017	0,017	0,018	0,018	0,017	0,017	0,017	0,018

Nei's Genetic Distance and Genetic Identity

OPA-04

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,485	0,000									P2
0,273	0,491	0,000								P3
0,651	0,479	0,267	0,000							P4
0,309	0,569	0,011	0,271	0,000						P5
0,607	0,569	0,417	0,558	0,452	0,000					P6
0,132	0,660	0,417	0,964	0,452	0,788	0,000				P7
0,763	0,392	0,823	1,216	0,859	0,348	0,491	0,000			P8
0,584	0,503	0,635	1,411	0,670	0,503	0,360	0,062	0,000		P9
0,187	0,485	0,283	0,404	0,318	0,318	0,318	0,753	0,871	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,616	1,000									P2
0,761	0,612	1,000								P3
0,522	0,620	0,765	1,000							P4
0,734	0,566	0,989	0,763	1,000						P5
0,545	0,566	0,659	0,572	0,636	1,000					P6
0,876	0,517	0,659	0,381	0,636	0,455	1,000				P7
0,466	0,676	0,439	0,296	0,424	0,706	0,612	1,000			P8
0,558	0,605	0,530	0,244	0,512	0,605	0,698	0,940	1,000		P9
0,829	0,615	0,753	0,667	0,727	0,727	0,727	0,471	0,419	1,000	P10

OPB-01

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,243	0,000									P2
0,644	0,326	0,000								P3
0,073	0,197	0,761	0,000							P4
0,688	1,141	0,748	0,624	0,000						P5
0,341	0,537	0,413	0,312	0,243	0,000					P6
0,007	0,227	0,692	0,063	0,726	0,381	0,000				P7
0,078	0,307	0,796	0,035	0,512	0,232	0,069	0,000			P8
0,158	0,327	0,848	0,040	0,488	0,234	0,148	0,020	0,000		P9
0,060	0,293	0,788	0,032	0,536	0,243	0,052	0,001	0,030	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,784	1,000									P2
0,525	0,722	1,000								P3
0,930	0,821	0,467	1,000							P4
0,503	0,320	0,473	0,536	1,000						P5
0,711	0,585	0,662	0,732	0,785	1,000					P6
0,993	0,797	0,501	0,939	0,484	0,683	1,000				P7
0,925	0,735	0,451	0,966	0,599	0,793	0,933	1,000			P8
0,854	0,721	0,428	0,960	0,614	0,792	0,863	0,980	1,000		P9
0,941	0,746	0,455	0,968	0,585	0,785	0,949	0,999	0,970	1,000	P10

OPB-04

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,366	0,000									P2
0,317	0,397	0,000								P3
0,302	0,060	0,481	0,000							P4
0,253	0,314	0,265	0,299	0,000						P5
0,617	0,534	0,330	0,646	0,286	0,000					P6
0,343	0,443	0,258	0,422	0,196	0,312	0,000				P7
0,405	0,416	0,605	0,339	0,247	0,315	0,443	0,000			P8
0,341	0,389	0,379	0,353	0,223	0,412	0,425	0,143	0,000		P9
0,325	0,577	0,410	0,550	0,167	0,447	0,307	0,353	0,253	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,694	1,000									P2
0,728	0,672	1,000								P3
0,740	0,941	0,618	1,000							P4
0,776	0,731	0,767	0,741	1,000						P5
0,540	0,586	0,719	0,524	0,751	1,000					P6
0,709	0,642	0,773	0,656	0,822	0,732	1,000				P7
0,667	0,660	0,546	0,713	0,781	0,730	0,642	1,000			P8
0,711	0,678	0,684	0,703	0,800	0,662	0,654	0,867	1,000		P9
0,723	0,562	0,664	0,577	0,846	0,639	0,736	0,702	0,777	1,000	P10

OPG-02

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,447	0,000									P2
0,583	0,658	0,000								P3
0,447	0,449	0,492	0,000							P4
0,534	0,672	0,072	0,567	0,000						P5
0,330	0,362	0,332	0,521	0,336	0,000					P6
0,249	0,543	0,297	0,352	0,319	0,272	0,000				P7
0,358	0,541	0,510	0,098	0,534	0,470	0,282	0,000			P8
0,358	0,454	0,548	0,144	0,577	0,523	0,301	0,063	0,000		P9
0,495	0,475	0,509	0,082	0,577	0,497	0,284	0,104	0,086	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,640	1,000									P2
0,558	0,518	1,000								P3
0,640	0,638	0,611	1,000							P4
0,586	0,511	0,930	0,567	1,000						P5
0,719	0,697	0,718	0,594	0,715	1,000					P6
0,779	0,581	0,743	0,703	0,727	0,762	1,000				P7
0,699	0,582	0,600	0,907	0,586	0,625	0,755	1,000			P8
0,699	0,635	0,578	0,866	0,562	0,593	0,740	0,939	1,000		P9
0,609	0,622	0,601	0,921	0,562	0,609	0,752	0,901	0,918	1,000	P10

OPZ-03

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,129	0,000									P2
0,205	0,056	0,000								P3
0,341	0,297	0,236	0,000							P4
0,442	0,330	0,158	0,239	0,000						P5
0,417	0,442	0,331	0,303	0,146	0,000					P6
0,182	0,388	0,431	0,369	0,449	0,446	0,000				P7
0,347	0,412	0,360	0,109	0,409	0,548	0,305	0,000			P8
0,370	0,456	0,436	0,181	0,468	0,630	0,282	0,058	0,000		P9
0,667	0,443	0,298	0,149	0,246	0,494	0,606	0,179	0,240	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,879	1,000									P2
0,815	0,945	1,000								P3
0,711	0,743	0,790	1,000							P4
0,643	0,719	0,854	0,788	1,000						P5
0,659	0,643	0,718	0,738	0,864	1,000					P6
0,833	0,679	0,650	0,691	0,638	0,640	1,000				P7
0,707	0,662	0,697	0,897	0,664	0,578	0,737	1,000			P8
0,691	0,634	0,647	0,834	0,626	0,532	0,754	0,943	1,000		P9
0,513	0,642	0,742	0,862	0,782	0,610	0,545	0,836	0,786	1,000	P10

