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Domestication Events of Grape (Vitis vinifera) from Antiquity and the Middle Ages in Hungary from Growers' Viewpoint

Morphological reconstruction of ancient grapes (*Vitis vinifera)* based on archaeological seed remains provide insight into the domestication and cultivation events of grapes in Hungary. Ancient grape seeds were excavated at Roman and Medieval archaeological sites in Hungary and analyzed by LM (Light Microscopy) and SEM (Scanning Electron Microscopy). Excavation sites included Budapest (*Aquincum*; 2nd–4th CENT. A.D. Hungary) and Keszthely (Fenékpuszta) of Roman Age (5th CENT. A.D., Hungary); and Gyır (Ece; 11–12th CENT. A.D., Hungary), Debrecen (13th CENT. A.D., Hungary) and the King's Palace of the Árpád Dynasty at the Castle of Buda, Budapest (15th CENT. A.D., Hungary) of the Middle Ages. Ancient seeds were compared to thirty current grape varieties of similar seed size, shape, and morphology. The modern grape variety *Vitis vinifera* cv. '*kék bakator*' (*syn.*: '*Blue Bocca d'Oro*'; '*aranybogyó*') was found most similar in seed morphology to one of the ancient samples (15th CENT. Debrecen, Hungary) which indicates the antiquity of this cultivar.

The genus Vitis

Species of the plant family *Vitaceae* are woody climbers comprising 13–17 genera: Acareosperma; Ampelocissus, Ampelopsis (pepper-vines); Cayratia; Cissus (treebines); Clematicissus; Cyphostemma; Leea; *Muscadinia*; Nothocissus; Parthenocissus; Pterisanthes; Pterocissus; Rhoicissus; Tetrastigma; Vitis (grapes); and Yua of about 700 species (Facsar 1970; Terpó 1976). The genus *Vitis* consists of about 60 inter-fertile species including about fifteen species of agronomic importance (Table 1). Of them, *V. vinifera* ($2n = 4\times = 38$) is the only species which is indigenous to Eurasia, with a relatively small nuclear (nuDNA) genome size of $0.475–0.5\times10^9$ DNA base pair (bp); and a 160,928 bp of chloroplast cpDNA (Jansen *et al*. 2006) and a regular size of higher plant mtDNA (1–400,000 bp). Most genera of family *Vitaceae* have 2n = 38 chromosomes (n = 19), but species of Muscadinia, Ampelocissus, Parthenocissus, and Ampelopsis have 2n = 40 (n = 20) chromosomes, and species of the genus Cissus has 2n = 24 (n = 12) chromosomes.

Changes in seed ('pip') shape, wild grapes have rounder pips with short beaks, while seeds of cultivated grape tend to be more elongated with longer beaks. Seed morphology indicates that domestication of grape (*Vitis vinifera*) began with the Eurasian wild grape (*V. sylvestris)* about 5,500–5,000 B.P. (before present) in southwest Asia and southern Transcaucasia (Armenia and Georgia). Seeds of *Vitaceae* are easily identified from a suite of unique and distinctive morphological characters (particularly a pair of ventral in folds and a dorsal chalazal scar).

The wild, dioecious ancestor form of *V. vinifera* ssp. *silvestris* (syn.: *V. silvestris*) still coexists with the cultivated, hermaphrodite flower form of *V. vinifera* ssp. v*inifera* (syn. *V. vinifera*) in Eurasia and North Africa (This *et al*. 2006). Today, thousands of cultivars have been developed which are generally classified in three main groups according to their final production, as wine grapes, table grapes including modern seedless grapes, and raisins.

Genetically, dioecy in wild grape is encoded by a single gene; female individuals are homogametic carrying homozygous recessive pistil-*su*ppressor alleles (su^msu^m) which suppress the development of anthers (and pollen). Male plants are heterozygous (SU^Fsu^m) carrying a dominant pistil-suppressing Su^F allele.

