

Grape (*Vitis vinifera*) seeds from Antiquity and the Middle Ages Excavated in Hungary - LM and SEM analysis

Macaristandaki kazılardan elde edilmiş Antik ve Orta Çağa ait üzüm (*Vitis vinifera*) tohumlarının Işık ve Elektron Mikroskop analizleri

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Özet

Bu çalışmada, Macaristandaki Roman ve Ortaçağ arkeolojik kazı bölgelerinden çıkarılmış üzüm (*Vitis vinifera*) tohumu kalıntıları Işık ve Tarayıcı Elektron Mikroskop kullanılarak analiz edilmiştir. Macaristan'da bulunan Roman dönemine ait Aquincum ve Fenékpuszt, ayrıca Győr, Debrecen ve yine Ortaçağa ait Buda kalesinde bulunan Árpád Dinasty'nin Kraliyet Sarayı kazı alanları içinde yer almaktadır. Fosil tohumlar benzer büyüklük, şekil ve morfolojideki günümüz otuz üzüm varyetesi ile karşılaştırılmış ve modern varyete *Vitis vinifera*, fosil tohumlardan birine morfolojik açıdan en yakını olarak tespit edilmiştir.

Anahtar kelimeler : Arkeolojik kazılar, Ortaçağ , SEM, fosil tohumlar, *Vitis*

Summary

Grape (*Vitis vinifera*) seed remains were excavated at Roman and Medieval archeological 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 (Fenékpuszt) of the Roman Age (5th CENT. A.D., Hungary); and Győr (Ece; 11-12th CENT. A.D., Hungary), Debrecen (13th CENT. A.D., Hungary) and the King's Palace of 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 (Szabó *et al.* 2007). 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.

Key words: Ancient grapes, SEM, *Vitis*

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1. INTRODUCTION

Species of the plant family *Vitaceae* are woody climbers comprising 13 – 17 genera (*Acareosperma*; *Ampelocissus*, *Ampelopsis* - pepper-vine; *Cayratia*; *Cissus* - treebine; *Clematicissus*; *Cyphostemma*; *Leea*; *Muscadinia*; *Nothocissus*; *Parthenocissus*; *Pterisanthes*; *Pterocissus*; *Rhoicissus*; *Tetrastigma*; *Vitis* - grape; and *Yua*) and about 700 species (Arnold *et al.* 2002; Chen and Manchester 2007). The genus *Vitis* consists of about 60 inter-fertile species including about fifteen species of agronomic importance (Table 1). Of them, *V. vinifera* (2n = 4x = 38; 0.475 x 10⁹ bp) is the only species which is indigenous to Eurasia, with a relatively small nuclear (nuDNA) genome size of 0.475 - 0.5 x 10⁹ DNA base pair

(bp); a 160,928 bp of chloroplast cpDNA (Jansen *et al.* 2006) and a regular size of higher plant mtDNA (1 - 400,000 bp) (http://megasun.bch.umontreal.ca/ogmp/projects/other/all_list.html). Most genera of the family *Vitaceae* have $2n = 38$ chromosomes ($n = 19$), species of *Muscadinia*, *Ampelocissus*, *Parthenocissus*, and *Ampelopsis* have $2n = 40$ chromosomes ($n = 20$), and species of the genus *Cissus* have $2n = 24$ chromosomes ($n = 12$).

Changes in seed ('pip') shape (wild grapes have rounder pips with short beaks, while seeds of cultivated grapes tend to be more elongated with longer beaks) indicate 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 or southern Transcaucasia (Armenia and Georgia) (Zohary and Hopf 2000).

The wild, dioecious ancestor form of *V. vinifera* ssp. *silvestris* (syn.: *V. silvestris*) still co-exists with the cultivated, hermaphroditic flower form of *V. vinifera* ssp. *vinifera* (syn. *V. vinifera*) in Eurasia and North Africa (This *et al.* 2006; Arnold *et al.* 2005). 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 grapes is encoded by a single gene; female individuals are homogametic carrying homozygous recessive pistil-suppressor alleles ($Su^m Su^m$) which suppress the development of anthers (and pollen). Male plants are heterozygous ($Su^F Su^m$) carrying a dominant pistil-suppressing Su^F allele.

