Ancient Citrullus DNA-unlocking Domestication Events

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Seed remains of watermelon (Citrullus lanatus lanatus) from the Middle Ages were excavated from two sites, Debrecen, Hungary (the 13th-14th Century A.D.) and Budapest, Hungary (the 15th Century A.D.). Seed remains were processed by floatation followed by seed sorting and identification in the laboratory. After seed morphological analysis aDNAs were extracted and then analyzed at eleven microsatellite (SSRs) loci with a final aim of sequence recovery and phenotype reconstruction. For comparative analysis, an herbarium sample from the 19th CENT. A.D. (Pannonhalma, Hungary) and forty-four current varieties were used. Molecular dendrograms based on microsatellite analysis revealed that middle age samples are close to current varieties with red flesh colour, which indicate the preferential cultivation of red-flesh and not yellow-flesh watermelon in the Middle Ages in Hungary. The 170-yr-old herbarium sample showed close molecular similarity to citron melon (Citrullus lanatus citroides), which also reflects the importance of citron melon as fodder in the Middle-Ages in Hungary. Results of seed morphology

were highly correlated with molecular data. Watermelon reached the New World after Columbus' second voyage in 1493 and dispersed quickly among American natives. One of the most ancient forms of small, round fruit with thin, green rind, red flesh and small black seeds has survived up to the recent times.

1. Introduction

The aDNA (*a*ncient DNA) recovered from excavated remains of plants and animals supply unique materials not only for the analysis of post-mortem DNA degradation (Brown 1999; Threadgold and Brown 2003), but also for tracing vegetation history and microevolution (Gugerli *et al*. 2005; Shlumbaum *et al.* 2007). Intact aDNA sequences (Szabó *et al.* 2005; Lágler *et al.* 2005; Gyulai *et al.* 2006) or the complete genome (Cooper *et al.* 2001; Pääbo *et al.* 2004) of the extinct organisms can be reconstructed in the case of optimal preservation conditions. In this study we present the analyses of seed morphology and the aDNA study of 700-, 600- and 170-year-old watermelons together with a comparison to modern cultivars.

2. Ancient-DNA (aDNA) analysis

Seed samples: Seed remains of watermelon (*Citrullus l. lanatus*) from the 13-14th CENT. were excavated in Debrecen, Hungary (Hajdu Zs. et al., Déri Museum, 2006, Debrecen; http://www.derimuz.hu/hirek/2006/kutak.html). In total, 95,133 seed remains of 206 plant species were identified. Of them, 251 watermelon seeds were determined to have the same morphological characters. At the 15th CENT. sites (8th well, Mansion Teleki, King's Palace of Árpád Dynasty, Buda Hill, Budapest; Hungary) (Nyékhelyi 2003), 54,415 watermelon seeds were excavated (Gyulai *et al.* 2006; Tóth *et al.* 2007ab, 2008). Wet-sieved sediment samples were processed by floatation followed by seed sorting and identification in the laboratory (Schermann 1966; Hartyányi and Nováky 1975). The 19th CENT. (ca. 1836) seeds were collected from the oldest botanical seed collection of Hungary (Pannonhalma, Hungary) (Vörös 1971), recently exhibited at the Hungarian Agricultural Museum, Budapest (Hungary). For comparative analysis, forty-four modern *Citrullus* species and varieties (Table 1) were included (Horváth *et al.* 2008).

Elimination of contamination: Seeds were incubated for seven days in an aseptic tissue culture medium to eliminate contamination before DNA extraction prior to washing with a detergent (3 min), rinsing three times with distilled water (3 min), surface sterilization with ethanol (70% v/v) for 1 min, using a bleaching agent (8% $Ca(OCl)_2$ w/v) for 1 min., followed by three rinses with sterile distilled water according to general aseptic culture techniques (Gyulai *et al.* 2006). Seeds of the modern varieties were also surface sterilized. Exogenously and endogenously contaminated seeds infected

by fungi and bacteria were eliminated from further analyses.

DNA extraction: Individual seeds were ground in an aseptic mortar with liquid nitrogen in a laminar air flow cabinet in the archaeobotanical laboratory of the St. Stephanus University, Gödöllő. aDNA was extracted by the CTAB (cethyltrimethylammonium bromide) method according to Biss *et al.* (2003), Yang (1997) and Cooper and Poinar (2000). Seed DNA of modern cultivars (0.1 g) was also extracted in CTAB buffer, followed by an RNase-A treatment (Sigma, R-4875) for 30 min at 37°C in each case. The quality and quantity of extracted DNA was measured (2 µl) by a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, USA – BioScience, Budapest, Hungary). DNA samples were adjusted to a concentration of 30 ng/µl with ddH_2O and subjected to PCR amplification.

PCR primers: For molecular analysis eleven nSSR (nuclear microsatellite) primer-pairs were used: *Cm*TC51 (att ggg gtt tct ttg agg tga / cca tgt cta aaa act cat gtg g); *Cm*TC168 (atc att gga tgt ggg att ctc / aca gat gga tga aac ctt agg); *Cm*ACC146 (caa cca ccg act act aag t / cga cca aac cca tcc gat aa) (Katzir *et al.* 1996; Danin-Poleg *et al.* 2001); and *bng*l161 (gct ttc gtc ata cac aca cat tca / atg gag cat gag ctt gca tat tt); *bng*l118-2 (gcc ttc cag ccg caa ccc t / cac tgc atg caa agg caa cca ac); *phi*118-2 (atc gga tcg gct gcc gtc aaa / aga cac gac ggt gtg tcc atc); *phi*121 (agg aaa atg gag ccg gtg aac ca / ttg gtc tgg acc aag cac ata cac); *bng*l339 (cca acc gta tca gca tca gc / gca gag ctc tca tcg tct tct t); *Cl*1-06 (cac cct cct cca gtt gtc att cg / aag gtc agc aaa gcg gca tag g); *Cl*2-23 (gag gcg gag gag ttg aga g / aca aaa caa cga aac cca tag c) and *Cl*2-140 (ctt ttt ctt ctg att tga ctg g / act gtt tat ccc gac ttc act a) (Jarret *et al.* 1997).