The shift, under domestication, to bisexual (hermaphrodism) flowers took place via a single mutation to SU^+_m which is also dominant over su resulting in two genotypes of hermaphroditc grape types (SU^+su^m and SU^+SU^+).

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Domestication events in the genus Vitis

The oldest (8,400 B.P.) wild grape (*Vitis sylvestris*) seeds (about 3 mm long) were excavated in Turkey, at Nevali Çori (NÇ) located near the Turkish city of Urfa (37°60'N, 38°70'E, 490 m above sea level) on the slope of a Euphrates side valley, Hilvan province. The first convincing evidence of *Vitis vinifera* seeds with indications of grape cultivation were also uncovered in Turkey at Kurban Höyük (5.700–5.200 B.P. non-calibrated radiocarbon time), followed by the early Bronze Age samples (3,200–1900 B.P.) along the Jordan Valley, at *Tell Shuna* (Jordan; Chalcoitic), *Jericho* (Cisjordan; early Bronze Age), and *Arad* (Israel, early Bronze Age) (Jacquat and Martinoli 1999). Ancient grape seeds were also excavated at Semma (Sudan) 3,500 B.P.

The earliest evidence of wine production (jars from Godin) was found in Iran (Hajji Firuz Tepe site in the Zagros Mountains) about 7,400–7,000 B.P. (This *et al*. 2006) and 5,500 4,900 B.P. Greek, Latin, and Egypt vine amphoras with gelyfied vine remains were found in the hulls of sunken ships sunk, similar to the famous shipwreck remains at Uluburun near Kas (Turkey). Grape cultivation gradually spread to Mesopotamia, Assyria, and Egypt (about 5,500–5,000 B.P.), and further west along the Mediterranean to Phoenicia, Greece, North Africa and then to the entire Roman Empire north to Pannonia (Hungary) and German tribes. Viticulture also spread eastward along the Silk Road and it reached China and Japan in 3,200 B.P.

Grape were introduced to the Americas by European colonists starting from the 16th CENT. after either the early Chinese explorer Zheng He (1405–1435), or Columbus voyages (first: Aug. 3 1492 to March 15 1493; second: Sept. 25 1493 to June 11 1495; third: May 30 1498 to Nov. 15 1500; fourth: May 11 1502 to Nov. 7 1504). The first plantations in North America were established on the West Coast by Spanish missionaries and later by Hungarian viticulturists like Ágoston Haraszty who is considered the '*father of California's grape-growing industry*'. Haraszty imported 200,000 grape cuttings from Europe from 1849, including grape varieties from his native Hungary. With the passing of time, Haraszty developed over half a million California acres to viticulture, making wine growing second to orange production in the state's agricultural economy. In recognition of his merits, Haraszty was named California's State Commissioner of Viticulture (Sisa 2006).

European grape formed hybrids with native *Vitis* species growing in North America. Some of these hybrids became resistant to *Phyloxera* (an insect pest), which devastated European vineyards in the 1880s, and supplied resistant rootstocks for replantations. This event indicates that the diversity of grape genome has been narrowed twice; first by the Biblical

flood, followed by the re plantation of Noah 'the first vintner' (Genesis 9) on Mount Ararat, and second by *Phyloxera* (This *et al*. 2002). Unlike the genome for dioecious *V. sylvestris*, genetic diversity of grape has been narrowing continuously as the result of vegetative propagation either by rooting of twigs, or by grafting.

In Hungary, the earliest wild grape (*Vitis sylvestris*) seed remains were found at Tiszapolgár (5,300 B.C.) and the earliest *Vitis vinifera* at Sopron (1,300 B.C.), which dates the origins of grape cultivations to the late Bronze Age *(Table 2, Fig. 1).*