The shift, under domestication, to bisexual (hermaphroditism) flowering was attained by a single mutation to Su^+ which is also dominant over Su^m resulting in two genotypes of hermaphroditic grape types ($Su^+ Su^m$ and $Su^+ Su^+$) (Zohary and Hopf 2000; McGovern 2004).

European grapes formed hybrids with native *Vitis* species growing in North America. Some of these hybrids became resistant to *Phylloxera* (an insect pest), which devastated European vineyards in the 1880s, and supplied resistant rootstocks for replanting. This event indicates that the grape genome diversity has been narrowed twice; first by the Biblical flood, followed by the replanting by Noah 'the first vintner' (Genesis 9) on Mount Ararat (McGovern 2004), and second by *Phylloxera* (This *et al.* 2002). Unlike the genome for dioecious *V. sylvestris*, the genetic diversity of grapes has been narrowing continuously as the result of vegetative propagation either by rooting of twigs, or by grafting.

In the study presented here, grape seeds excavated from the Roman Age and Medieval time were analyzed and compared to thirty current grape varieties.

2. MATERIALS AND METHODS

Excavated and wet-sieved sediment samples were processed by floatation followed by seed sorting and identification in the laboratory according to Gyulai *et al.* (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. They were then desiccated in an acetone concentration series (10-50-70-90-100 %), dehydrated at the CO₂ critical point (Blazers CDC 020), and covered with gold (30 nm). Samples were examined and photographed using a TESLA BS-300 scanning electron microscope (Fig. 2) as described by Gyulai *et al.* (1992). For LM analysis (Greguss 1967), a Leica microscope (# 301-371.010) was used (Satkhov *et al.* 2007). Seeds of thirty current *Vitis* (Fig. 3) were used for comparative analyses (Chen and Manchester 2007; Hardie *et al.* 1996; Schermann 1966; Mangafa and Kotsakis 1996; Facsar 1970).

3. RESULTS AND DISCUSSION

The oldest wild grape (*Vitis sylvestris*) seeds (about 3 mm long) were excavated in Turkey at Nevalı 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 (8,400 B.P.) (Hauptman 1997; Pasternak 2008). The first convincing evidence of seeds of *Vitis vinifera* with indications of grape cultivation were also uncovered in Turkey at Kurban Höyük (5.700 – 5.200 B.P. non-calibrated radiocarbon time) (Zohary 2000; McGovern 2004), and the early Bronze Age (3,200 – 1900 B.P.) sites 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. (Zeist 1983)

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. (McGovern 2004). Later, Greek, Latin, and Egyptian amphoras with gelified wine remains were also found in the hulls of shunken ships (McGown 2004), similar to the famous shipwreck remains at Uluburun near Kas (Turkey) (Ward 2003). 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 the German tribes. Viticulture also spread eastward along the Silk Road and it reached China and Japan in 3,200 B.P. (Rivera and Walker 1989).

Grapes were introduced to the Americas by European colonists starting from the 16th CENT. after either the early Chinese explorer Zheng He (1405 – 1435), or the 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 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, he developed over half a million acres in California to viticulture, making wine growing second only 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).

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.; Hungary), which dates the origins of grape cultivations to the late Bronze Age (Table 2, Fig. 1).

The earliest wine residue dates back to 700 B.C. (at Fehérvárcsurgó, Hungary), which places the beginnings of wine making to the Iron Age (Szabó *et al.* 2007). Thus, there is evidence that both grape cultivation and wine making date to well before to the Roman period in Hungary. Seeds of *Vitaceae* are easily identified from a suite of unique and distinctive morphological characters (particularly a pair of ventral infolds and a dorsal chalazal scar) (Chen and Manchester 2007).

