Diversity of leaf morphometric parameters in natural Greek populations of *Arbutus unedo*

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Abstract

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This paper investigates leaf morphology variation of the strawberry tree *(Arbutus unedo)* within and between two natural contrasting populations of significant latitudinal difference (Kassandreia, Chalkidiki and Ancient Olympia, Peloponnese). This study employed 11 leaf size and shape parameters, recorded by image processing and analyzing software. The results showed that in the measurements of central tendency (parameter means) the northern population of Kassandreia presented the highest values, while in contrast the highest values in the measurements of spread were found in the southern population of Ancient Olympia. Moreover, statistically significant differences between populations were detected in leaf size, but not in leaf shape parameters. Results are discussed in the context of their value in studying quantitative population differentiation and laying the basis of more advanced studies.

Keywords

contrasting sites, morphology, natural variation, strawberry tree

Introduction

The strawberry tree (*Arbutus unedo* L.) belongs to the Ericaceae family and grows on dry slopes with well-drained and relatively acidic soils. Rich and deep, its root system takes advantage of soil moisture, while providing protection from soil erosion. Its height usually ranges from 2–3 m, but it can reach up to 12 meters (Korakis, 2015). It has a circum-Mediterranean distribution (Fig. 1, Caudullo et al., 2017; Oliveira et al., 2011), while it is also found in western France and Bulgaria with a northern limit reaching southwestern Ireland (Kelleher, 2013). The northern populations are remnants of a formerly wider distribution linked to earlier warmer and wetter periods.

Fig. 1. Natural distribution of *Arbutus unedo* (Caudullo et al., 2017).

Due to their availability and a wide range of morphological variation, leaf size and form have been a rich source of plant data since the classifications of Theophrastus. Leaf morphometric differences are due to the genetic control of morphogenesis, degree of heterozygosity, epigenetics, plastic responses to contrasting environmental conditions during development, normal developmental patterns (leaf heteroblasty), shoot types, etc. (Aravanopoulos, 2005; 2010). Leaves are the primary site for photosynthesis in terrestrial ecosystems, generating growth and underpinning ecosystem function. Leaves are multiple function plant organs and their interplay with different environmental pressures and conditions has resulted in a great morphological diversity arising from

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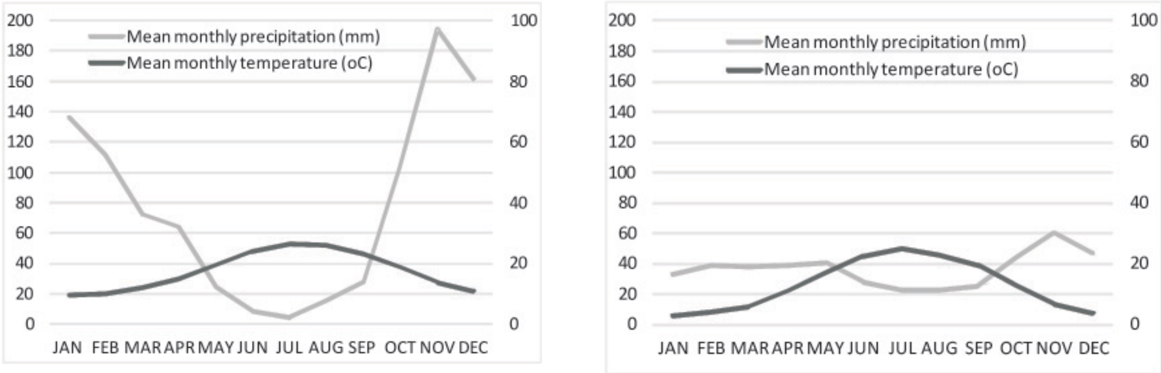

Fig. 2. Walter Lieth (ombrothermic) diagrams from the vicinity of Ancient Olympia (Pyrgos Weather Station; left) and Kassandreia (Mikra Weather Station; right) (HNMS, 2022).

phylogenetic and adaptive processes over evolutionary time (Benavides et al., 2021; Nicotra et al., 2011).

Morphological characters usually exhibit a greater degree of inter-population differentiation than genetic markers (Hamrick, 1983) and it has been suggested that different evolutionary forces may be acting upon these different sets of characters (Mitton, 1978). Population leaf variation within species may reveal both plastic and genetic responses to environmental gradients, and thus indicate where local adaptation has occurred (Leimu and Fischer, 2008; Benavides et al., 2021). Therefore, studies of population relationships and population differentiation (Dickinson, 1986; Aravanopoulos, 2005; Kourmpetis and Aravanopoulos, 2010), or hybridization (Neophytou et al., 2007) via morphometric analysis, form an important component in the study of any species.