Table 1. *Vitis species (1–27), hybrids (1–9) and gene bank samples (1–12)*

Vitis species	*Vitis* hybrids*	*Vitis* gene bank samples
1. *Vitis acerifolia*	1. V. arizonica x V. rupestris	1. Vitis sp.
2. Vitis aestivalis	2. V. berlandieri x V. riparia	2. Vitis sp. 196-17
3. Vitis amurensis	3. V. berlandieri x V. rupestris	3. Vitis sp. 216-N
4. Vitis arizonica	4. V. berlandieri x V. vinifera	4. Vitis sp. 44-53M
5. Vitis bashanica	5. V. cinerea x V. riparia	5. Vitis sp. 8007
6. Vitis berlandieri	6. V. cinerea x V. rupestris	6. Vitis sp. 8658
7. Vitis betulifolia	7. V. labrusca x V. vinifera	7. Vitis sp. cv. 'Norton'
8. Vitis bryoniifolia	8. V. pseudoreticulata x V. vinifera	8. Vitis sp. CWD 96.70
9. Vitis cinerea *(downy grape)*	9. V. riparia x V. rupestris	9. Vitis sp. Nie 372
10. Vitis davidii		10. Vitis sp. Nie 415
11. Vitis flexuosa	* Interspecific hybrids registered in Hungary (2006):	11. Vitis sp. NL-
12. Vitis heyneana		12. Vitis sp. Qiu
13. Vitis kelungnsis		
14. Vitis labrusca *(Concord grape)*	*'Bianka'; 'Csillám'; 'Duna gyöngye'; 'Esther'; 'Fanny'; 'Göcseji zamatos'; 'Kunleány'; 'Medina; 'Nero'; 'Odysseus'; 'Orpheus'; 'Platina'; 'Pannon frankos'; 'Pölöskei muskotály'; 'Refrén'; 'Taurus'; 'Teréz'; 'Viktória gyöngye'; 'Zalagyöngye'.*	
15. Vitis piasezkii		
16. Vitis popenoei *(totoloche grape)*		
17. Vitis pseudoreticulata		
18. Vitis quinquangularis		
19. Vitis riparia *(riverbank grape)*		
20. Vitis rotundifolia *(fox grape)*		
21. Vitis rupestris *(rock grape)*		
22. Vitis shuttleworthii *(callose*		
23. Vitis sinocinere		
24. Vitis thunbergi		
25. Vitis tiliifolia		
26. Vitis vinifera *(wine grape)*		
27. Vitis yeshanensis		

The earliest wine residue in Hungary dates back to 700 B.C. (at Fehérvárcsurgó), which places the beginnings of wine making to the Iron Age. Thus, there is evidence that both grape cultivation and wine making date to well before to the Roman period in Hungary.

How to recover ancient DNA

Excavated and wet-sieved sediment samples of the study presented were processed by flotation followed by seed sorting and identification in the laboratory according to Gyulai *et al.* (2001, 2006). For SEM analysis, seeds were air dried, fixed in glutaraldehyde (5% w/v in phosphate buffer 0.07 M, pH 7.2) and washed three times in the same buffer for 10 minutes. Samples were then desiccated in acetone concentration series (10–50–70–90–100%), dehydrated at the CO_2 critical point (Blazers CDC 020), and covered with gold (30 nm). Seeds were examined and photographed using a TESLA BS-300 scanning electron microscope *(Fig. 2)* as described by Gyulai *et al.* (2006). For LM analysis, a Leica microscope (# 301–371.010) was used. For comparative analysis seeds of thirty current *Vitis* cultivars *(Fig. 3)* were applied.

Morphological reconstruction of ancient grapes

Ancient grape seeds of the study presented were compared to current grape varieties of similar seed size, shape, and anatomy, and analyzed by LM and SEM *(Fig. 2)*.