Ancient grape seeds in the study presented here 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 with obviously 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 samples from the 11th - 12th CENT. (#3 Fig. 2) showed incomparably unique morphology. 8

Ancient DNA (aDNA) was 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).

Acknowledgement: The author thanks Prof. L Kovács (Missouri, USA) for their comments on this manuscript, and Claudine Jenda (Auburn, AL USA) and for Richard Lágler (PhD Students) for the assistance of the manuscript preparation. The project was supported by grants of EU SOCRATES Student Exchange Program.

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Table 1. The most important species (1-27), hybrids (1-9) and NCBI (Altschul *et al.* 1997) gene bank (NCBI) samples (1-12) of the genus *Vitis*.

| <i>Vitis</i> species | <i>Vitis</i> hybrids* | <i>Vitis</i> gene bank samples |
|---|--|----------------------------------|
| 1. <i>V. acerifolia</i> | 1. <i>V. arizonica</i> x <i>V. rupestris</i> | 1. <i>Vitis</i> sp. |
| 2. <i>V. aestivalis</i> | 2. <i>V. berlandieri</i> x <i>V. riparia</i> | 2. <i>Vitis</i> sp. 196-17 |
| 3. <i>V. amurensis</i> | 3. <i>V. berlandieri</i> x <i>V. rupestris</i> | 3. <i>Vitis</i> sp. 216-N |
| 4. <i>V. arizonica</i> | 4. <i>V. berlandieri</i> x <i>V. vinifera</i> | 4. <i>Vitis</i> sp. 44-53M |
| 5. <i>V. bashanica</i> | 5. <i>V. cinerea</i> x <i>V. riparia</i> | 5. <i>Vitis</i> sp. 8007 |
| 6. <i>V. berlandieri</i> | 6. <i>V. cinerea</i> x <i>V. rupestris</i> | 6. <i>Vitis</i> sp. 8658 |
| 7. <i>V. betulifolia</i> | 7. <i>V. labrusca</i> x <i>V. vinifera</i> | 7. <i>Vitis</i> sp. cv. 'Norton' |
| 8. <i>V. bryoniifolia</i> | 8. <i>V. pseudoreticulata</i> x <i>V. vinifera</i> | 8. <i>Vitis</i> sp. CWD 96.701 |
| 9. <i>V. cinerea</i> (downy grape) | 9. <i>V. riparia</i> x <i>V. rupestris</i> | 9. <i>Vitis</i> sp. Nie 372 |
| 10. <i>V. davidii</i> | | 10. <i>Vitis</i> sp. Nie 415 |
| 11. <i>V. flexuosa</i> | * Interspecific hybrids registered in Hungary (2006): | 11. <i>Vitis</i> sp. NL-2003 |
| 12. <i>V. heyneana</i> | | 12. <i>Vitis</i> sp. Qiu 94046 |
| 13. <i>V. kelungensis</i> | 'Bianka'; 'Csillám'; | |
| 14. <i>V. labrusca</i> (Concord grape) | 'Duna gyöngye'; 'Esther'; | |
| 15. <i>V. piasezkii</i> | 'Fanny'; 'Göcseji zamatos'; | |
| 16. <i>V. popenoei</i> (totoloch grape) | 'Kunleány'; 'Medina; | |
| 17. <i>V. pseudoreticulata</i> | 'Nero'; 'Odysseus'; | |
| 18. <i>V. quinqueangularis</i> | 'Orpheus'; 'Platina'; | |
| 19. <i>V. riparia</i> (riverbank grape) | 'Pannon frankos'; | |
| 20. <i>V. rotundifolia</i> (muscadine), 2n=40 | 'Pölöskei muskotály'; 'Refrén'; | |
| 21. <i>V. rupestris</i> (rock grape) | 'Taurus'; 'Teréz'; | |
| 22. <i>V. shuttleworthii</i> (callose grape) | 'Viktória gyöngye'; 'Zalagyöngye' | |
| 23. <i>V. sinocinerea</i> | | |
| 24. <i>V. thunbergii</i> | | |
| 25. <i>V. tiliifolia</i> | | |
| 26. <i>V. vinifera</i> (wine grape) | | |
| 27. <i>V. yeshanensis</i> | | |