The aim of this study was to analyze leaf morphometric variation in two *Arbutus unedo* populations from contrasting regions (Ancient Olympia, Peloponnese and Kassandra, Chalkidiki, Greece) and to test the null hypothesis (H_O) of the absence of population differences in leaf morphometric (size and shape) parameters. This is the first pertinent study of this species using populations from Greece.

Materials and methods

Sampling areas

Arbutus unedo leaves were collected from Ancient Olympia Peloponnese (X: 37.63616, Y: 21.62500) at an average altitude of 245 m (N = 28 trees) and Kassandreia, Chalkidiki (X: 40.02293, Y: 23.43341), at an average altitude of 120 m (N = 18 trees). According to Walter Lieth diagrams from the two closest weather stations of the respective areas (Fig. 2), the former site presents a lengthier dry season (HNMS, 2022). Sampling took place from large natural populations. Fruiting trees were sampled at a minimum distance of 30 m to avoid sampling filial structures, with the restriction of at least one neighboring tree being present within 25 m, to avoid sampling inbred individuals. Leaves were stored in nylon bags and then treated as herbarium samples. From each tree, lateral branches covering its periphery were collected and from those five fully developed leaves with no defects were selected at random for measurements.

Fig. 3. Scanned leaves (population Ancient Olympia).

Image and data analysis

Each batch of five leaves was electronically scanned with a HP Photosmart C4485 All-in-one printer-scanner-copier (Fig. 3). Scanned leaves were analyzed using the *ImageJ* software/ Fiji version/ (Abrumoff et al., 2004; Ferreira and Rasband, 2012). This is one of the most commonly used open scientific image analysis software for such studies (Blazakis et al., 2017). ImageJ was used for segmentation (Meijering et al., 2009), while the leaf image analysis protocol followed Blazakis et al. (2017). The following leaf parameters were recorded selecting the measurement area: (1) leaf area (cm^2), (2) perimeter (cm), (3) leaf length (cm), (4) leaf width (cm), (5) circularity, (6) feret (cm), (7) leaf length/leaf width (aspect ratio), (8) roundness and (9) solidity. Moreover, (10) petiole length (cm) and the ratio (11) leaf length/petiole length were measured according to the software instructions (without use of automated commands). The measurements were made using the "straight line" option, having set the scale in a previous step, so that all measurements were consistent to each other. A total of four parameters (circularity, leaf length/leaf width, roundness and solidity) are considered as leaf shape parameters, while the rest as leaf size parameters. Data normality was investigated by using the Shapiro-Wilk test. In the absence of data normality, trimming of outlier values was used to achieve a normal distribution. Population differences were evaluated with t-tests. The SPSS statistical software (IBM, 2020) was used.

Results and discussion

Descriptive statistics

From each sampled tree, 11 leaf morphology parameters were estimated for each of the five leaves. In total, 230 leaves were studied, and 2,530 data points were recorded. The relevant descriptive statistics are presented in Fig. 4. Leaves are larger in the population of Kassandreia as this population presents higher values in the corresponding leaf parameters (Fig. 4). For population variation, both the standard deviation (SD) and especially the coefficient of variation (CV), show mostly medium levels of variation (10 < CV < 30; Fig. 4). Moreover, the population of Kassandreia can be regarded as showing greater phenotypic variability in leaf size-related parameters and lower for leaf shape-related parameters, compared to the population of Ancient Olympia, based on the values of the measures of spread observed (Fig. 4).

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Population comparisons

The populations appear to present significant differences in leaf trait parameters, as in the majority of the parameters (73%; Fig. 4) statistically significant differences were found. The Kassandreia population presents higher values in leaf size parameters in a statistically significant manner when compared to the Ancient Olympia population. This assertion does not extend to leaf shape parameters. Overall, the two populations differ significantly in leaf size, but not in leaf shape; the postulated HO is rejected for leaf size and cannot be rejected for leaf shape.