Based on seed morphology, the 15th CENT. seeds (Budapest, Hungary) were similar to the currently grown grape variety '*kék bakator*' ('Blue Bocca d'Oro') *(Fig. 2, Fig. 3),* which is one of the oldest varieties grown in Hungary and Italy, as the etymology of its name *Bocca d'Oro* (*aranybogyó*) suggests. Other seed samples of obvious ancient type with short seed beaks from the Roman (2nd–4th CENT. A.D., #1 and #2 Fig. 2) and medieval age (13th CENT., Debrecen, #4 *Fig. 2*) showed no such similarity to any of the thirty currently grown grape varieties analyzed (Fig 3). Seed sample from the 11th –12th CENT. (#3 Fig. 2) showed incomparably unique genotype. Ancient DNA (aDNA) were also extracted from the seeds according to Gyulai *et al.* (2006), Szabó *et al.* (2005), and Lágler *et al.* (2005) and amplified by WGA (Genomplex, Whole Genome Amplification, Sigma WGA-2) with a 5–9 fold amplification rate of total genomes, and analyzed by *Vitis*-specific primer pairs (results presented elsewhere).

Table 2. *Vitis (V. sylvestris, V. vinifera and V. sp.) and wine remains (pieces #) excavated in Hungary*

Ages	Excavation sites (Hungary)		*Vitis vinifera*	*Vitis sylvestris*	*Vitis* sp.
5,300 - 4,700 B.C. (Middle Neolithic)	1.	Magyaratád			10C
4,700 - 4,300 B.C. (Late Neolithic)	2.	Tiszapolgár-Csőszhalom		1C	
3,500 - 3,000 B.C. (Late Copper Age)	3.	Szombathely-Sé		1C	
	4.	Kompolt-Kistéri tanya		18C	
1,300 - 900 B.C. (Late Bronze Age)	5.	Békés-Várdomb			10C
	6.	Dunakeszi-Székesdűlő		2	
	7.	Ludas, Varjú-dűlő		2C	
	8.	Mosonmagyaróvár-Németdűlő		3	
	9.	Sopron-Krautacker 1	1		
900 - 500 B.C. (Early Iron Age; Hallstatt)	10.	Fehérvárcsurgó-Eresztvény	W		
	11.	Sopron-Krautacker3	2	1	
	12.	Sopron-Krautacker6	9		
	13.	Zagersdorf	3		
5th - 1st CENT. A.D. (Late Iron Age; La Tène)	14.	Budapest-Corvin tér			1C
1st – mid 5th CENT. A.D. (Roman age)	***15.***	***Budapest (Aquincum), Kaszásdűlő***	6,108		
	16.	Budapest (Kunigunda str)	W		
	17.	Budapest (Bécsi str 69-71)			i
	18.	Budapest (Vörösvári str 20-22)			13; 1C
	19.	Dunaújváros (***Intercisa***)	W		
	20.	***Keszthely-Fenékpuszta***	33C		1C
	21.	Budapest (Bécsi str 44)	3		
	22.	Szekszárd	W		
	23.	Tác-Fövenypuszta (*Gorsium*)	3		x
1st - mid 5th CENT. A.D. (Barbaricum)	24.	Budapest (XIV. reg. Paskál park)	1C		
	25.	Gyomaendrőd (Endrőd 170)		1C	
	26.	Kiskundorozsma-Nagyszék		1C	
6th - 8th CENT. A.D. (Awarian Age)	27.	Keszthely-Fenékpuszta	2C		
	28.	Budakalász	L		
	29.	Fonyód-Bélatelep	255		
	30.	Fényed-Szegerdő			10C
8st - 9th CENT. A.D (Late Migration periode; Caroling Age)	31.	Zalavár-Vársziget parkoló	127C		
895 A.D. - 1,301 A.D. (Hungarian conquest - Árpád Age)	**32.**	**Győr (Ece)**	28P		
	33.	Gyomaendrőd (Endrőd 170)		1C	
	34.	Rákoskeresztúr		3	
	35.	**Debrecen (Kölcsey Cultur-Centre)**	24		
1,301 – 15th CENT. A.D. (Hungarian Kingdom)	***36.***	***Budapest (St György sq Teleki Palace)***	210686		
	37.	Budapest (Kapucinusok 16)	192151		
	38.	Budapest (Bécsi str 34-36)	14773		
	39.	Budapest (Honvéd FŐP)	43170	1	
	40.	Budapest (Hunyadi János str 22)	1341		
	41.	Budapest (Dísz sq 8)	1233		
	42.	Budapest (Hess András sq 1)	365		
	43.	Budapest (Úri str 40)	66		
	44.	Budapest (Színház str)	4		
	45.	Baj-Öregkovács-Hill	3		
	46.	Budapest (Dísz sq 10)	x		
16th - 17th CENT. A.D. (Late Medieval; Hungarian Kingdom to Turkish occupation)	47.	Budapest (Medve str 13)	4954		
	48.	Hollókő-Castle	47C		150C
	49.	Kereki-Fehérkő Castel	x		
	50.	Külsővat	2P		
	51.	Lászlófalva-Szentkirály	5		
	52.	Nagyvázsony-Csepely	65C		12C
	53.	Pápa (Deák Ferenc str)	x		
	54.	Pápa (Hantai str)	12		
	55.	Pécs (Med School)	22C		
	56.	Sopron (L str 7)			8
	57.	Sopron (Templom str 14)			10C
	58.	Sümeg (Castle)	xx		
	59.	Szarvasgede	xC		
	60.	Szécsény-Plébániatemplom	693; 42F		
	61.	Székesfehérvár (Palotai str 5)	15C		
	62.	Vác (Széchenyi str 3-7)	44349P		
	63.	Visegrád (*Mathias Palace*)	52		