PCR amplification: Hot Start PCR (Erlich *et al.* 1991) was combined with Touchdown PCR (Don *et al.* 1991) using AmpliTaq Gold™ Polymerase. Reactions were carried out in a total volume of 25 µl (containing genomic DNA of 30-50 ng, 1 x PCR buffer (2.5 mM $MgCl_2$), dNTPs (200 µM each), 20 pmol of each primer and 1.0 U of *Taq* polymerase. Touchdown PCR was performed by decreasing the annealing temperature by 1.0 °C / per cycle with each of the initial 12 cycles (PE 9700, Applied Biosystems), followed by a 'touchdown' annealing temperature for the remaining 25 cycles at 56 °C for 30 s with a final cycle of 72 °C for 10 min (transgene detection) and held at 4 °C. A minimum of three independent DNA preparations of each sample was used. Amplifications were assayed 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 TBE buffer. Each successful reaction with scorable bands was repeated at least twice. Transilluminated gels were analyzed by the 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 the PCR runs. Fragments were purified in a spin column (Sigma 5-6501) according to the manufacturer's protocol and subjected to sequencing.

DNA Sequencing: Fragments were subjected to automated fluorescent DNA sequencing (ABI PRISM 3100 Genetic Analyzer). Sequence alignments were analyzed by BioEdit Sequence Alignment Editor (NCSU, USA) software programs. For BLAST (Basic Local Alignment Search Tool) analysis a NCBI (National Center for Biotechnology Information) computer program was used.

Data analysis: Cluster analysis was carried out by the SPSS-11 program package using the Average Linkage within group based on the presence versus absence of SSR alleles.

3. Ancient Cucurbits

The oldest plant remains with proven human activity have revealed only cereal seeds of wild barley (*H. spontaneum*) and wild emmer (*Triticum dicoccoides*) from 19,000 B.P. at Ohalo II., river Jordan (Nadel *et al.* 2004, 2006; Piperno *et al.* 2004). The 17,310±310 B.P. site in Korea (Chungbuk National University, South Korea) revealed the first ancient rice (*Oryza sativa*) seed remains with an extractable amount of aDNA (Suh *et al.* 2000).

The first *Cucurbit* seeds were excavated from the Spirit Cave (Hoabinh, Thailand) including cucumber type *Cucumis* seeds at least 9,180 ± 360 as analyzed by C^{14} of bamboo charcoal (Gorman 1969).

Radiocarbon dating is generally used to determine the age of carbonaceous materials up to about 60,000 years based on the the naturally occurring isotope carbon-14 (^{14}C) (Plastino *et al.* 2001). The technique was discovered by Libby (Arnold and Libby 1949), and awarded the Nobel Prize in 1960. The methodology of radiocarbon dating is based on the fact that carbon has two stable, nonradioactive isotopes (^{12}C), (^{13}C) and one unstable isotope (^{14}C) with a half-life of 5,568±30 years (expressed in Libby half-life) or 5,730 years (in Cambridge half-life). Practically, the small amount of ^{14}C would have vanished from Earth long ago if the cosmic rays which enter the atmosphere did not continuously generate it from nitrogene molecules (N_2) of the air according to the classical nuclear reaction, as n (neutron) + $^{14}N_7 \rightarrow$ $^{14}C_6$ + p (proton). The highest rate of ^{14}C production takes place at altitudes of 9 to 15 km, but it spreads evenly throughout the atmosphere, producing at a constant rate, and the proportion of radioactive to non-radioactive carbon, which is constant, is ca. 1 ^{14}C / 600 billion atoms/mole. ^{14}C reacts with oxygen to form CO_2, which enters plants by photosynthesis, and then goes from plants into animals. When organisms (plants or animals) die the incorporation of ^{14}C stops, and its amount gradually decreases in the cadaver

through radioactive decay by turning back the generative reaction producing $^{14}N_7$ according to the reaction: n (neutron) + $^{14}C_6 \rightarrow {}^{14}N_7 + e^- + \nu_e$ (anti neutrino). This decay is used to measure how long ago a piece of once-living material died and is expressed as years B.P. (before present), and calibrated as 1950 A.D.

4. 3,000-yr old watermelon seeds in pyramid of Pharaoh Tutankhamum (ca. 3,330 B.P.)

The monotypic genus *Citrullus* is comprised of only four diploid (2n = 4x = 22; 4.25 - 4.54 x 10^8 bp; 0.42 pg DNS) species, including the annual watermelon (*Citrullus lanatus*), the perennial colocynth (*syn.*: bitter apple) (*Citrullus colocynthis*), two wild species growing in the Kalahari Desert, Africa, as the *Citrullus ecirrhosus* with bitter-tasting fruit, and the annual *Citrullus rehmii* with pink and olive green spotted, mandarin sized, non-edible fruits (Robinson and Decker-Walters 1997; Dane and Liu 2006).

Unlike the genus *Citrullus*, the species watermelon (*Citrullus lanatus*) comprises diverse varieties, subspecies, mutants, and feral forms such as the cultivated watermelon (*C. lanatus lanatus*) (syn.: *C. vulgaris*) with its ancient form of citron melon (*syn*.: African tsamma) (*Citrullus lanatus citroides*), and the Mediterranean seed mutant egusi type watermelon (*C. lanatus mucospermum*) (Kanda 1951; Gusmini *et al*. 2004). Watermelon, citron and colocynth have a history of production in Europe (Wasylikowa and Veen 2004; Creamer 2005; Tóth *et al.* 2008).

The primary gene centre for watermelon is not known. The excavations of five and six thousand year-old seed remains of *C. lanatus* in Egypt and Libya imply that domestication might have occurred in Northern Africa (Dane and Liu 2006).