Table 2. Grapes (*V. sylvestris*, *V. vinifera* and *V. sp.*) and wine remains (pieces #) excavated in Hungary (1-63). (^Ccarbonized seeds; ^Fgrape fragments; ^Ppetrified seeds; i - imprints; L - leather wine holder; W - wine residues); (x: number of pieces 1-10) (Gyulai F. *et al.* 1992). (Excavation sites studied are indicated in bold)

| Ages | Excavation sites in Hungary | <i>Vitis vinifera</i> | <i>Vitis sylvestris</i> | <i>Vitis sp.</i> | |
|--|---|---------------------------|-------------------------|--------------------|------------------|
| 5,300 - 4,700 B.C. (Middle Neolithic) | 1. Magyaratád | | | 10 ^C | |
| | 2. Tiszapolgár-Csőszhalom | | 1 ^C | | |
| 4,700 - 4,300 B.C. (Late Neolithic) | 3. Szombathely-Sé | | 1 ^C | | |
| 3,500 - 3,000 B.C. (Late Copper Age) | 4. Kompolt-Kistértanya | | 18 ^C | | |
| | 5. Békés-Várdomb | | | 10 ^C | |
| 1,300 - 900 B.C. (Late Bronze Age) | 6. Dunakeszi-Székesdűlő | | 2 | | |
| | 7. Ludas, Varjú-dűlő | | 2 ^C | | |
| | 8. Mosonmagyaróvár-Németdűlő | | 3 | | |
| 900 - 500 B.C. (Early Iron Age; Hallstatt) | 9. Sopron-Krautacker 1 | 1 | | | |
| | 10. Fehérvárcsurgó-Eresztvény | W | | | |
| | 11. Sopron-Krautacker3 | 2 | 1 | | |
| | 12. Sopron-Krautacker6 | 9 | | | |
| | 13. Zagersdorf | 3 | | | |
| 5 th - 1 st CENT. A.D. (Late Iron Age; Celtic Age) | 14. Budapest-Corvin tér | | | 1 ^C | |
| | 15. Budapest (Aquincum), Kaszásdűlő | 6,108 | | | |
| 1 st - mid 5 th CENT. A.D. (Roman age) | 16. Budapest (Kunigunda str) | W | | | |
| | 17. Budapest (Bécsi str 69-71) | | | i | |
| | 18. Budapest (Vörösvári str 20-22) | | | 13; 1 ^C | |
| | 19. Dunaújváros (<i>Intercisa</i>) | W | | | |
| | 20. Keszthely-Fenekpuszta | 33 ^C | | 1 ^C | |
| | 21. Budapest (Bécsi str 44) | 3 | | | |
| | 22. Szekszárd | W | | | |
| | 23. Tác-Fövenypuszta (<i>Gorsium</i>) | 3 | | x | |
| | 24. Budapest (XIV. reg. Paskál park) | 1 ^C | | | |
| | 25. Gyomaendrőd (Endrőd 170) | | 1 ^C | | |
| 1 st - mid 5 th CENT. A.D. (Barbaricum) | 26. Kiskundorozsma-Nagyszék | | 1 ^C | | |
| | 27. Keszthely-Fenekpuszta | 2 ^C | | | |
| | 28. Budakalász | L | | | |
| | 29. Főnyed-Szegerdő | | | 10 ^C | |
| 6 th - 9 th CENT. A.D. (Late Migration periode; Avarian Age) | 30. Fonyód-Bélatelep | 255 | | | |
| | 31. Zalavár-Vársziget parkoló | 127 | | | |
| | 32. Győr (Ece) | 28 ^P | | | |
| 895 A.D. - 1301 A.D. (Hungarian conquest - Árpád Age) | 33. Gyomaendrőd (Endrőd 170) | | 1 ^C | | |
| | 34. Rákoskeresztúr | | 3 | | |
| 1301 - 15 th CENT. A.D. (Late Medieval; Hungarian Kingdom) | 35. Debrecen (Kölcsey Cultural Centre) | 24 | | | |
| | 36. Budapest (St György sq, Teleki Palace) | 210686 | | | |
| | 37. Budapest (Kapucinusok sq 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 | | | |
| | 47. Visegrád (Mathias Palace) | 52 | | | |
| | 48. Budapest (Medve str 13) | 4954 | | | |
| | 16 th - 17 th CENT. A.D. (Late Medieval; Hungarian Kingdom to Turkish occupation) | 49. Hollókő-Castle | 47 ^C | | 150 ^C |
| | | 50. Kereki-Fehérkő Castel | x | | |
| 51. Külsővat | | 2 ^P | | | |
| 52. Lászlófalva-Szentkirály | | 5 | | | |
| 53. Nagyvázsony-Csepely | | 65 ^C | | 12 ^C | |
| 54. Pápa (Deák Ferenc str) | | x | | | |
| 55. Pápa (Hantai str) | | 12 | | | |
| 56. Pécs (Med School) | | 22 ^C | | | |
| 57. Sopron (L str 7) | | | | 8 | |
| 58. Sopron (Templom str 14) | | | | 10 ^C | |
| 59. Sümeg (Castle) | xx | | | | |
| 60. Szarvasgede | x ^C | | | | |
| 61. Szécsény-Plébániatemplom | 693; 42 ^F | | | | |
| 62. Székesfehérvár (Palotai str 5) | 15 ^C | | | | |
| 63. Vác (Széchenyi str 3-7) | 44349 ^P | | | | |