(Ccarbonized seeds; Fberry fragments; Ppetrified seeds; i – imprints; L – leather wine holder; W – wine residues); (x: pieces 1–10). (excavation sites studied are indicated in bold)

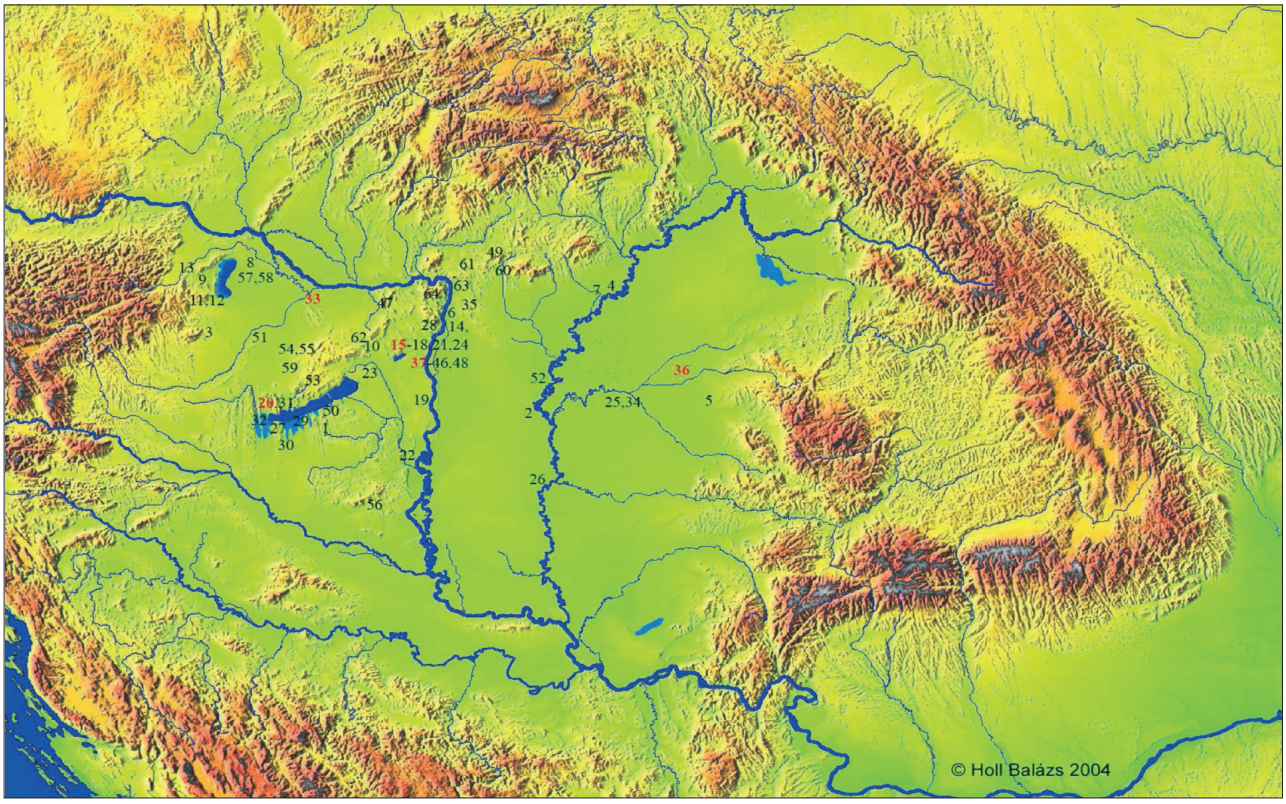


Figure 1: *Archaeological sites of Hungary where Vitis seeds were excavated listed in Table 2.*