The oldest watermelon (*Citrullus l. lanatus*) seeds (6,000 years old) were excavated in Helwan (Egypt, Africa), at a site in 4.000 B.C. (Barakat 1990). About 5,000-yr old seed remains were excavated in Uan Muhuggiag, Libya, Africa at a site from 3000 B.C. (Wasylikowa and Veen 2004). Several watermelon seeds were found in the Pharaoh's tombs in Thebes (New Kingdom: 1,550-1,070 B.C.; stored in Agricultural Museum, Dokki, Giza, Egypt) (Warid 1995) and in the pyramid of Tutankhamum ca. 1330 B.C. (Hepper 1990; Kroll 2000; Vartavan and Amorós 1997) (***Fig. 1***).

The Greeks and Romans traveling to Egypt must have known of watermelon probably without discriminating it from colocynth and citron melon. Pliny II. wrote about a wild one (probably the current colocynth) and two types (one with pale green, and the other with grass green rind) of cultivated colocynth (probably the current watermelon), as it has been written: „…*Another kind of wild gourd is called Colocynthis. The fruit is smaller than the cultivated one, and full of seeds. The pale variety is more useful than the grass-green one…*" (Pliny 23-79) (***Fig. 2***).

The Codex *De Materia Medica* (produced not too long after the time of Pliny) provides nearly 400 color paintings of different plants but no watermelon illustrations, only a precise colour painting of colocynth which looks very much like the current forms of colocynth (*Citrullus colocynthis*) (Dioscorides 1st CENT. and the second 'edition' with colour paintings from 512 A.D.) (***Fig. 2***).

Six hundred years later, when the Iberian Peninsula was conquered by the Berbers (Moors) led by Tarik Ibn Ziyad in 711 A.D., new watermelon types might have entered Europe as recorded in the ancient *Book of Agricuture* (Al-Awwam 1158). In this book, two cultivated forms were compared, a black seed type (with dark-green rind which turns black when it ripens) and a red seed type (with green rind which turns to yellow) (Blake 1981). Watermelon might have also been introduced to Europe through Crusaders (Fischer 1929) led by either Richard I. The Lion-Hearted (the 3rd Crusade, 1190-1199), or the Hungarian King, Endre II. of the Árpád Dynasty (the last, 6th Crusade, 1228-1229). Watermelon spread through Europe quickly and became a very popular and commonly cultivated fruit in Renaissance Europe, with the first illustration on the frescos in the *Villa Farnesina*, Rome, Italy, 1517 B.C. (painted by Giovanni Martini da Udine) (Janick and Paris 2006). By 800 A.D., watermelons had been introduced to India and by 1100 A.D. to China. It reached the New World after Columbus' second voyage in 1493 and dispersed quickly among American natives (Blake 1981). One of the most ancient forms of small, round fruit with thin, green rind, red flesh and small black seeds (***Fig. 3***) has survived up to recent times (Gilmore 1919).

One of the oldest watermelon herbarium samples is available from G Bauhin's (1560-1624) collections (about a hundred years earlier than Linnaeus's), who named it *Anguira citrullus* (personal communication, Mark Spencer, The Natural History Museum, London, UK). No watermelon herbarium sample remained from the C Linnaeus (1753) collections, who named watermelon *Cucurbita citrullus*, and colocynth *Cucumis*

Figure 1. The most ancient seed remains of watermelon (Citrullus l. lanatus). (1) 6,000-yr old B.P. seed from Helwan, Egypt (Barakat 1990); (2) 5,000-yr old B.P. seed from Uan Muhuggiag (Libya) (Wasylikowa and Veen 2004) (1mm size bar); (3) 3,550-3,070-yr old B.P. seeds Pharaoh's tomb, Thebes (New Kingdom; stored in Agricultural Museum, Dokki, Giza, Egypt) (Warid 1995).

colocynthis (personal communication, Arne Anderberg, The Linnean Herbarium**,** Swedish Museum of Natural History, Sockholm, S) (**Fig. 2**).

5. Citrullus seed remains from the Middle Ages

Watermelon seeds excavated at both medieval sites analyzed in this study appeared to be extremely well preserved due to anaerobic conditions in the slime of a deep well covered by water, apparently used as dust holes in the Middle Ages (Gyulai *et al.* 2006). The herbarium sample seeds from the 19th cent. were stored under precise conditions in glass containers (Vörös 1971) (***Fig. 4***).

Eleven microsatellite probes were used in the study and presented for morphological reconstruction of the ancient watermelons. Allelic diversity of microsatellites were also reliably detected in aDNAs of 300 – 1,100-yr old seagrass (*Posidonia oceanica*) (Raniello and Procaccini 2002). SSRs were also used to morphologically reconstruct 600-yr old melon (*Cucumis staivus*) (Szabó et al. 2005) and millet (*Panicum miliaceum*) (Lágler *et al.* 2005; Gyulai *et al*. 2006). SSR analysis was also applied to herbarium samples of the common reed (*Phargmites australis*), about 100 years old, to track plant invasion in North America (Saltonstall 2003).

