Szent István University
Gödöllő

Archaeogenetics of melon (*Cucumis melo*): ITS and SSR sequence heterogenity from a medieval sample to current varieties

Ph.D. thesis

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1. Introduction and aims

Melon is an important vegetable crop of the tropical, subtropical and temperate climates. Problems and questions on intraspecific classification and domestication haven't been cleared absolutely yet. Our information on cultivation and spread of melon in Hungary, are based on archaeological records.

DNA analysis opened up new perspectives and possibilities on studing evolution of species. We are able to isolate DNA from conserved plant remaines (hundreds or thousands year old samples), and amplify the biomolecules from a single copy using PCR based methods. DNA sequences are useful to compare extinct or endangered organisms with their living relatives, to analyse the genetical distance to closely related species.

Seed remains of melon (*Cucumis melo* L.) excavated from a 15th century sediment layer in a well at the King's Palace of Budapest, Buda Hill, Hungary. The well-preserved status of the seeds gives us the possibility to isolate and ampify DNA during different molecular analysis on archaeological samples of Hungarian melon cultures. The morphological and molecular comparison of 47 melon varieties (variety, landrace) can give us data for the variety identification, and the cluster analysis on exact intraspecific relationships.

The aims of our work were to isolate and amplify the conserved DNA from 600 years old melon seeds, to determinate the DNA degradation rate, to select the edogenous and exogenous contaminated seeds. Further aim was to study 47 current cultivars (11 varieties, 20 landraces, and 16 samples of genbank collection) using 26 morphological traits and specie specific microsatellite markers to estimate the diversity and to provide insight to reconstruct an extinct melon landrace in Hungary recovered from the midle ages.

2. Materials and Methods

Seed remains of melon (*Cucumis melo* L.) were excavated from a 15th century sediment layer in a well at the King's Palace of Budapest, Buda Hill, Hungary. Medieval seeds were surface sterilize by NaOCL (7 %) for 20 min, and incubated on aseptic tissue culture medium for three months. Seeds of 47 modern melon cultivars were selected form gene bank of Agrobotanical Institute Tápiószele, Hungary and sown in compost media in glass houses. Seedlings at two-three leaf stage were transplanted and grown up in 5 m x 5 m field plots in duplicate experiments.

Morphological characterization was carried out according to the combined standards of Descriptor Lists of ABI (AgroBotanical Institute, Tápiószele, Hungary) and the National Institute for Quality Control (OMMI, Budapest, Hungary). In total, 23 traits were recorded by visual inspection.

Medieval seed remains which showed no contamination after surface sterilization and a three month incubation period in aseptic tissue culture media F6 were subjected for DNA isolation. Several DNA isolation methods were tested on the current and archaeological samples. Total DNA of aseptic medieval seed remains and modern cultivars (0.1 g) in each case was extracted in CTAB, cethyltrimethylammonium bromide, buffer followed by an RNase-A treatment (Sigma, R-4875) for 30 min at 37 ◦C. The quality and quantity of extracted DNA were measured by a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, USA – Bio- Science, Budapest, Hungary). DNA samples were adjusted to concentration of 30 ng/μl with ddH_2O and subjected to PCR amplification where at least three independent DNA preparations of each sample was used.

For ITS analysis medieval melon sample was compared to modern varieties using the primer pair 5_-tcg taa caa ggt ttc cgt agg tg-3_, and 5_-tcc tcc gct tat tga tat gc-3_ complementary to the evolutionary conserved spacer regions of the nuclear ribosomal (rDNA) gene cluster including ITS1-5.8S-ITS2 (internal transcribed spacer).

The maximum fragment size of reliable DNS amplicons were verified with 60 RAPD primers (OP-A; OP-AB; OP-M) in the comparative molecular analysis of 10 modern varieties and medieval melon samples.

Each successful reaction with scorable bands was repeated at least twice. Amplifications were assayed (RAPD, SSR) prior to ALF-SSR analysis by agarose (1.8 %, SeaKem LE, FMC) gel electrophoresis (Owl system), stained with ethidium bromide (0.5 ng/µl) after running at 80 V in 1 X TAE buffer. Transilluminated gels were analyzed by ChemilImager v 5.5 computer program (Alpha Innotech Corporation - Bio-Science Kft, Budapest, Hungary). A negative control which contained all the necessary PCR components except template DNA was included in PCR runs.

