GRAPES (*Vitis vinifera* ssp. L.) FROM TWO ARCHAEOLOGICAL SITES IN SLOVENIA: WILD OR CULTIVATED? – TESTING WITH A DNA METHOD

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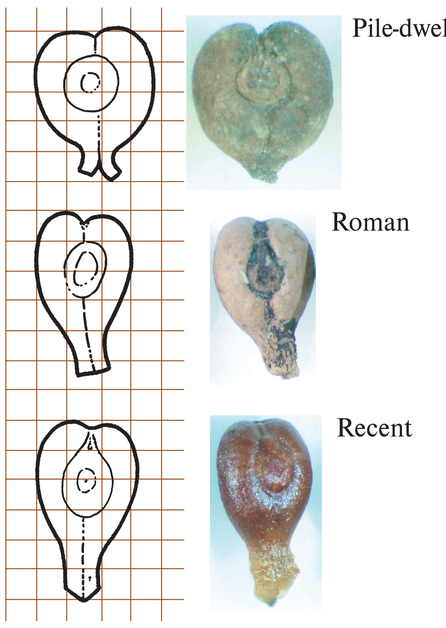
Introduction

The oldest seeds of grape (*Vitis vinifera ssp. L.*) from Slovenian archaeological sites were found at the Eneolithic pile-dwelling settlement Hočevarica at Ljubljansko barje, Slovenia, dated to the 36th century B. C. (Jeraj 2004).

Different biometric studies on grape seeds from Hočevarica, and from a Roman settlement in Vrhnika, Slovenia, dated to the 1st century A. D. (Horvat and Mušič 2006), showed that the morphologic characteristics of the seeds from the Roman site are different from the Pile-dwelling seeds and from the Recent cultivated seeds (Korenčič, Korošec-Koruza and Velušček in press). The range of Stummer index (Breadth/Length) for the Roman grapes is somewhere between the Pile-dwelling and the Recent grapes (*fig. 1*).



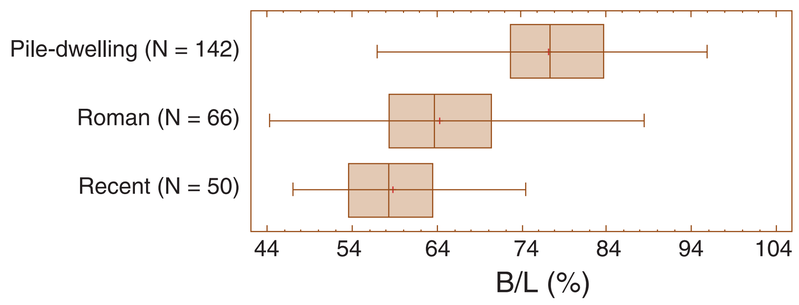
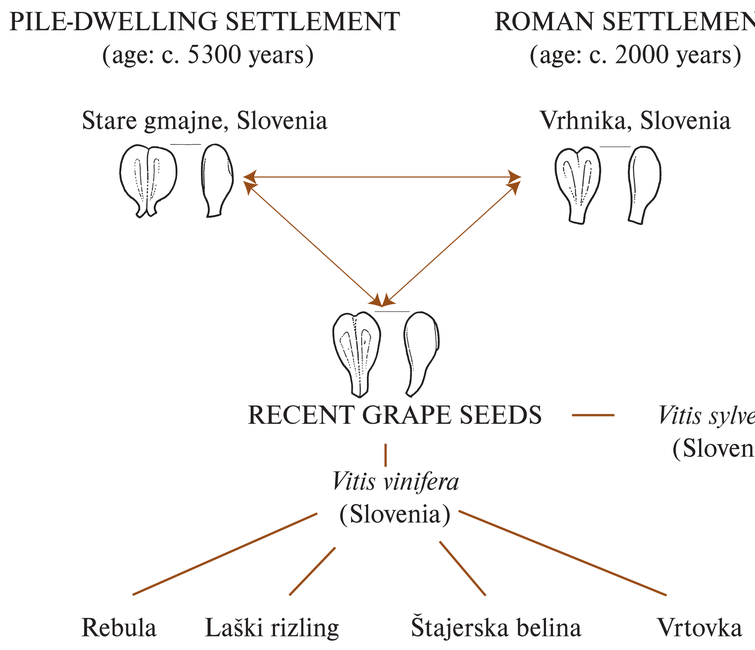


Fig. 1: Stummer index (Breadth/Length) for 3 research groups of grape seeds.

