Examinations of 600-year-old seeds by means of archaeobotanical and genetical methods

Bacsó R.[1], Facsar G.[2], Gyulai F.[3], Bisztray Gy.D.[1] and Velich I.[1],

[1]*Corvinus University of Budapest, Faculty of Horticultural Sciences, Department of Genetics and Horticultural Plant Breeding, Budapest, Hungary*
[2]*Corvinus University of Budapest, Faculty of Horticultural Sciences, Department of Botany, Budapest, Hungary*
[3]*Agrobotanical Institute, Tápiószele, Hungary*

Summary: About 600-year-old plant seeds were discovered in a well of a mediaeval cellar in the course of an excavation in Budapest. After the archaeobotanic purification seed of 16 species were found in large quantities. Seeds preserved in the best state were selected from each group. The existence of endosperm was analysed in these subfossils, which turned to be successful mainly in the case of grapes (*Vitis vinifera*) and cornels (*Cornus mas*). Seeds of these two species contained the most endosperm and remains of the embryo. DNA was extracted with the help of DNEasy Plant Mini Kit and analysed by RAPD-PCR method. The amplification of DNA extracted from cornel seeds resulted in detecting a 1500 bp fragment, which makes the comparison of these samples with present-day cornels possible.

Key words: mediaeval seeds, fruits, cornel, grape, RAPD-PCR

Introduction

The study of a DNA sequence from remains was presented for the first time in 1984 (*Higuchi*, 1984). It was the analysing of an extinct equid, to attempt the extraction of DNA. One year later Svante Pääbo discovered DNA fragments in a mummy, that seemed to be well-preserved (*Pääbo* 1985). In 1985. Rollo could amplify short fragments for 3300 years old cress seeds. Nowadays, the research workers are able to analyse many remains, in Switzerland, *J.-F. Manen* works with archaeological grape seeds. He analysed them with microsatellite markers (*Manen*, 2003).

In Hungary, *Heszky* et al. analysed 700-year-old sediments (2001), and studied them with Cluster and MDS analysis.

We examined 600-year-old remains that were discovered in a mediaeval well with genetical and archaeological methods to explore their nature.

Our target was to determine the species and subspecies of these seeds. We wanted to extract some intact DNA fragments, amplify and detect them. Our final goal was to compare these remains with current species and subspecies. With the help of our examinations, we might offer essential proof of the cultivated and collected plants of mediaeval Hungary.

At the same time these genotypes might contain several genes that are missing from present-day species, thus by reconstructing the mediaeval genotypes and by selecting these genes from the current gene banks we may support plant breeding strategies.

Material and methods

These subfossils were first cleaned with high-pressure water wash, then we started to separate the species into glass dishes (*Gyulai*, 2001).

We took 50 samples from the remains, and sterilized them with alcohol and flame to kill the microbes. The endosperm of the cornel (*Cornus mas*) and grape (*Vitis vinifera* ssp. *vinifera*) seeds seemed to be in good condition, so we started to examine these species.

First, we cut them up in the sterile box with sterile tools, and scraped out the endosperm into a sterile Eppendorf tube.

To avoid the contaminations we extracted the DNA another day from the remains and also from current seeds. We used the Qiagen's DNEasy Plant System Mini Kit for the

Table 1. The primers used.

Primer	The name of the plant
OPA-12	*Vitis vinifera* ssp. *vinifera*
OPA-05	*Cornus mas*
OPC-05	*Cornus mas*
OPC-06	*Cornus mas*
Primer combinations	
OPA-03 + OPO-14	*Cornus mas*
OPO-17 + OPO-14	*Cornus mas*
OPB-20 + OPO-14	*Cornus mas*
OPO-18 + OPO-14	*Cornus mas*
OPO-10 + OPO-14	*Cornus mas*
OPO-10 + OPAU-10	*Cornus mas*

DNA extraction (*Qiagen*, 2000), than we amplified the DNA with RAPD-PCR method (*Deák*, et al., 2003). For the primers used see *Table1*.

Results and discussion

We were able to identify 16 species, out of 66,500 seeds. In the first sample we found 33,463 pieces of seeds, this sample was the richest in seeds. We examined 3 different samples of the seeds found in the well. Components of the first sample are shown in ***Figure 1***. We examined these remains with morphological methods and we found 2 different subspecies of grape seeds with the help of Facsar's works (*Facsar* 1970, 1972, 1975).

Table 2. Components of the first sample

The name of plant	*Quantity (piece)*	*Weight (g)*	*g/100 piece*
Vitis vinifera ssp. *vinifera*	30 057	901.7	3333.37
Prunus persica	6 fragment	4.9	
Citrullus lanatus	584	52.6	1110.27
Cucumis sp.	1 231	35.1	3507.12
Juglans regia	29 fragment	3.5	
Cerasus avium/vulgaris	593	130.6	454.06
Prunus domestica	75+9 fragment	34.8	
Cornus mas	149	46.7	31.34
Prunus amygdalus	12	5.7	210.53
Corylus avellana	2 fragment		
Mespilus germanica	74	9	822.22
Prunus spinosa	16	1.6	1066.67
Setaria viridis/verticillata	1		
Malus/Pyrus	193 whole and fragment	5.6	2.9
Morus nigra	671	4.7	14276.6
Summary	33 463	1 651.00	

We were able to amplify large DNA fragments from the archaeological cornels and with the simultaneous use of two primers (OPO 10 + OPO 14), we amplified a fragment that was as long as one fragment of the current species.

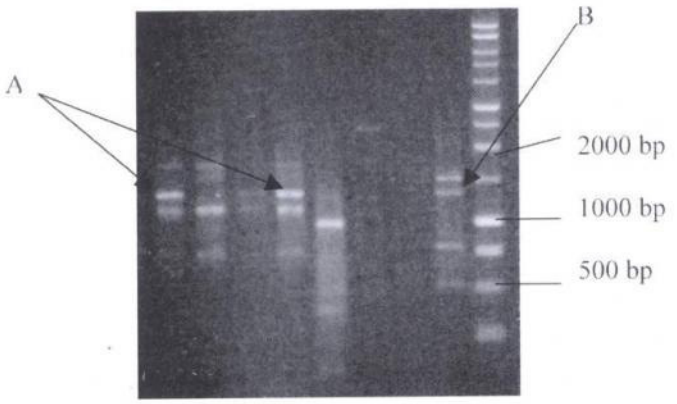

Figure 1. We separated the DNA fragments on agarose gel and dyed them with ethidium bromide

Among the grape seeds, we found two different subspecies that were similar to present-day Hungarian subspecies.

We started to compare the DNA fragments of these seeds with each other, and there were many similar and some different fragments *(Figure 1., A & B arrow)*. The arrow B shows fragments of mediaeval cornels.

Conclusions

We were able to classify 66,500 pieces of seeds into 16 species and 2 different subspecies of grape seeds using morphological methods. Later we could extract and amplify the DNA from these seeds, and found them in good condition.

In case of some samples the quantity and quality of the extracted DNA make the comparison of mediaeval and present-day species possible by means of molecular markers. We could amplify current cornel DNA fragments the size of which correspond to the fragments of 600 years old cornels. Further on, we would like to compare these species with the help of the hybridization and sequence analyses of similar fragments.

These examinations make the comparing analyses easier in the fields of both DNA-degradation and comparison of early and current species.

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