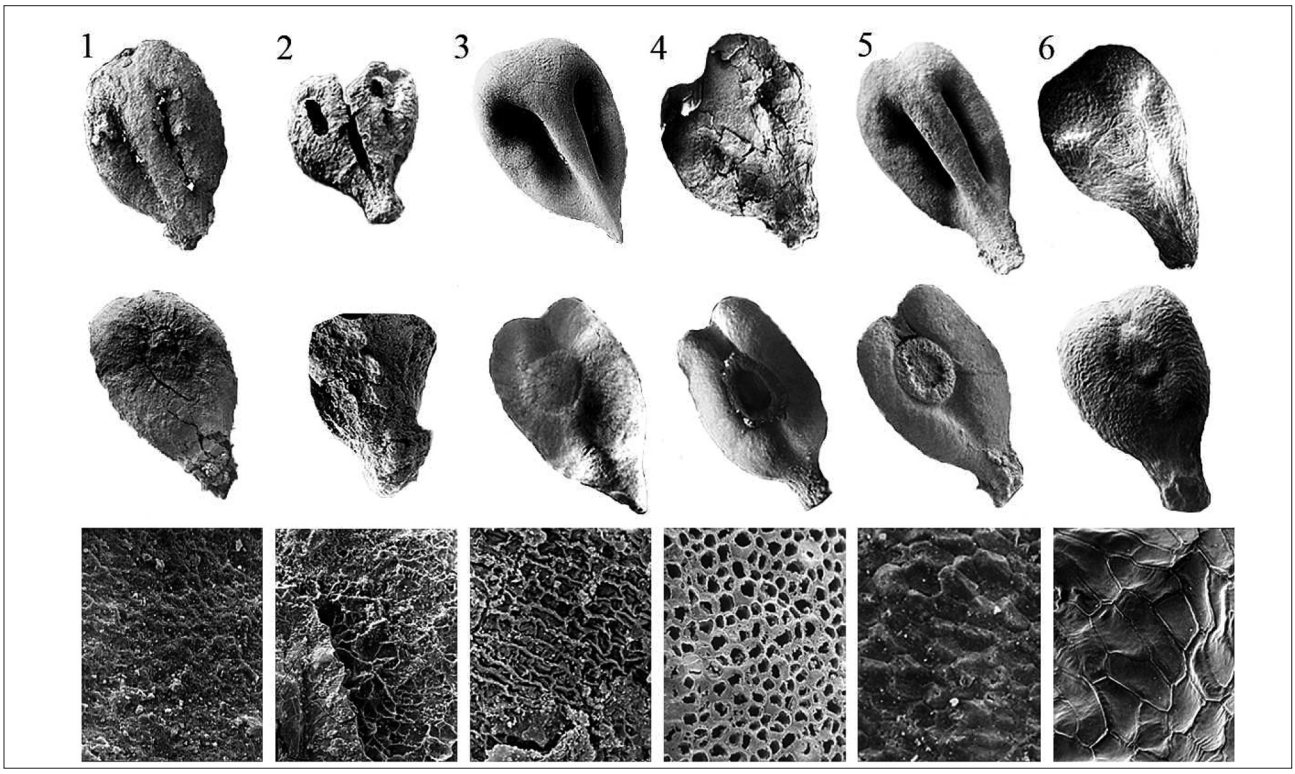


Figure 2: *Morphology of ancient Vitis seeds excavated in Hungary. SEM micrographs of seeds excavated at a 4th Roman Villa at Budapest (Aquincum, Hungary) (2nd – CENT., A.D.)* ***(1);*** *and Keszthely (5th CENT. A.D., Fenékpuszta, Hungary)* ***(2);*** *a vineyard site near Gyır (Ece, Hungary) (11–12th CENT.)* ***(3);*** *Debrecen (Hungary) (13th CENT.)