As morphological identification of subspecies (wild – morphologically rounded or cultivated – morphologically pyriform) is questionable (Korenčič, Korošec-Koruza, Velušček in press), we completed the classical ampelo-morphological methods and initiated molecular (DNA) methods to help answer these questions.

Material



Methods

DNA Extraction

Several precautions have been performed during DNA isolation to prevent contamination of samples with any "in-lab" DNA contamination. As negative sample, isolation without any plant material have been performed in the same way as for seed samples. Classical CTAB extraction has been performed for DNA isolation from grape seeds, followed by silica-milk suspension cleaning. Briefly, 3 seeds have been soaked in pre-warmed CTAB buffer (68 ° C) [2 % CTAB, 100 mM Tric-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 0.2% b-mercaptoethanol] and crushed to a fine powder, incubated for up to 2 hours at 68 ° C and extracted two times by mixture of chloroform : isoamylalcohol (24:1). DNA was precipitated by isopropanol, washed in 70 % ethanol and dissolved in 60 µl of TE buffer [10 mM Tris-HCl, 1 mM EDTA, pH 8.0]. Dissolved "DNA" was cleaned by silica-milk suspension according to manufacturer's recommendations (Fermentas). DNA was eluted in final volume of 50 µl. The presence of isolated DNA have been checked by 0.8% gel electrophoresis.

PCR amplification

Amplification of two ***loci*** have been employed to test if any DNA of amplifiable quality has been isolated:
1. ITS region as representative of genomic DNA with ITS4 (`TCCTCCGCTTATTTGATATGC`) and ITS5 (`GGAAGTAAAAGTCGTAACAAGG`) primer pair and
2. trnL-F region with C (`ATTTGAACTGGTGACACGAG`) and F (`CGAAATCGGTAGACGCTACG`) primer pair as representative of chloroplast DNA (cpDNA).
The PCR consisted of following components: 5 µl of silica-eluted grape DNA, 1x PCR buffer, 1.5 mM MgCl2 (ITS), 2.5 mM MgCl2 (cpDNA), 0.2 mM of each dNTP, 0.5 µM of each region specific primer. PCR reaction was carried out for 30 cycles at 61°C of annealing temperature and results were checked by 1.3% gel electrophoresis.

Results and discussion

Initial tests for suitability of archaeological seed grapes for DNA isolation has been conducted. Classical CTAB isolation, suitable for isolation from vast variety of plant tissues, have been used. For archaeological seeds, colored (brownish) samples have been obtained, while for present seeds high amount of starch co-precipitated with DNA. Glass-milk cleaning procedure successfully removed coloration and starch. Electrophoresis confirmed presence of genomic DNA in recent samples, while for Roman samples high amount of smeared signal was noted, although probably it was not due to the degraded DNA (data not shown). First attempt showed that cpDNA can be successfully amplified from recent seed samples, while for archeological seeds no amplification was obtained (*fig. 2*). Amplification of ITS region was unsuccessful for all samples and need further optimization (data not shown).

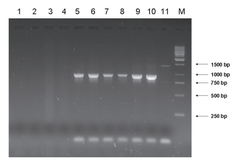


Fig. 2: Amplification of cpDNA region revealed expected fragment in present grapes (5. Štajerska belina, 6. Rebula, 7. Laški rizling, 8. Vrtovka, 9. and 10. reference DNA of Vitis vinifera cultivated), while no amplification was observed in archeological samples (1. Pile-dwelling rounded, 2. Pile-dwelling pyriform, 3. Roman rounded, 4. Roman pyriform).

Conclusions

Several reports are available, were DNA has been successfully isolated from archeological samples of vinegrape seeds (Manen et al. 2003). Some modifications as use of charcoal or PVP to obtain better DNA are currently taking place.

Beside the amplification of ITS and cpDNA, microsatellite markers and sequencing of these regions will be further used to help answer questions of archeological grapes.

Acknowledgement

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