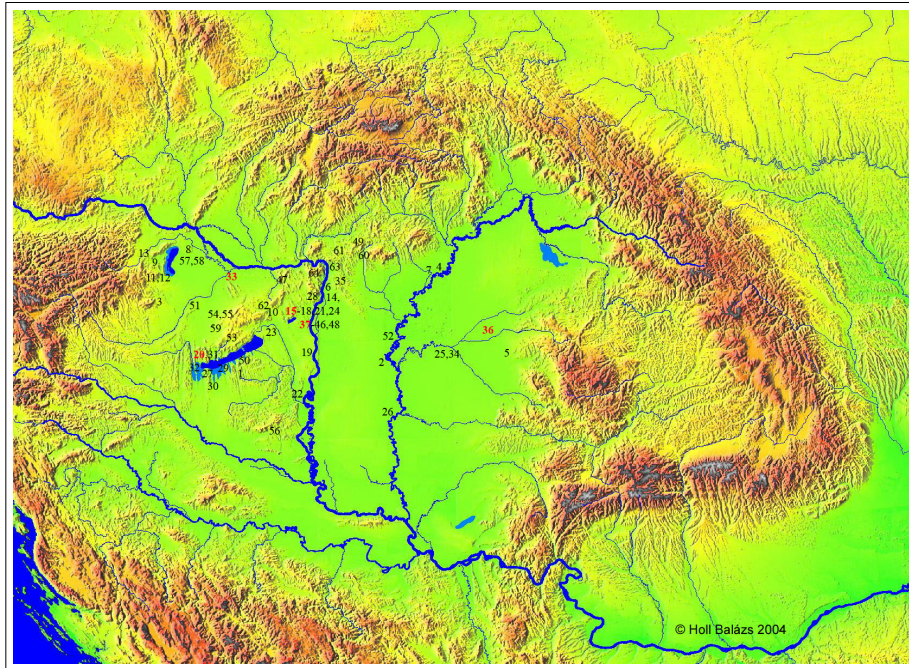


Figure 1. Archaeological sites of Hungary where *Vitis* seeds were excavated (listed in Table 2.)