CB-09

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,496	0,000									P2
0,520	0,370	0,000								P3
0,544	0,188	0,438	0,000							P4
0,570	0,453	0,174	0,488	0,000						P5
0,410	0,351	0,184	0,330	0,103	0,000					P6
0,410	0,405	0,526	0,522	0,441	0,412	0,000				P7
0,816	0,675	0,376	0,562	0,484	0,440	0,540	0,000			P8
0,720	0,718	0,529	0,561	0,650	0,441	0,548	0,113	0,000		P9
0,552	0,540	0,564	0,633	0,910	0,668	0,528	0,314	0,138	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,609	1,000									P2
0,594	0,691	1,000								P3
0,580	0,829	0,645	1,000							P4
0,565	0,636	0,840	0,614	1,000						P5
0,664	0,704	0,832	0,719	0,902	1,000					P6
0,664	0,667	0,591	0,593	0,643	0,663	1,000				P7
0,442	0,509	0,686	0,570	0,616	0,644	0,583	1,000			P8
0,487	0,488	0,589	0,571	0,522	0,643	0,578	0,893	1,000		P9
0,576	0,583	0,569	0,531	0,403	0,513	0,590	0,730	0,871	1,000	P10

CB-12

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,192	0,000									P2
0,532	0,454	0,000								P3
0,375	0,359	0,381	0,000							P4
0,382	0,407	0,287	0,411	0,000						P5
0,364	0,334	0,269	0,374	0,194	0,000					P6
0,219	0,377	0,483	0,290	0,344	0,339	0,000				P7
0,529	0,456	0,515	0,172	0,455	0,562	0,273	0,000			P8
0,463	0,596	0,792	0,323	0,297	0,532	0,372	0,310	0,000		P9
0,312	0,458	0,863	0,388	0,277	0,381	0,406	0,560	0,145	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,826	1,000									P2
0,587	0,635	1,000								P3
0,688	0,698	0,683	1,000							P4
0,682	0,665	0,751	0,663	1,000						P5
0,695	0,716	0,764	0,688	0,824	1,000					P6
0,804	0,686	0,617	0,748	0,709	0,713	1,000				P7
0,589	0,634	0,598	0,842	0,634	0,570	0,761	1,000			P8
0,629	0,551	0,453	0,724	0,743	0,588	0,689	0,733	1,000		P9
0,732	0,633	0,422	0,678	0,758	0,683	0,666	0,571	0,865	1,000	P10

CB-13

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,159	0,000									P2
0,261	0,222	0,000								P3
0,338	0,280	0,328	0,000							P4
0,302	0,322	0,243	0,344	0,000						P5
0,105	0,111	0,138	0,273	0,195	0,000					P6
0,192	0,148	0,175	0,284	0,170	0,113	0,000				P7
0,194	0,214	0,177	0,220	0,231	0,110	0,161	0,000			P8
0,282	0,314	0,386	0,357	0,265	0,226	0,271	0,234	0,000		P9
0,232	0,226	0,293	0,345	0,257	0,146	0,187	0,172	0,103	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,853	1,000									P2
0,770	0,801	1,000								P3
0,713	0,756	0,721	1,000							P4
0,739	0,725	0,784	0,709	1,000						P5
0,900	0,895	0,871	0,761	0,823	1,000					P6
0,825	0,863	0,840	0,753	0,843	0,893	1,000				P7
0,823	0,807	0,838	0,803	0,794	0,895	0,851	1,000			P8
0,754	0,731	0,680	0,700	0,767	0,798	0,762	0,792	1,000		P9
0,793	0,798	0,746	0,708	0,773	0,864	0,829	0,842	0,902	1,000	P10

CB-15

Pairwise Population Matrix of Nei Genetic Distance

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
0,000										P1
0,150	0,000									P2
0,215	0,150	0,000								P3
0,133	0,030	0,133	0,000							P4
0,143	0,071	0,109	0,060	0,000						P5
0,298	0,225	0,073	0,202	0,193	0,000					P6
0,233	0,416	0,355	0,403	0,360	0,243	0,000				P7
0,300	0,382	0,462	0,400	0,461	0,326	0,283	0,000			P8
0,383	0,362	0,443	0,372	0,433	0,329	0,347	0,074	0,000		P9
0,323	0,316	0,447	0,348	0,391	0,433	0,396	0,190	0,129	0,000	P10

Pairwise Population Matrix of Nei Genetic Identity

P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
1,000										P1
0,861	1,000									P2
0,807	0,861	1,000								P3
0,876	0,970	0,876	1,000							P4
0,867	0,932	0,896	0,942	1,000						P5
0,742	0,798	0,929	0,817	0,825	1,000					P6
0,792	0,660	0,701	0,669	0,698	0,784	1,000				P7
0,741	0,683	0,630	0,670	0,631	0,722	0,754	1,000			P8
0,682	0,696	0,642	0,689	0,649	0,720	0,707	0,929	1,000		P9
0,724	0,729	0,640	0,706	0,676	0,648	0,673	0,827	0,879	1,000	P10