For comparative microsatellite analysis on 47 melon cultivars and landraces SSRs detected by ALFexpress II including a Helium-Neon laser (2.5 mW output) can excite Cy5 dye with 632.8 nm laser bean and detect the emitted signal at 660-670 nm with a high resolution range of 50 amol (5 x 10^{-14} M) to 45 fmol (4.5 x 10^{-11} M) was used. 8 microsatellite primers were selected from the tests of 34 locus. DNA amplification was carried out on non-contaminated DNA samples of medieval seeds in duplicate experiments.

ITS and SSR fragments were isolated from agarose gel purified using the QiaQuick PCR purification kit (Qiagen, Crawley, West Sussex, UK, Cat.No. 28106) and cloned into the vector pGEM-T Easy Vector System II (Promega, Southhampton, UK, Cat no A1380), and transformed into Jm109 competent cell (Promega, Cat No. L2001) using the Promega protocols. Inserts were cut out by *EcoR*I. Three independent clones of the fragments (ITS and SSR) were subjected to automated fluorescent DNA sequencing (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). Sequences were analyzed by computer program ChromasPro version 1.11 (Technelysium Pty Ltd, USA). Sequence alignment was computed by BioEdit Sequence Alignment Editor (NCSU, USA). Cluster analysis was carried out by the SPSS11 program package using the Jaccard Index (Average Linkage, within group) based on the presence versus absence of morphological characters, RAPD fragments, or SSR alleles. PIC value (polymorphism index content) of each SSR was calculated by the formula of Anderson.

3. Results and discussion

600 years old archaeological plant remains containing 3 000 000 seeds of 88 species were recovered, and classified (**Figure 1.**).

Figure 1. Seed remains of melon (*Cucumis melo* L.) and other species excavated from 15th century (King's Palace of Budapest, Buda Hill, Hungary).

250 well preserved seeds were recovered from the plant remains of 28117 melon seeds in total. Seeds showing no physical damage were selected and classified using seed morphological characters. Medieval seeds were successfully surface sterilize by $HgCl_2$, or Na-OCL (8 %) for 20 min, and incubated in semi-steril condition (room temperature), or on aseptic tissue culture medium (MS, 1/2MS, F6) for three months. We were able to keep 40% of the 250 melon seeds steril during in vitro incubation, but the induction of callus, or the isolation of living cells were not successfull. We could

not get positive results from the seed germination or callus proliferation from single putative surviving seed cells, according to the surroundings of seedconservation. The diversity of 47 melon varieties **(Figure 2.; Figure 3.)** and landraces were morphologically characterized. Classification of melon types were difficult partly due to the combining of genetic material by breeders and to the free hybridization between wild and cultivated germplasm.