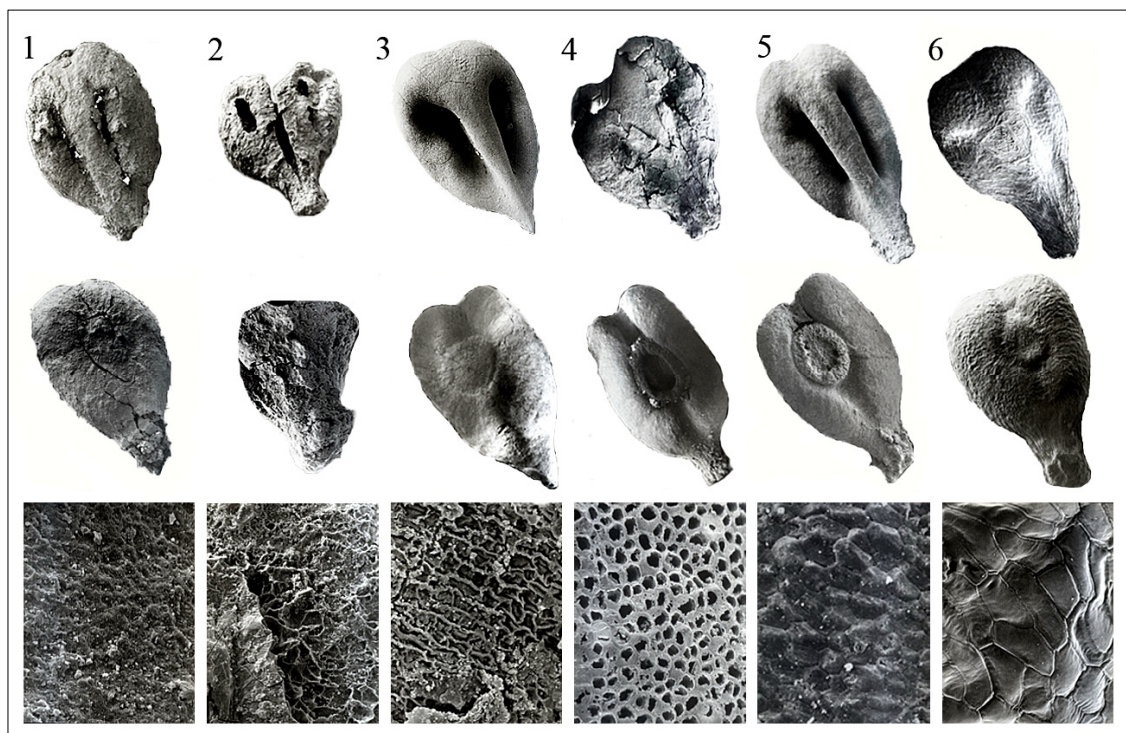


Figure 2. Morphology of ancient *Vitis* seeds excavated in Hungary. SEM micrographs of seeds excavated at a Roman Villa in Budapest (*Aquincum*, Hungary) (2nd - 4th CENT., A.D.) (1); and Keszthely (5th CENT. A.D., Fenékpusztá, Hungary) (2); a vineyard site near Győr (Ece, Hungary) (11-12th CENT.) (3); Debrecen (Hungary) (13th CENT.) (4); and at the King's Palace of Árpád Dynasty 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 20X magnification. The bottom row shows seed coat textures at 500x magnification (SEM processed by G Gyulai).