In agreement to this study, Lopes et al. (2012) showed that Portuguese A. unedo genotypes presented high within and between population variations in leaf morphology. Significant variation in quantitative traits (pomological characteristics) that allowed superior genotype selection was also evident in *A. unedo* in Turkey (Sulusoglu et al., 2011; Celikel et al., 2008). Similar results of significant natural population variation in leaf morphometrics was also found in other woody perennials, for instance in *Crataegus* sp. (Dickinson, 1986), *Castanea sativa* (Aravanopoulos, 2005), in *Juglans regia* (Kourmpetis and Aravanopoulos, 2010), in *Alnus sabcurdata* (Akbarian et al., 2011), in *Fagus sylvatica* (Hatziskakiet al., 2011; Mohebi Bijarpasi et al., 2019; Stojnic et al., 2016) in *Pinus brutia* (Christou and Aravanopoulos, 1997) and *Pinus strobus* (Rajora et al., 1991), and in the oaks *Quercus alnifolia* and *Q. coccifera* (Neophytou et al., 2006), *Q. petraea* and *Q. robur*, (Kleinschmit et al., 1995), as well as in *Q. gambelii* and *Q. grisea* (Williams et al., 2001).

Despite the strawberry tree's many biologically beneficial properties (Oliveira et al., 2011) (edible fruit usually processed and consumed as jam, drink or honey, use in folk and mainstream medicine), pertinent studies are scarce. This is the first study on strawberry tree leaf morphometrics in Greece using two natural populations that differ in altitude, latitude and therefore climate. Leaf size and shape variables are sensitive to climate perturbations (Peppe et al., 2011, Wright et al., 2017) while leaf morphological variation is a key indicator of plant response to climatic change (Li et al., 2020). Generally, leaf size changes over time are usually positively correlated with precipitation (Li et al., 2020). In the present case, notable

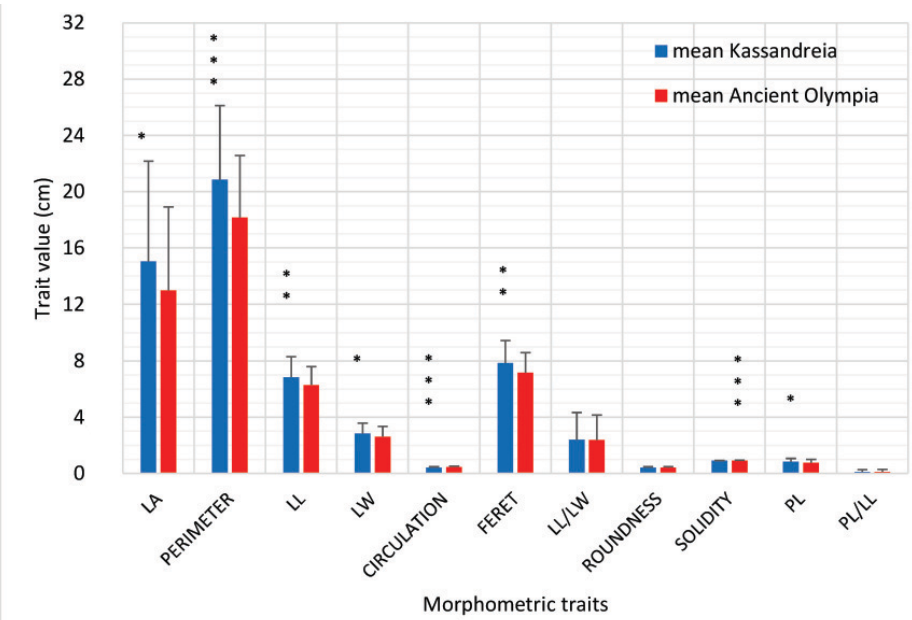

Fig. 3. Descriptive statistics for mean and coefficient of variation (CV), of the populations studied in 11 leaf parameters and ratios of Arbutus unedo (LA: leaf area, PL: petiole length, LL: leaf length, LW: leaf width; * notations indicate statistically significant population differences of t-test results (*: p<0.05, **: p<0.01, ***: p<0.001).

morphometric variation was found, while statistically significant differences were detected in leaf size (higher values in Kassandreia), but not in leaf shape parameters. Different environmental conditions and isolation by distance account for such findings. For instance, Ancient Olympia is characterized by a larger dry period than Kassandreia (Fig. 2), while during this period average precipitation in the former is about 4× less than in the latter. The detection of such phenotypic variation is promising and should trigger future studies for mapping both phenotypic and genetic variation of strawberry tree peripheral populations which may possess interesting gene complexes (Fady et al., 2016), with the long-term goal of artificial selection in a breeding program for pharmaceutically important traits such as arbutin.

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