



# A study on the diversity of natural *Arbutus unedo* Hellenic populations



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## Background

The strawberry tree (*Arbutus unedo*) is well known for the use of its leaves, fruits, bark and roots in traditional medicine and more recently in the therapy of hypertension, diabetes, inflammatory and cardiovascular diseases. The plant contains several antioxidant compounds.

## Materials & Methods

The diversity of natural *A. unedo* populations from Greece was studied with leaf morphometrics and DNA markers. Five natural populations spanning from east (Lesvos island 39°12' N, 26°05' A) to west (Igoumenitsa 39°30' N, 20°15' E), and from north (Arnea, 40°29' N, 23°38' E) to south (Ancient Olympia 37°38' N, 21°46' E), were sampled. The fifth population was that of Kassandreia (40°01' N, 23°26' E), and the average sample size per population was N=20 trees. Morphometric population variation was studied by employing 11 leaf size and shape parameters recorded by image processing. Three DNA extraction methods were evaluated: (a) CTAB [1], (b) Custom CTAB [2], (3) Nucleospin Plant II Mini commercial kit (Macherey – Nagel, Germany).

## Results (2/2)

Table 1. Quantity & Quality statistics of DNA extracted from four *A. unedo* populations using CTAB based methods or commercial kit.

Population	Mean DNA conc. (ng/μl)	Mean OD (260/280)	Mean OD (260/230)	Method	Sampling time
Igoumenitsa	59,290	1,480	0,806	KIT	October.
Arnea	23,330	1,765	0,080	CTAB*	March
Kassandreia	139,294	1,605	0,180	CTAB*	April
Lesvos	84,112	1,980	0,115	CTAB*	August

\* CTAB & Custom CTAB employed

## Results (1/2)

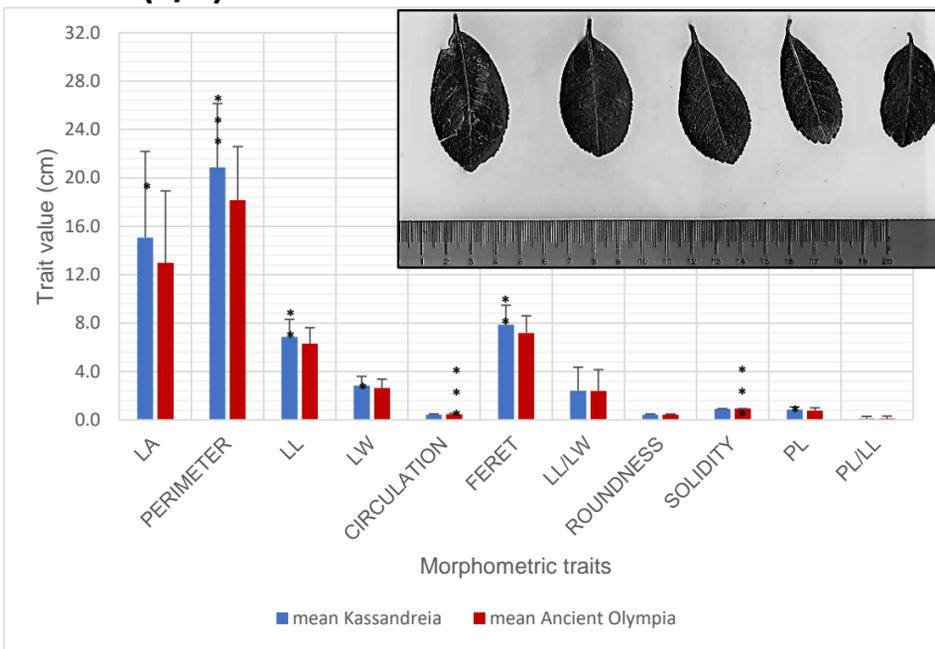


Fig. 1. Descriptive statistics of the populations studied in 11 leaf parameters and ratios of *Arbutus unedo* (LA, leaf area; PL, petiole length; LL, leaf length; LW, leaf width); \* notations indicate statistically significant population differences of t-test results (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001).

## Discussion

In contrasting north/south population comparisons it was found that regarding measurements of central tendency the northern population (Kassandreia) presented the highest values, while in contrast in the measures of spread the highest values were found in the southern population (Ancient Olympia). Furthermore, statistically significant population differences were found in leaf size, but not in leaf shape parameters. In agreement with this study, Lopes et al. [3] showed that Portuguese *A. unedo* genotypes presented high within and between population variation in leaf morphology.

DNA extraction and isolation was a challenge due to high amounts of phenolics present in leaves (arbutin, catechin, ethyl gallate), and among the protocols studied the NucleoSpin® Plant II Mini Kit provided the best results for downstream applications. Sampling time seems to also play a role in the amount of trace phenols post-extraction.

## Conclusions

The combination of DNA markers and morphometric analyses provides a foundation for diversity studies and characterization of *A. unedo* populations for downstream applications in population genetics studies, genetic conservation, as well as in medicinal and natural products research.

## Literature

- Doyle, J.J.; Doyle, J.L. A Rapid Dna Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochem Bull* 1987, 11–15.
- Sá, O.; Pereira, J.A.; Baptista, P. Optimization of DNA Extraction for RAPD and ISSR Analysis of *Arbutus Unedo* L. Leaves. *Int. J. Mol. Sci.* 2011, 12, 4156–4164.
- Lopes, L.; Sá, O.; Pereira, J.A.; Baptista, P. Genetic Diversity of Portuguese *Arbutus Unedo* L. Populations Using Leaf Traits and Molecular Markers: An Approach for Conservation Purposes. *Sci. Hortic.* 2012, 142, 57–67.