Molecular dendrograms of the study, based on 737 ALF fragments identified at eleven nuclear microsatellite (nSSR) loci, revealed that middle age samples show close lineages to ancient varieties currently growing in Hungary (***Fig. 5***) with red flesh colour. Results of seed morphology correlated strongly with molecular results (***Fig. 6***). The 13th -14th cent. sample

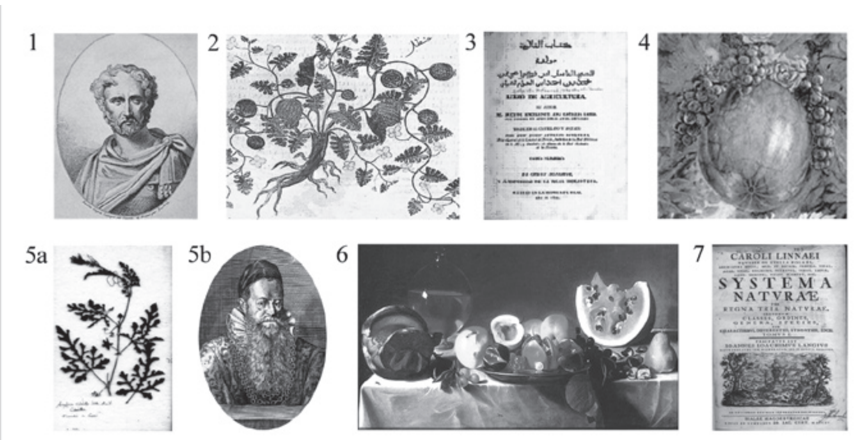

Figure 2. References and illustration of ancient Citrullus: (1) Pliny (Plinius) II (23-79; 'cultivated Colocynth'); (2) Dioscorides (1st cent, and 512 a.d.; the first colour painting of C. colocynthis); (3) Ibn Al-Awwam (1158): 'Book of Agricuture'; (4) Fresco in the Villa Farnesina, Rome, Italy (1517) painted by Giovanni Martini da Udine (Janick and Paris 2006); (5a) Herbarium sample of watermelon, G. Bauhin (1560-1624) (5b); (6) Caravaggio, 1603, Still Life with Melons and Carafe of White Wine; (7) Linnaeus (1740) Sytema Naturae.

(Debrecen) was similar to cv. '*Kecskeméti vörösbájú*'; the 15th CENT. sample (Budapest) was similar to cv. '*Belyj dlinnij*' (# 12) (***Fig. 7*** and ***8***). These results also reflect the preferential cultivation of red-flesh and not yellow-flesh watermelon in the Middle Ages in Hungary. Red flesh watermelon also appeared in the painting *Still Life with Melons and Carafe of White Wine* (1603 B.C.) painted by Caravaggio (Fig. 2). Molecular data obtained might provide further tools for watermelon breeders. The 170-yr-old herbarium sample (Pannonhalma, Hungary) showed close molecular similarity to citron melon (*Citrullus lanatus citroides*) cv.'*Újszilvás*' which reflects the importance of citron melon as fodder in the Middle-Ages in Hungary.

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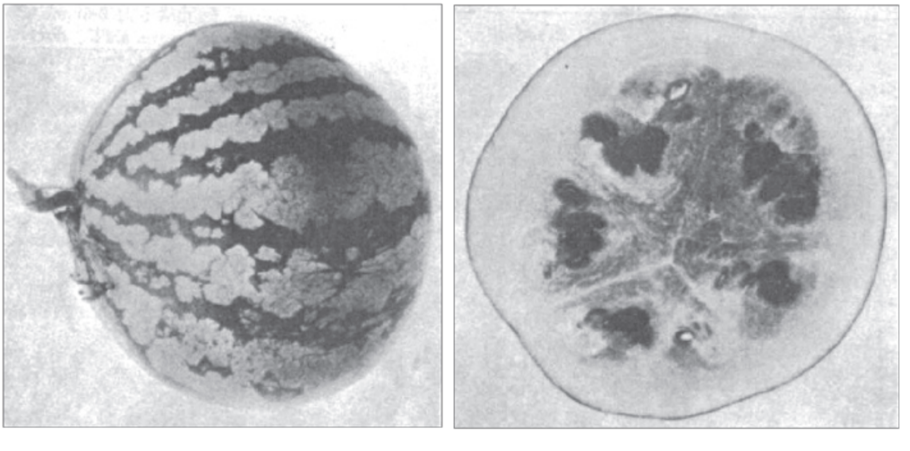

Figure 3. Ancient American watermelon grown from seed obtained from Penishka, an American native of Ponca tribe (Gilmore 1919).

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Figure 4. Map of Europe showing the excavation sites from the 13th-14th CENT. A.D. Debrecen (1), the 15th CENT. A.D. Budapest (2); and the 19th CENT. herbarium sample at Pannonhalma (Hungary) (3).