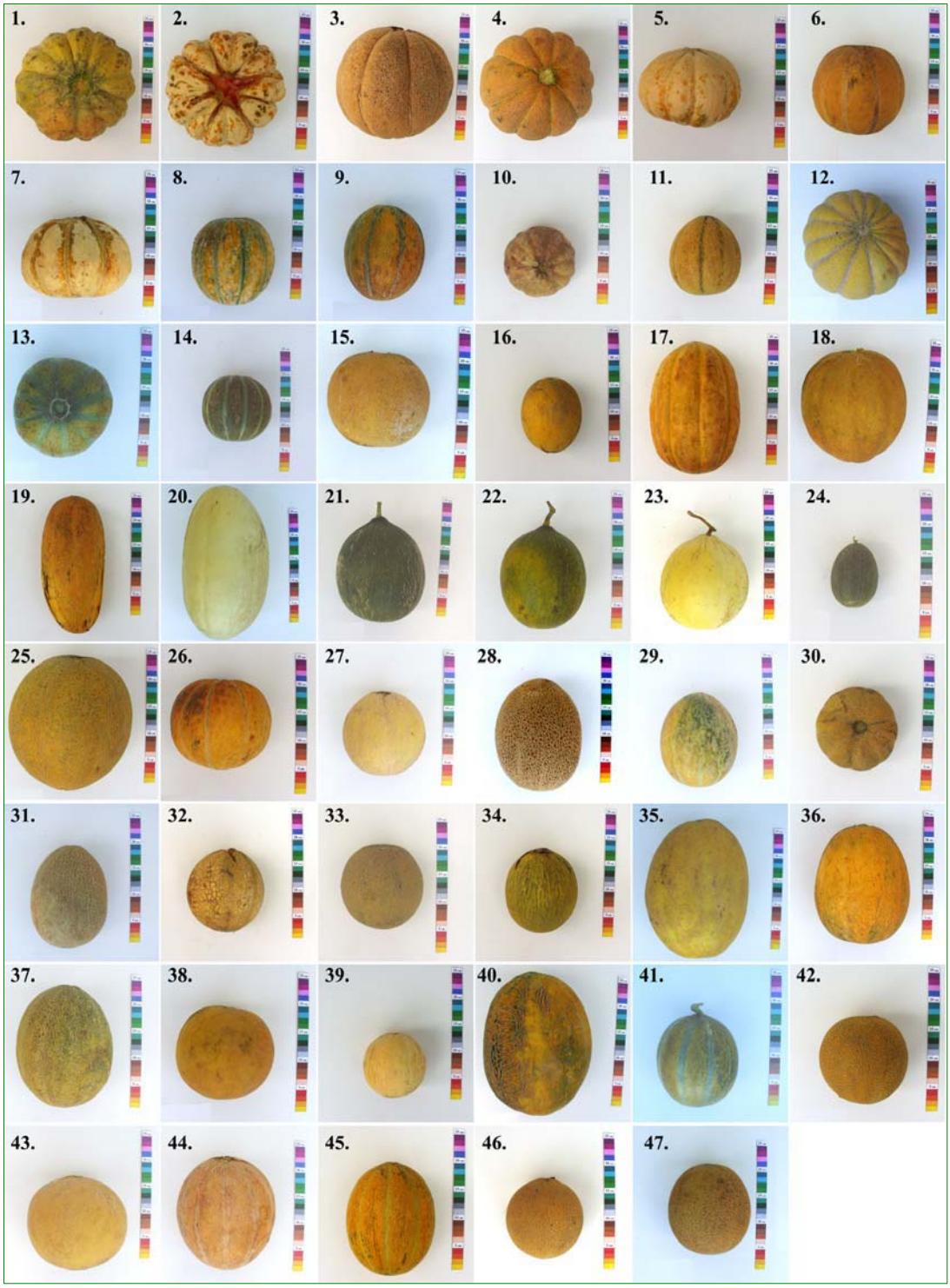

Figure 2. Fruit of the current melon (*Cucumis melo* L.) cultivars and landraces (1 to 47) examined in the present study.

Figure 2. Fruit of the current melon (*Cucumis melo* L.) cultivars and landraces (1 to 47) examined in the present study, and the medieval seeds (48).

The weighted cluster analysis based on the morphological markers (1 to 23) using Jaccard-index grouped the 47 varieties into three subclusters, non of the varieties showed transition types. Two old Hungarian landraces of *'Túrkeve'* and '*Hógolyó*' were grouped in one subcluster. We could not

distinguish the variety ' Ezüst Ananász' from ' Sweet Ananász' and the landrace 'Kisteleki' showed high similarity with the variety 'Nagycsenkeszi'.

The quality, quantity and degradation of extracted DNA were verified on agarose gel (0.8 %). aDNA samples extracted from individual seed remains showed different level of degradation probably due to preservation conditions compared to the compact DNA samples of modern cultivars. DNA of the 15th century samples showed median degradation with high molecular weight (100-1000bp) still present as a result of cold, humid and anaerobic conditions in the medieval well (Hungary) covered with water.