* ***(4);*** *and at the King's Palace of Árpád Dinasty in the Castle of Buda (Budapest, Hungary) (15th CENT.)* ***(5).*** *The SEM micrograph of seeds of the contemporary Vitis vinifera cv. 'kék bakator' is also shown (6). Upper (ventral view) and middle (dorsal view) rows show seeds morphology at 20 x magnification. Bottom row shows seed coat textures at 500 x magnification*

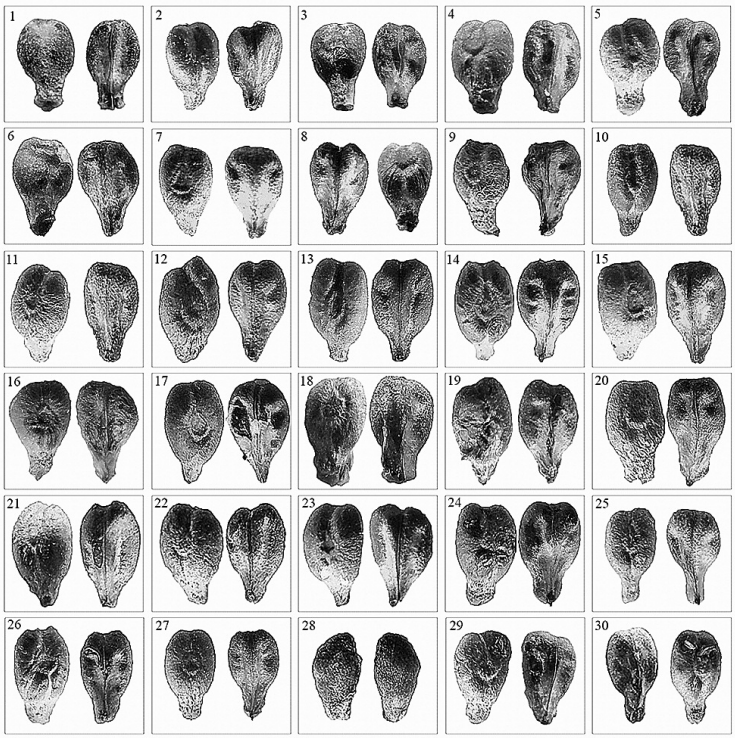
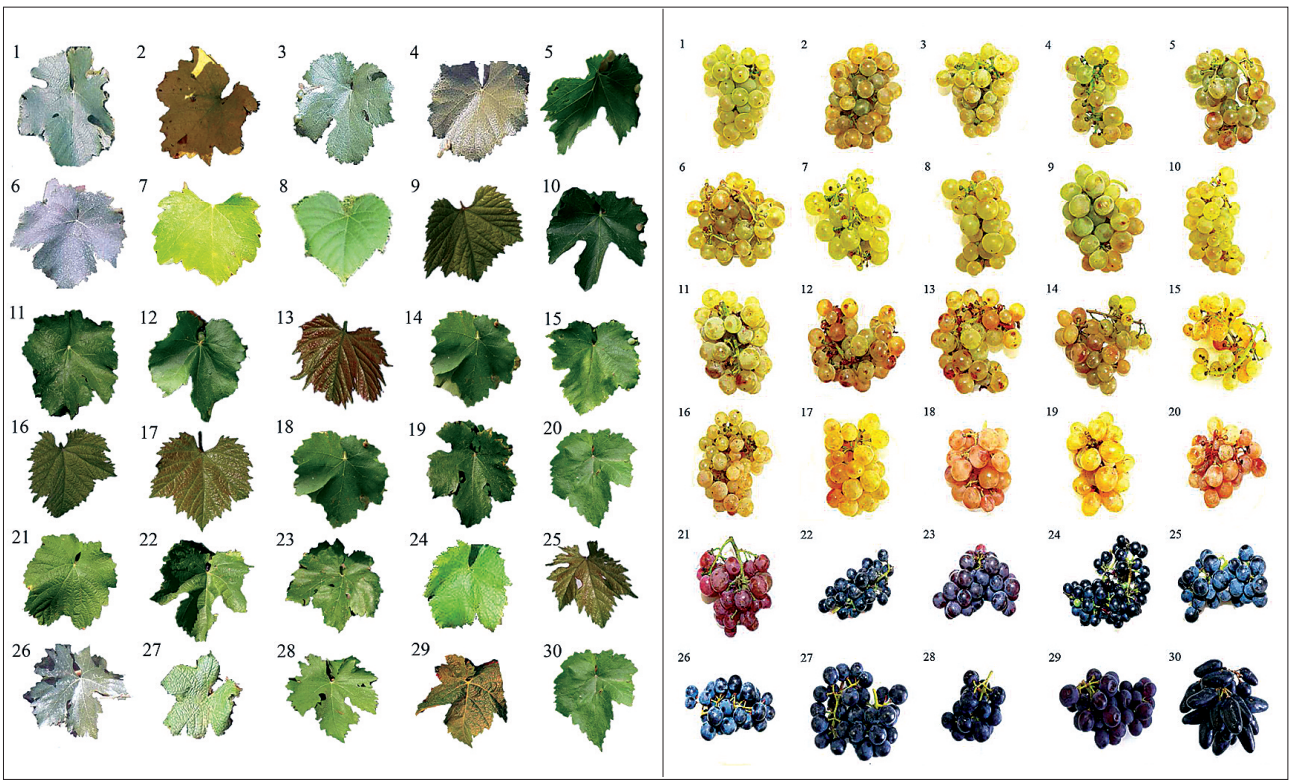