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#	Cultivars	Short name	Latin name	Code (Tápiószele)
1	Finn 168	Fin.	Citrullus colocynthis	RCAT036168
2	Belga 172	Bel.	Citrullus colocynthis	RCAT036172
3	Portugál 547	Prt.	Citrullus colocynthis	RCAT035547
4	Szeged 099	Szg.	Citrullus l. citroides	RCAT036099
5	De Bánát 235	Rom.	Citrullus l. citroides	RCAT035235
6	Újszilvás 816	Újs.	Citrullus l. citroides	RCAT055816
7	Bácsbokod 917	Bác.	Citrullus l. lanatus	RCAT035917
8	Napsugár 257	Nap.	Citrullus l. lanatus	00257/05
9	Sándorfalva 105	Snd.	Citrullus l. lanatus	RCAT036105
10	Dévaványa 101	Dév.	Citrullus l. lanatus	5101/02
11	Szentesi sugárhasú 260	Sts.	Citrullus l. lanatus	00260/05
12	Belyj dlinnij 152	Bed.	Citrullus l. lanatus	RCAT036152
13	Ráckeve 812	Rác.	Citrullus l. lanatus	RCAT055812
14	Csárdaszállás 113	Csr.	Citrullus l. lanatus	RCAT035113
15	Tura 389	Tur.	Citrullus l. lanatus	RCAT035389
16	Biri 114	Bir.	Citrullus l. lanatus	RCAT035114
17	Klondike R7 096	Kln.	Citrullus l. lanatus	RCAT036096
18	Charleston gray 263	Chg.	Citrullus l. lanatus	00263/05
19	Taktaharkány 790	Tkt.	Citrullus l. lanatus	RCAT034790
20	Túrkeve 112	Trk.	Citrullus l. lanatus	RCAT035112
21	Ukrainskij 545 149	Ukr.	Citrullus l. lanatus	RCAT036149
22	Szirma 782	Szr.	Citrullus l. lanatus	RCAT034782
23	Marsowszky 256	Mar.	Citrullus l. lanatus	00256/05
24	Háromfa 754	Hár.	Citrullus l. lanatus	RCAT034754
25	Debrecen 111	Deb.	Citrullus l. lanatus	RCAT035111
26	Sibiriak 098	Sib.	Citrullus l. lanatus	RCAT036098
27	Nagyecsed 775	Ngye.	Citrullus l. lanatus	RCAT034775
28	Nagykálló 785	Ngyk.	Citrullus l. lanatus	RCAT034785
29	Hevesi 258	Hev.	Citrullus l. lanatus	00258/05
30	Nagyvárad 767	Ngyv.	Citrullus l. lanatus	RCAT034767
31	Nyírbátor 155	Nyrb.	Citrullus l. lanatus	RCAT035155
32	Oros 862	Oro.	Citrullus l. lanatus	RCAT035862
33	Rákóczifalva 145	Rák.	Citrullus l. lanatus	RCAT035145
34	Kömörő 762	Köm.	Citrullus l. lanatus	RCAT034762
35	Nyíregyháza 778	Nyre.	Citrullus l. lanatus	RCAT034778
36	Kecskeméti vöröshúsú 259	Kev.	Citrullus l. lanatus	00259/05
37	Ilk 236	Ilk	Citrullus l. lanatus	RCAT035236
38	Pusztadobos 146	Pusz.	Citrullus l. lanatus	RCAT035146
39	Gyöngyös 969	Gyn.	Citrullus l. lanatus	RCAT034969
40	Crimson sweet 262	CrS.	Citrullus l. lanatus	00262/05
41	Kibéd 172	Kib.	Citrullus l. lanatus	5172./02
42	Sugar baby /Génbanki 261	SuB.	Citrullus l. lanatus	00261/05
43	Lipót 970	Lip.	Citrullus l. lanatus	RCAT034970
44	Korai kincs 255	Kok.	Citrullus l. lanatus	00255/05

Table 1. List of the current cultivars of colocynth ('sártök') (Citrullus colocynthis, 1-3); citron melon ('takarmány dinnye') (Citrullus lanatus citroides 4-6) and watermelon ('görögdinnye') (Citrullus lanatus lanatus, 7-44) analyzed.

Figure 5.a.

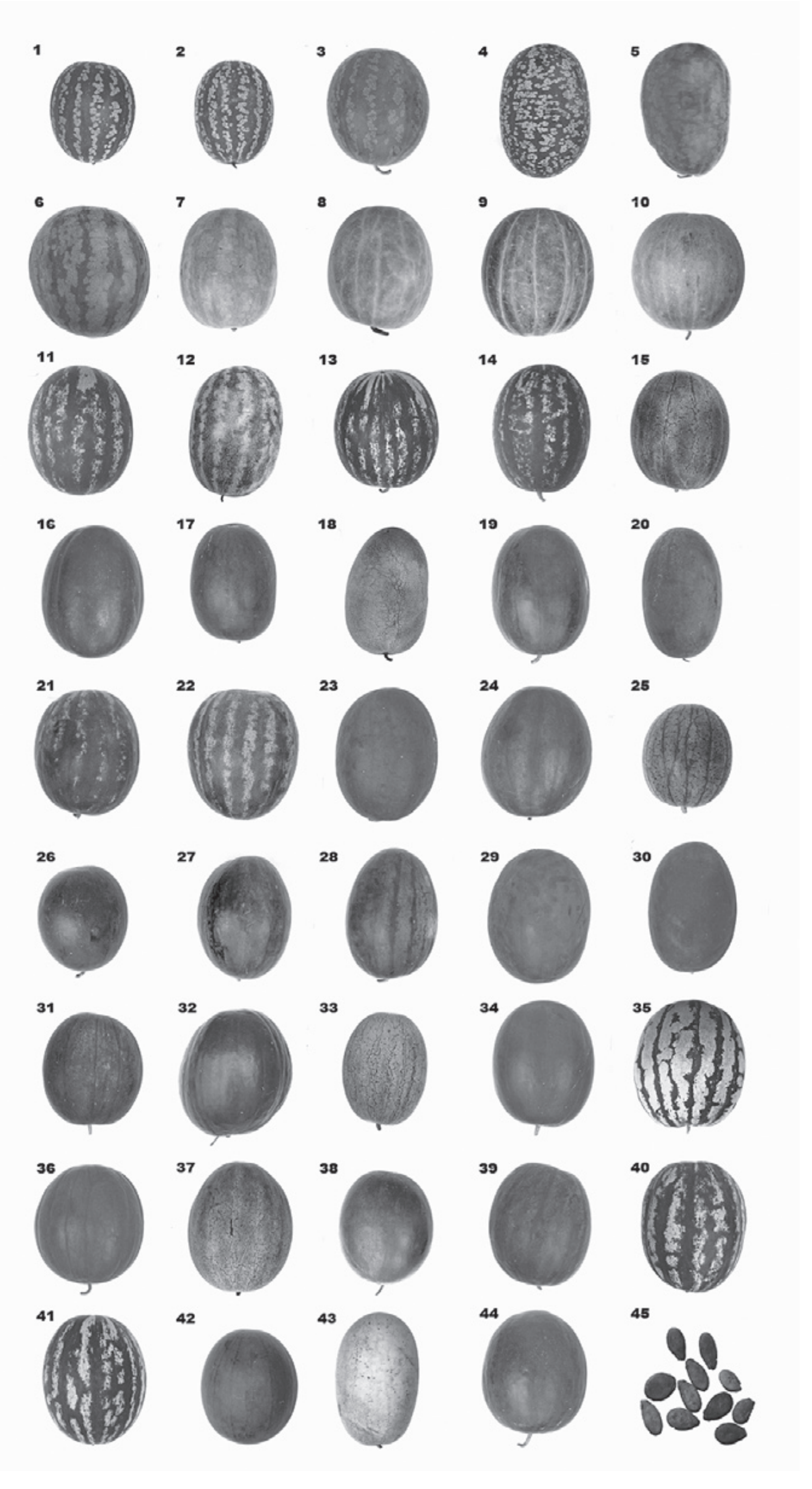
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Figure 5.b.