Melon specific ITS fragment with 667 bp length were amplified in the medieval DNA sample and in control modern varieties. The ITS region sequence obtained from a single PCR product for each variety gave a smaller ITS1 region (217 bp) and a ITS2 region (238 bp). The length of the 5.8S ribosomal DNA region remained constant with a value of 163 bp. Six of the 29 surface sterilized medieval seeds showed endogenous contamination with an extra ITS fragment with 579 bp length, which, after sequencing, was found to be an *Aspergillus nidulans* (NCBI: AF455505). DNA samples of these contaminated seeds were excluded from further molecular analysis. None of the modern varieties showed endogenous contamination in ITS. The non-contaminated fragments were sequenced after either reamplification or cloning from the medieval and current samples.

Twelve of the previously tested sixty RAPD primers (OP-A; OP-AB; OP-M), amplified reproducible polymorphic bands in medial samples and modern varieties. Seven primers did not produced amplicons. Several primers showed monomorphic patterns, nevertheless after the analysis of 373 RAPD fragments 14 were polymorphic in the archaeological sample out of 105 scored. The variety 'Ezüst Ananász' were 85% similar to 'Sweet Ananász'. Medieval samples showed 65% of homology with 'Hógolyó' and great distance to 'Ezüst Ananász'. The method was useful for verifying the quality of aDNA and contamination free PCR amplification.

Eight of the twenty SSR primer pairs selected from the 34 during primertests amplified 34 microsatellite alleles from current melon varieties and 600 hundred years old relatieves in identical fragment ranges. A total of 485 alleles were detected in the 47 melon cultivars separated with 1-2 base fidelity on ALF fluorometer (**Table 1a.; 1b.**). Size variation among the alleles for a single SSR locus ranged from 4 to 44 base pairs. We could not distinguish the variety 'Ezüst Ananász' from 'Sweet Ananász', and the highest genetic distance were found between the variety 'Ezüst Ananász' and 'Kősárga'.

Table 1a: SSR variation at eight microsatellite loci of melon (*C. melo*) cultivars and landraces (1 to 47) compared to an extinct medieval melon (48)