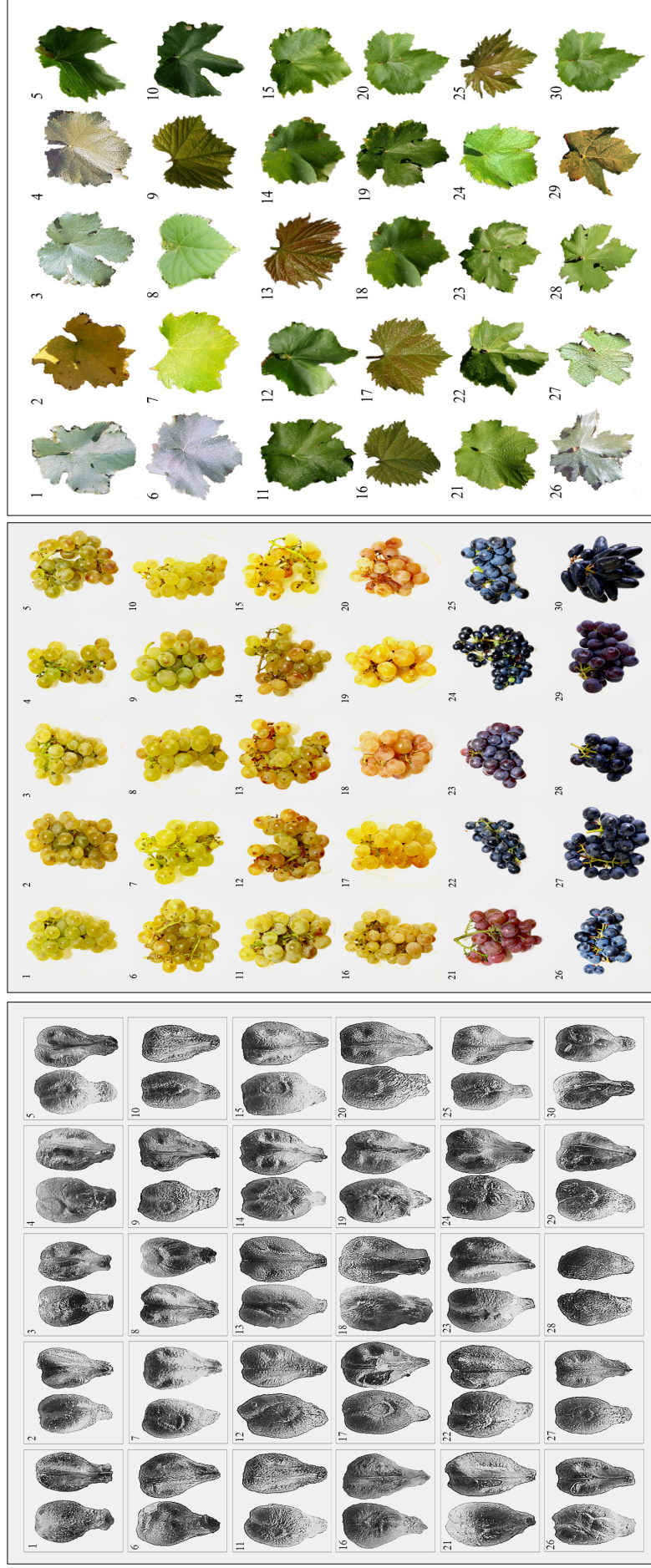
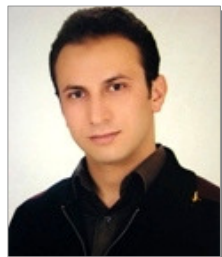


Figure 3. Seed, grape and leaf morphology of current grape cultivars (*Vitis vinifera*, but #8 and #21) grown in Hungary used for comparative analysis (from the collection of Keszthely University, Hungary).

1. 'Rajnai rizling' NI-378; 2. 'Leányka'; 3. 'Zöldsziváni'; 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. vinifera* 'Aramon' x *V. riparia* I43 B'; 22. 'Bakó'; 23. 'Visnivi rami';
 24. 'Kékfrankos'; 25. 'Kék bakator'; 26. 'Opotó'; 27. 'Sziürkebarát D 34'; 28. 'Kismis vatkana'; 29. 'Piros szlanka';
 30. 'Süvenir'.

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