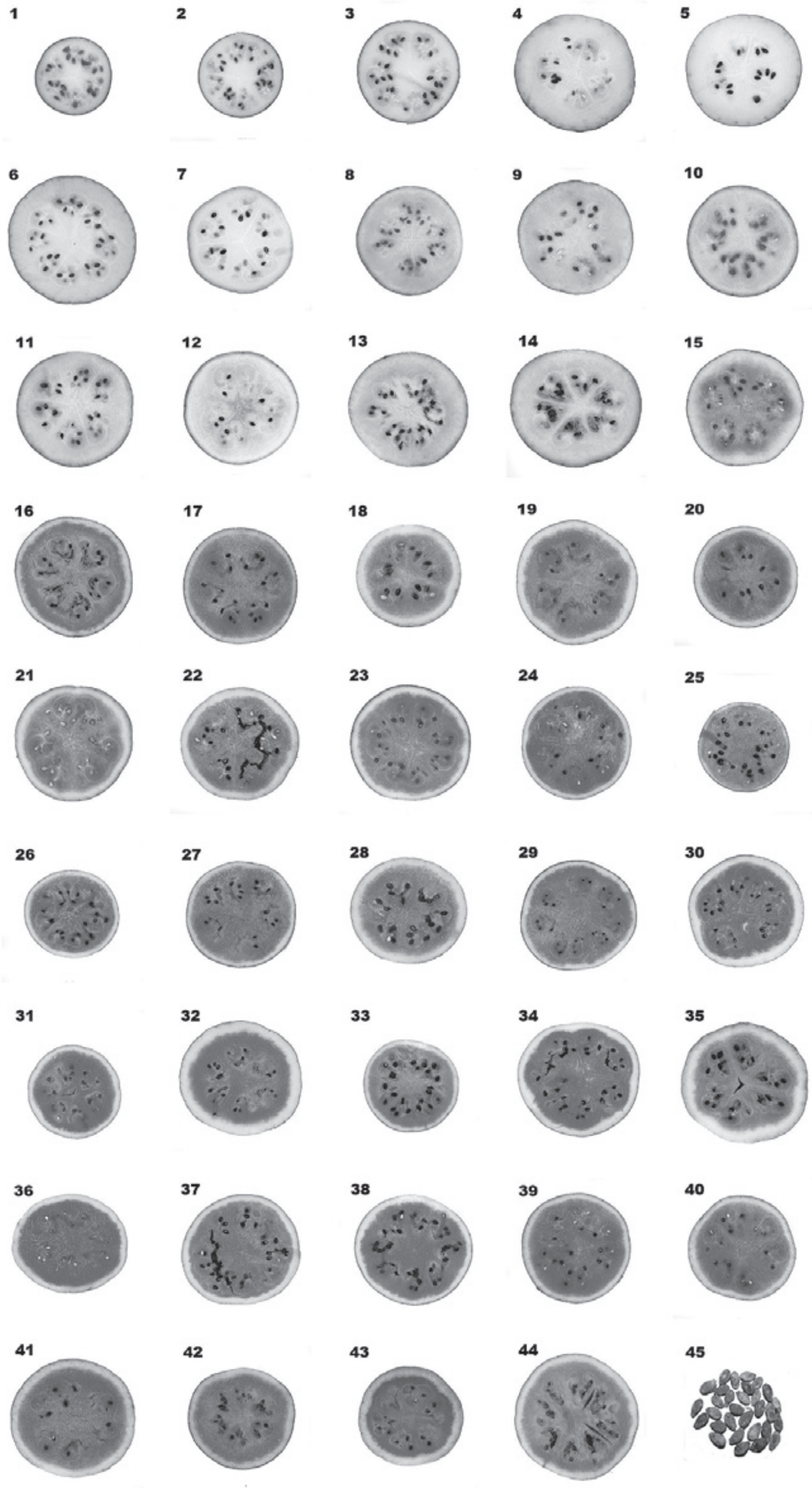
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Figure 5.c. Rind (5.a) flash (5.b) and seed (5.c) types of current Citrullus species and cultivars at ripening time (see Table 1) used for comparative analyses. Ancient seeds of 13th-14th CENT. A.D. Debrecen (in a), 15th CENT. A.D. Budapest (b); and 19th CENT. Pannonhalma (c) are included.