			Mikroszatellit marker																					
SSR lókusz			CMCT44		CMAG59					CMGA104					CMCT134				CMTA134					
Ismétlődő szekvencia			$(CT)_{10}TG$ $TT(CT)_3$		$(GA)_2A(AG)_8$					$(GA)_{14}AA(GA)_3$					$(TA)_2(CT)_8(AT)_7$				$(TA)_{12}$					
Allél(bp)			100	104	124	126	128	130	132	113	117	123	125	129	108	124	150	152	147	155	157	159	161	163
Méret tartomány			100-104		124-132					113-129					108-152				147-163					
PIC érték			0,15		0,67					0,67					0,70				0,66					
N.	Var.	T.																						
1	PaMa	C	-	104	124	-	-	-	-	113	-	-	-	-	-	124	150	-	147	-	-	-	-	163
2	SwAn	C	-	104	124	-	-	-	-	113	-	-	-	-	-	-	-	152	147	-	-	-	-	-
3	Nyir	C	-	104	124	-	-	-	-	-	-	123	-	-	-	124	-	-	147	-	-	-	-	163
4	JavZ	C	-	104	124	-	-	-	132	113	-	123	-	-	-	-	150	-	147	-	-	-	-	-
5	Tura	C	-	104	124	-	-	-	-	113	-	-	-	-	-	-	-	152	147	-	-	-	-	-
6	Heve	C	-	104	-	-	-	-	132	-	-	123	-	-	-	-	150	-	147	-	-	-	-	-
7	EzAn	C	-	104	124	-	-	-	-	113	-	-	-	-	-	-	-	152	147	-	-	-	-	-
8	Bele	C	-	104	124	-	-	-	-	113	-	-	-	-	-	124	-	152	147	-	-	-	-	-
9	SICa	C	-	104	124	-	-	-	-	-	-	-	-	-	-	124	150	-	147	-	-	-	-	163
10	Alta	C	-	104	124	-	-	-	-	-	-	-	-	129	-	124	-	-	147	-	-	159	-	-
11	PlBa	C	-	104	124	-	-	-	-	-	-	123	-	-	-	124	-	-	-	-	-	159	-	-
12	Pusz	C	-	104	124	-	-	-	-	113	-	123	-	-	-	124	150	-	147	-	-	-	-	-
13	Musk	C	-	104	124	-	-	-	-	-	-	123	-	-	-	124	-	-	-	-	-	-	161	-
14	Csar	C	-	104	124	-	-	-	-	-	-	123	-	-	-	124	150	-	147	-	-	159	-	-
15	Topa	C	-	104	-	126	-	-	132	-	-	123	-	-	-	-	-	152	147	-	-	-	-	-
16	Melo	C	100	-	-	-	-	130	132	-	-	-	125	-	108	-	150	-	147	155	-	-	-	-
17	Kisk	C	-	104	-	-	-	-	132	113	-	-	-	-	108	124	-	-	-	155	-	-	161	-
18	ZiJa	I	-	104	124	-	-	-	132	113	-	-	-	-	-	124	-	-	-	-	-	159	-	163
19	Sopo	I	-	104	124	-	-	-	-	-	117	123	-	-	-	124	-	-	-	-	-	159	-	163
20	Peny	I	-	104	124	-	-	-	132	-	-	-	-	-	108	124	-	-	-	-	-	159	-	-
21	Tuke	I	-	104	-	-	128	-	-	-	-	123	-	129	-	-	-	152	147	-	-	-	-	-
22	Pion	I	-	104	-	126	-	-	132	113	-	-	125	-	-	-	150	-	147	-	-	-	-	-
23	Hogo	I	100	-	-	-	128	-	-	-	-	-	-	129	-	-	-	152	147	-	-	-	-	-
24	Afgh	I	100	104	124	-	-	-	132	-	-	-	125	-	-	124	150	-	-	-	-	-	-	163
25	Turk	R	-	104	-	-	-	130	132	-	-	123	-	-	-	-	150	-	147	-	-	-	-	-
26	Tarn	R	-	104	124	-	-	-	-	113	-	123	-	-	-	124	150	-	147	-	-	-	-	163
27	HoRo	R	-	104	124	-	-	-	-	-	-	-	-	-	-	124	-	-	-	-	-	159	-	-
28	Halb	R	-	104	124	-	-	-	-	-	-	123	-	-	-	124	-	-	147	-	-	-	-	163
29	Limo	R	-	104	124	-	-	-	-	113	-	-	-	129	-	-	-	152	147	-	-	-	-	-
30	Tete	R	-	104	124	-	-	-	-	-	-	123	-	-	-	124	-	-	147	-	-	159	-	-
31	Muhi	R	-	104	-	-	-	-	132	-	117	123	-	-	-	124	-	-	147	-	-	-	-	163
32	Kosa	R	-	104	-	126	-	-	-	-	-	-	-	129	-	-	-	152	147	-	-	-	-	-
33	Hegy	R	-	104	124	-	-	-	-	113	-	123	-	-	-	-	-	152	147	-	-	-	-	-
34	Togo	R	-	104	124	-	-	-	-	113	-	123	-	-	-	-	150	-	147	-	-	-	-	-
35	Kist	R	-	104	-	126	-	-	132	-	-	123	-	-	108	-	150	-	147	-	157	-	-	-
36	Nagy	R	-	104	-	-	-	-	132	-	-	123	-	-	-	124	-	-	-	-	-	159	-	-
37	NyBa	R	-	104	124	-	-	-	-	-	-	123	-	-	-	124	-	-	147	-	-	-	-	163
38	Szir	R	-	104	124	-	-	-	-	113	-	123	-	-	108	124	-	-	-	-	-	159	-	-
39	CaPa	R	-	104	-	126	-	-	132	-	-	123	-	-	-	124	-	-	-	-	-	159	-	-
40	Gb-1	R	-	104	124	-	-	-	132	113	-	-	125	-	108	-	150	-	147	155	-	-	-	-
41	Fort	R	-	104	-	126	-	-	-	-	-	123	-	-	-	-	-	-	147	-	-	159	-	-
42	Kalo	R	-	104	-	126	-	-	-	-	117	123	-	-	108	124	-	-	-	-	-	159	-	163
43	Takt	R	-	104	124	-	-	-	-	113	-	-	-	-	-	-	150	-	147	-	-	-	-	-
44	Sara	R	-	104	124	-	-	-	-	113	-	123	-	-	108	124	-	-	-	-	-	159	-	-
45	Dese	R	-	104	-	-	-	-	132	-	-	123	-	-	-	-	150	-	147	-	-	159	-	-
46	MaKi	R	-	104	-	126	-	-	-	-	-	123	-	-	-	124	-	-	-	-	-	-	-	163
47	Gali	R	-	104	124	-	-	-	-	113	-	123	-	-	-	124	150	-	147	-	-	159	-	-
48	MAC		100	-	-	-	128	-	-	-	-	-	-	129	-	-	-	152	147	-	-	-	-	163
Num			4	45	30	8	3	2	16	19	3	28	4	6	8	27	17	11	34	3	1	16	2	13
Σ			49		61					60					63				69					