Figure 3: *Seed, grape and leaf morphology of current grapes (Vitis vinifera, but #8 and #21) grown in Keszthely (Hungary) used for comparative analysis.*
1. *'Rajnai rizling' NI-378;* ***2.*** *'Leányka';* ***3.*** *'Zöldszilváni';* ***4.*** *'Ezerfürtű';* ***5.*** *'Juhfark';* ***6.*** *'Chasselas blanc K-15';* ***7.*** *'Kunleány';* ***8.*** *V. riparia x V. rupestris;* ***9.*** *'Narancsízű';* ***10.*** *'Fehér lisztes'* ***11.*** *'Mirkpvacsa';* ***12.*** *'Hárslevelű';* ***13.*** *'Változó góhér';* ***14.*** *'Sárfehér';* ***15.*** *'Kéknyelű';* ***16.*** *'Csabagyöngye';* ***17.*** *'Mátyás király';* ***18.*** *'Fehér járdovány';* ***19.*** *'Kossuth';* ***20.*** *'Piros gohér';* ***21.*** *V. vinivera 'Aramon' x V. riparia 143 B';* ***22.*** *'Bakó';* ***23.*** *'Visnivi rami';* ***24.*** *'Kékfrankos';* ***25.*** *'Kék bakator';* ***26.*** *'Oportó';* ***27.*** *'Szürkebarát D 34';* ***28.*** *'Kismis vatkana';* ***29.*** *'Piros szlanka';* ***30.*** *'Suvenir'*



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