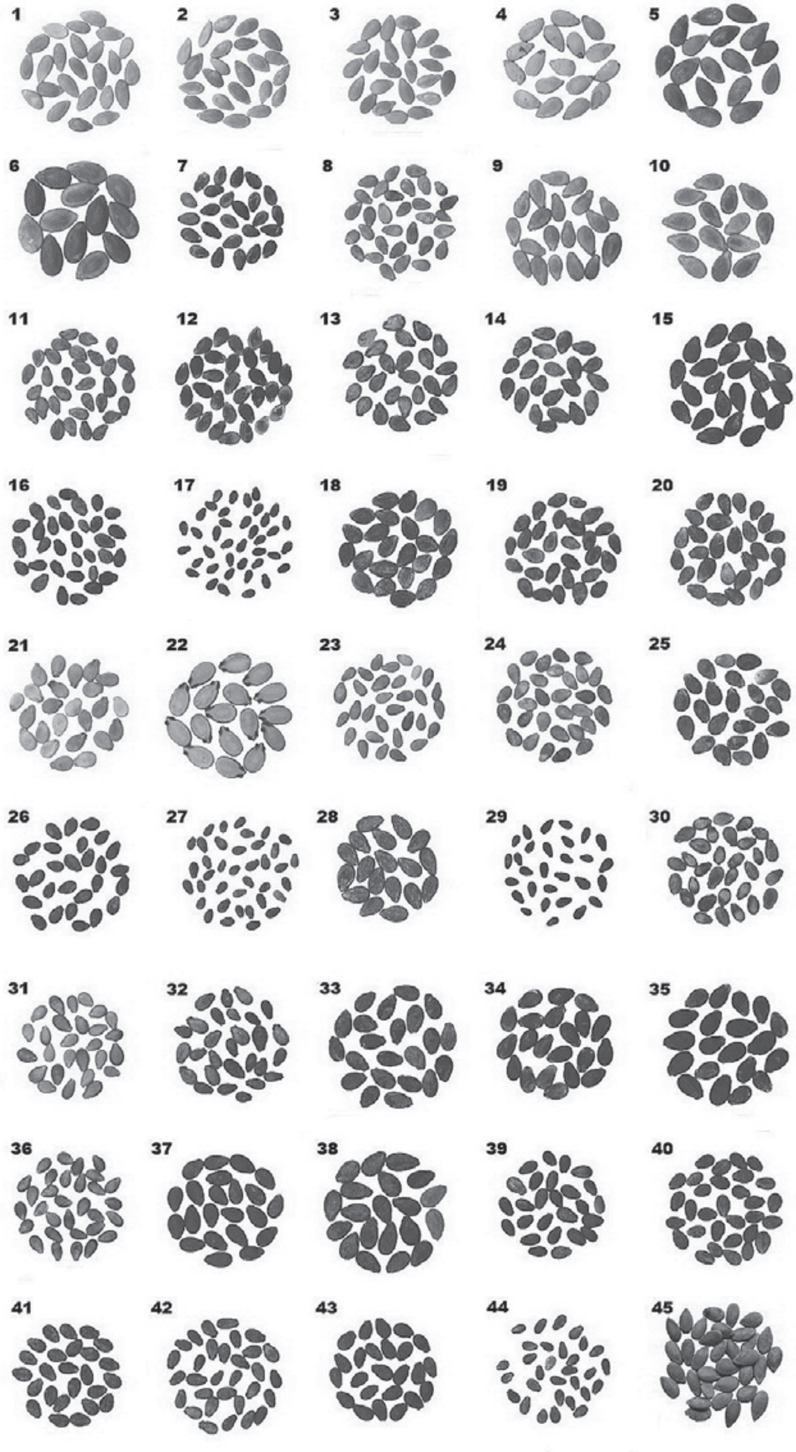

Figure 6. Individual samples of ancient Citrullus seeds compared to current varieties (a – dorsal and b – ventral view). Ancient watermelon (C. l lanatus) seeds excavated at the 13th-14th CENT. A.D. site, Debrecen (1); the 15th CENT. A.D. site, Budapest (2); and the 19th CENT. herbarium sample of citron melon (C. l . citroides) from Pannonhalma (3). Comparative samples of current Citrullus cultivars similar to 13th-14th CENT. A.D. sample, Debrecen (4 and 7: # 36. cv. 'Kecskeméti vöröshúsú' and # 17. cv. 'Klondike´); 15th CENT.. sample, Budapest (5 and 8: # 12. cv. 'Belyj dlinnij', and # 14. cv. 'Csárdaszállás'); and 19th CENT. herbarium sample of citron melon (6 and 9: # 5. cv. 'De Bánát', and # 6. cv. 'Újszilvás') (size bar indicate 1mm).

#	cv.	126/cmct168	190/cl2-140	124/bngl118-2	113/bngl339	146/bngl161	124/cmacc146	130/ phi121	139/bngl161	132/phi118-2	306/cmct51	145//cl1-06	136/phi118-2	124/phi121	130/cl1-06	178/cl2-23	131/bngl339	150//cl1-06	187/cl2-23	309/cmct51	132/bngl118-2	183/cl2-23	136/cmacc146	135/ phi121	129/phi118-2	140/bngl339	Total
1 Fin.	C.c.	●	●	●	●	●	●	●	●			●	●			●	●			●	●				●		15
2 Bel.	C.c.	●	●	●	●	●	●	●	●			●				●	●			●							12
3 Prt.	C.c.	●	●	●	●	●		●	●			●		●		●	●		●	●							13
4 Szg.	C.l.c.	●	●	●	●	●	●			●	●	●				●	●			●	●			●			14
5 Rom.	C.l.c.	●	●	●	●	●	●	●	●	●	●	●	●			●	●			●	●	●					17
6 Újs.	C.l.c.	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●			●	●		●				18
7 Bác.	C.l.l.	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●				●		●	●		19
8 Nap.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●				●	●								15
9 Snd.	C.l.l.	●	●	●	●		●	●	●		●	●	●	●			●										12
10 Dév.	C.l.l.	●	●	●	●	●	●	●	●	●	●		●	●	●			●									14
11 Sts.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●		●	●	●		●	●						●	●	18
12 Bed.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●		●	●		●	●	●	●	●						18
13 Rác.	C.l.l.	●	●	●	●	●	●		●	●	●	●		●			●										12
14 Csr.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●		●	●	●	●		21
15 Tur.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●			●				●	●						15
16 Bir.	C.l.l.	●	●	●	●	●	●	●	●	●	●		●	●	●			●	●								15
17 Kln.	C.l.l.	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●					●		19
18 Chg.	C.l.l.	●	●	●	●	●	●		●	●	●	●	●	●	●	●		●									15
19 Tkt.	C.l.l.	●	●	●	●	●	●		●	●	●	●	●	●	●	●					●	●					16
20 Trk.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●			●			●	●	●	●	●		●		19
21 Ukr.	C.l.l.	●	●	●	●	●	●	●		●	●		●	●	●		●	●									14
22 Szr.	C.l.l.	●	●	●	●	●	●	●		●	●	●	●	●	●						●	●					15
23 Mar.	C.l.l.	●	●	●	●		●	●	●	●	●	●	●	●		●			●	●	●		●				17
24 Hár.	C.l.l.	●	●	●	●	●	●		●	●	●	●	●	●	●			●									14
25 Deb.	C.l.l.	●	●	●	●	●	●	●		●	●		●	●	●			●					●	●			15
26 Sib.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●					18
27 Ngye.	C.l.l.	●	●	●	●	●	●	●	●	●	●		●	●			●	●									14
28 Ngyk.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●							●					15
29 Hev.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●		●		●	●						●		17
30 Ngyv.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●				●					●	17
31 Nyrb.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●		●	●						●						14
32 Oro.	C.l.l.	●	●	●	●		●	●	●	●	●	●	●	●			●								●		14
33 Rák.	C.l.l.	●	●	●	●	●	●		●	●	●	●	●	●								●					13
34 Köm.	C.l.l.	●	●	●	●	●	●	●	●	●	●		●	●	●			●									14
35 Nyre.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●		●	●						●						14
36 Kev.	C.l.l.	●	●	●	●	●	●	●		●	●	●	●	●		●	●	●	●								16
37 Ilk	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●									●			15
38 Pusz.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●			●				●						15
39 Gyn.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●				●		●			18
40 CrS.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●	●		●			●		18
41 Kib.	C.l.l.	●	●	●	●	●	●	●	●	●	●		●	●	●		●	●									15
42 SuB.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●		●								16
43 Lip.	C.l.l.	●	●	●	●	●	●	●	●	●	●	●	●	●				●				●	●			●	17
44 Kok.	C.l.l.	●	●	●	●	●	●	●		●	●		●	●				●	●			●		●		●	17
13-14.c.Deb.		●	●	●	●	●	●	●				●		●	●	●	●		●	●							14
15.c.Buda		●	●	●	●	●	●	●	●	●	●	●	●		●	●	●			●		●	●	●	●		20
19.c.Pannonh.		●	●	●	●	●	●	●	●	●	●	●	●	●		●	●										15
Total		47	47	47	47	44	46	41	39	42	43	38	39	40	26	21	25	20	14	15	14	14	7	8	10	4	737