The medieval sample was 75% similar to 'Hógolyó', and showed the lowest similarity to 'Ezüst Ananász'. The number of alleles per marker including CMCT44 ($(CT)_n$, 2 alleles), CMAG59 ($(AG)_n$, 5 alleles), CMGA104 ($(GA)_n$, 5 alleles), CMCT134 ($(CT)_n$, 4 alleles), CMTA134 ($(TA)_n$, 6 alleles), CMCTT144 ($(CTT)_n$, 7 alleles), CMTC168 ($(TC)_n$, 6 alleles) and CMCT170 ($(CT)_n$, 5 alleles) ranged from 2 to 7 with an average of 5.7 and the corresponding PIC values ranging from 0.15 to 0.71.

Table 1b: SSR variation at eight microsatellite loci of melon (*C. melo*) cultivars and landraces (1 to 47) compared to an extinct medieval melon (48)

			Mikroszatellit marker																	
SSR lókusz			CMCTT144							CMTC168						CMCT170				
Ismétlődő szekvencia			$((CTT)_{10}CTAC(CTT)_6$							$(TC)_{14}$						$(CT)_8$				
Allél (bp)			184	190	193	202	211	214	217	186	190	192	194	200	202	158	162	164	166	168
Mérettartomány			184-217							186-202						158-168				
PIC érték			0,71							0,68						0,70				
N.	Var.	T.																		
1	PaMa	C	-	-	193	-	-	-	-	-	-	-	-	-	202	-	-	164	-	-
2	SwAn	C	-	-	193	-	-	-	-	186	-	-	194	-	-	-	162	-	-	-
3	Nyir	C	-	-	193	-	-	-	-	-	-	-	-	-	202	-	-	-	-	168
4	JavZ	C	-	-	193	-	-	-	-	-	-	-	194	-	202	-	162	-	-	168
5	Tura	C	184	-	-	-	-	-	-	-	-	-	194	-	-	-	162	-	-	168
6	Heve	C	-	-	193	-	-	-	-	-	-	-	194	-	202	-	-	-	166	-
7	EzAn	C	-	-	193	-	-	-	-	186	-	-	194	-	-	-	162	-	-	-
8	Bele	C	184	-	-	-	-	-	-	-	-	-	194	200	-	-	162	-	-	-
9	SICa	C	-	-	-	-	-	-	217	-	-	-	-	200	-	-	-	-	-	168
10	Alta	C	-	-	193	-	-	-	-	-	-	-	194	-	202	158	162	-	-	-
11	PlBa	C	184	-	-	-	-	-	-	-	-	-	194	-	-	-	162	-	-	-
12	Pusz	C	-	-	193	202	-	-	-	-	-	-	194	-	-	-	162	-	-	168
13	Musk	C	-	-	193	-	-	-	-	-	-	-	-	200	-	-	162	-	-	-
14	Csar	C	184	-	-	-	-	-	-	-	-	-	194	200	-	-	162	-	-	168
15	Topa	C	-	-	193	-	-	-	-	-	-	-	-	-	202	-	-	164	-	-
16	Melo	C	184	-	193	-	-	-	-	-	190	-	-	-	-	-	-	-	-	168
17	Kisk	C	-	-	193	-	-	-	-	-	-	-	-	200	-	-	162	-	-	168
18	ZiJa	I	-	-	193	-	211	-	-	-	-	-	194	-	-	-	-	-	-	168
19	Sopo	I	184	-	-	-	-	-	-	-	-	-	194	-	202	-	-	-	-	168
20	Peny	I	184	-	-	-	-	-	-	-	-	-	194	-	-	-	-	-	-	168
21	Tuke	I	-	-	193	-	-	-	-	-	-	-	-	-	202	-	-	-	-	168
22	Pion	I	-	-	193	-	-	-	-	-	-	-	-	-	202	-	-	-	-	168
23	Hogo	I	184	-	-	-	-	-	-	-	-	-	-	-	202	-	162	-	-	-
24	Afgh	I	-	190	-	-	-	-	-	-	-	-	194	-	202	-	-	-	-	168
25	Turk	R	184	-	-	-	-	-	-	-	-	-	194	200	-	-	162	-	-	-
26	Tarn	R	184	-	-	-	-	-	-	-	-	-	194	-	-	-	-	-	166	-
27	HoRo	R	184	-	193	-	-	-	-	-	-	-	-	-	202	-	-	-	166	-
28	Halb	R	-	-	-	-	-	-	217	-	-	192	-	200	-	-	-	-	-	168
29	Limo	R	-	-	193	-	-	-	-	-	-	-	-	-	202	-	162	-	-	168
30	Tete	R	184	-	-	-	-	-	-	-	-	-	-	-	202	-	-	-	-	168
31	Muhi	R	184	-	-	-	-	-	-	-	-	-	-	-	202	-	162	-	-	-
32	Kosa	R	-	-	-	-	-	-	217	-	-	192	-	-	-	-	-	164	-	-
33	Hegy	R	184	-	-	-	-	214	-	-	-	-	-	-	202	-	162		-	-
34	Togo	R	-	-	193	-	-	-	-	-	-	-	-	-	202	-	162	-	-	-
35	Kist	R	184	-	-	-	-	214	-	-	-	-	194		202	-	-	-	-	168
36	Nagy	R	-	-	193	-	-	214	-	-	-	-	-	-	202	-	162	-	-	-
37	NyBa	R	184	-	-	-	-	214	-	-	-	-	-	-	202	-	-	-	-	168
38	Szir	R	-	190	-	-	-	-	-	-	-	-	194	200	-	158	-	-	-	168
39	CaPa	R	-	-	-	-	-	-	217	-	-	-	194	-	-	-	-	164	-	168
40	Gb-1	R	-	-	193	-	-	-	-	-	-	-	-	-	202	-	-	164	-	-
41	Fort	R	-	-	193	-	-	214	-	-	-	-	-	200	-	-	162	-	-	-
42	Kalo	R	-	-	193	-	-	-	-	-	-	-	-	-	202	158	-	164	-	-
43	Takt	R	-	-	-	-	-	214	217	-	-	-	194	-	-	-	162	-	-	-
44	Sara	R	-	-	193	-	211	-	-	-	-	-	194	-	202	158	-	-	-	168
45	Dese	R	184	-	-	-	-	-	-	-	-	-	-	200	202	-	-	-	-	168
46	MaKi	R	184	-	193	-	-	-	-	-	-	-	-	-	202	-	-	164	-	-
47	Gali	R	184	-	193	-	-	-	-	-	-	-	-	-	202	-	162	-	-	-
48	MAC		-	-	193	-	-	-	-	-	-	-	-	-	202	-	-	-	166	-
Num			19	3	25	1	2	6	5	2	1	2	21	10	27	4	21	7	4	23
Σ			61							63						59				

The alleles per marker showed different fragment lengths according to changes in the number of core sequence repeats. 7 markers amplified rare, in diversity studies most valuable alleles that were presented only in 10% of genotypes: CMCT44 (100bp allel) and, CMAG59 (128bp, 132bp alleles), CMGA104 (117bp, 125bp alleles), CMTA134 (155bp, 157bp, 161bp alleles), CMCTT144 (190bp, 202bp, 211bp alleles), CMTC168 (186bp, 190bp, 192bp alleles) and CMCT170 (158bp, 166bp alleles).