Figure 7.

Allele diversity at eleven microsatellite loci in the current Citrullus species and cultivars (1-44), compared to medieval (13th-14th and 15th centuries) and herbarium samples (19th CENT.).

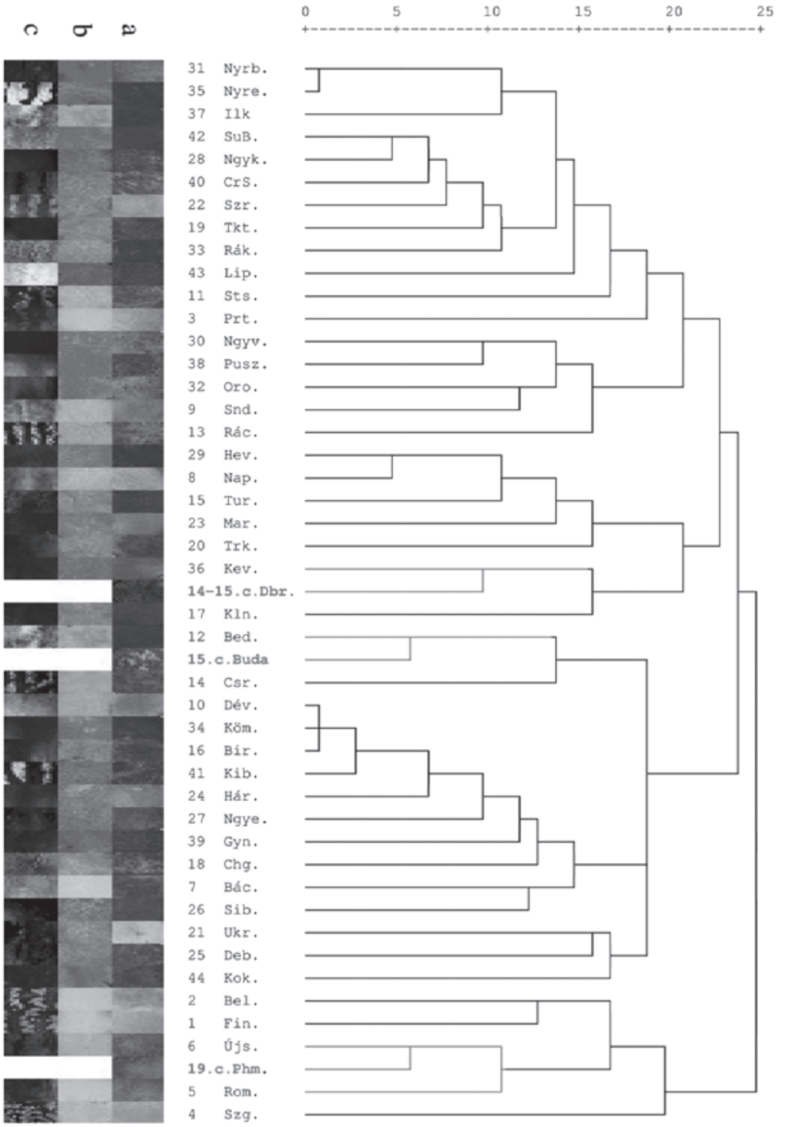

Figure 8. Molecular dendrogram (Rel Genet Dist, 0 – 25) of current varieties of colocynth ('sártök') (Citrullus colocynthis, 1 - 3), citron melon ('takarmány dinnye') (Citrullus lanatus citroides, 4 - 6) and watermelon ('görögdinnye') (Citrullus lanatus lanatus, 7 - 44) (see Table 1) compared to archaeological and herbarium samples. Colors of seed coat (a), flesh (b) and rind (c) are indicated.