The ITS sequence analysis was used to classify 30current melon varieties and landraces compared to the medieval sample using the detected (previosly published) SNPs in ITS1-5.8S-ITS2 region of rDNA (ribosomal DNA). SNPs were observed at the 94-95 bp (GC to either RC, RS or AG) of ITS1; and at 414 bp (A-to-T substitution), 470 bp (T to Y or C), 610 bp (A to R or G) and 633 bp (A-to-G transition) of ITS2. The ITS region showed very low sequence heterogeneity, as was expected from previous results that reported only a 2–3% intraspecific variation in *Cucumis.*

The intraspecific ITS sequence variation in our study was less than 1%, with a 1 bp difference (666 bp / 667 bp) between 'MAC' and 'Hógolyó', and a 5 bp difference (662 bp / 667 bp) between 'MAC' and 'Topáz'. We identified 3 nucleotide changes (94, 95, 99 bp) in the sequence of 'MAC' compared to the sequence Z48805 obtained from NCBI genbank

Sequence analysis of the microsatellite alleles showed different fragment lengths depending on changes in the number of unit of core sequences. The length of SSRs did not show a time dependent variation in lengths as expected results of microevolution, as medieval melon showed intermediate sizes in SSRs compared to modern cultivars. The nucleotide sequences of the CMCTT144 and CMCT170b alleles from 'MAC' and modern varieties showed different fragment lengths according to changes in the number of core sequence repeats of the microsatellite $(CTT)_{10}CTAC(CTT)_6$ and $(CT)_8$. The numbers of trinucleotide repeat $(CCT)_n$ showed a range from 8, 11, and 19 to 20. The medieval samples amplified differrent alleles (193 bp 166 bp) as the inodorus types. Surprisingly, one of the oldest varieties, 'Hógolyó', showed the shortest allele length at the CMCTT144 locus with eight repeats of core sequence $[(CCT)_8]$ compared to the longest in 'Hales Best' (one of the newest F1 hybrid varieties) OMMI (2003) with a 21 repeat number $(CCT)_{21}$, while 'MAC' had a middle size repeat number $(CCT)_{11}$. After cloning and sequencing the fragments we do not observed any deletion or insertion due to the slippage of Taq-polimerase.

The dendrogram of comparative morphological and molecular analysis produced by SPSS11 based on the presence versus absence of SSR alleles and sequence differences in ITS revealed that medieval melon had the closest genetic similarity to a registered melon cultivar 'Hógolyó' selected from an old Hungarian melon landrace which has apparently showed ancient characteristics of indeterminate growing habit and wine development.

3.1 New Scientific Results

1. Two hundred and fifty medial melon seeds (15. century) were excavated, selected and identified.
2. aDNA of 600 years old conserved seeds were isolated (6,3-8,6 ng DNS/ 0,1 g seed), and the DNA degradation was estimated by UV-spectrofotometry.
3. The 221 exogen and 6 endogen contaminated medieval seeds or aDNA samples were selected on aseptic tissue culture or using ITS sequences.
4. In total 105 fragments produced by 60 RAPD primers were amplified. The maximum fragment size in the PCR amplification of degradated DNA was determined (857 bp)
5. The genotype of middle age melo was identified in the comparative molecular analysis with 47 current melon varieties using 8 microsatellite markers. In total 485 SSR alleles were identified.
6. The sequence heterogenity of microsatellite fragments of current and medieval melon were analysed and no correlation was found between the heterogenity and the 600 years of microevolution
7. A phenotype reconstruction made by morphological and molecular clusters revealed that that medieval melon was an *idodorus* type melon with small size.

4. Conclusions and Recommendations

Our work on regeneration and comparative molecular analysis of 600 years old seed remains were unique in hungarian archeogenetic research. Exogen contaminated seeds could be selected on tissue culture, and the endogen contamination were exclude with ITS analysis. We have isolated aDNA samples from the medieval melon seeds with several methods of RAPD, ITS, and SSR.

In a comparative SSR analysis with 47 current melon varieties revealed a diverse sequence heterogenity of a total of 485 SSR alleles. Three main types of melon were identified: inodour, reticultaus and cantaloupe. Genes preserved in the aDNA samples preserved in plant tissues and organs can provide crutial data for tracing plant domestication and evoution. These results also indicated that cloned DNA sequences recovered from aDNA samples can be used for molecular breeding of modern melon cultivars via gene transfer.

5. Publications

Selected Papers Related to the Thesis

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