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Variation of Morphological Traits in Natural Populations of *Arbutus unedo* L. (Ericaceae) in Morocco

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ABSTRACT

Arbutus unedo L. (Arbutus, Ericaceae) is a plant of economic, ecological and therapeutic importance. The present study aims to investigate the intra- and inter-population morphological variability of *Arbutus* in twelve natural populations in Morocco from different biogeographical regions, namely: the North West, Pre-Rif, Central Rif, Western Rif, Central Plateau, High and Middle Atlas. This study includes a biometric and morphological study of trees, leaves, fruits and seeds. The analysis of variance (ANOVA) of morphological traits revealed significant genetic variability within and between the natural populations studied, which belong to different geographic areas with varying bioclimate. Analyses showed high correlation between the traits. Principal component analysis (PCA) and hierarchical clustering using the UPGMA method separated these populations into two groups, independent of their geographical origin. This study showed that Moroccan *Arbutus* is a rich source of variation for economic characters. The morphological variability revealed during this study can provide a good basis for conservation and selection of efficient genotypes with the required traits.

Key words: *Arbutus unedo* L., Genetic diversity, Geographical origin, Morphological variability

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Introduction

Arbutus unedo L. (Arbutus, family Ericaceae) is a wild shrub of the Mediterranean region. The species has been used as a source of food and for therapeutic purposes since the Greek age (Font Quer, 2016). In recent years, these applications have led to an ever-increasing interest among scientists and even the general public. The berries of *A. unedo* contain significant amounts of phytochemicals, including polyphenols and trace minerals (Kivçak et al., 2001; Celikel et al., 2008; Oliveira et al., 2009; Ruiz-Rodríguez et al., 2011; Abbas, 2015; Boussalah et al., 2018; Faida et al., 2019). In folk medicine, the species is used for its antiseptic, diuretic and vascular laxative properties (Pallauf et al., 2008). Fruits are processed into jam, wine, distillates and liqueurs (Ayaz et al., 2000; Alarcao-Silva et al., 2001). *A. unedo* contributes to the maintenance of wildlife biodiversity, helping in stabilizing the soils by avoiding erosion, and has a high capacity for regeneration after fires and survives fairly well in poor soils (Mesléard & Lepart, 1989).

The increased demand for the use and consumption of Arbutus requires conservation and selection programs. The implementation of these programs is based on studies of genetic diversity within and between populations. Several studies on the diversity of the Arbutus have been carried out using diverse markers morphological (Celikel et al., 2008 ; Sá, 2011; Boussalah et al., 2018), biochemical (Kivçak et al., 2001; Ozcan & Hacıseferogullari, 2006; Oliveira et al., 2009; Ruiz-Rodríguez et al., 2011; Boussalah et al., 2018; Naceiri Mrabti, 2018), isozyme (Takrouni et al., 2012), and molecular (Takrouni & Boussaid, 2010; Sá et al., 2011; Lopes et al., 2012; Gomes et al., 2013; Ribeiro et al., 2017; Fazenda et al., 2019). In part, these authors revealed low to moderate levels of genetic diversity within populations, and a high degree of genetic differentiation among Arbutus populations in Portugal, Algeria and Tunisia. In Morocco, there has been one study of variation in biochemical markers (Naceiri Mrabti, 2018), but none of morphological or molecular. A few studies deal with variation in biochemical characters.

Morphological information is essential to make a first inventory in order to identify the most discriminating and informative characteristics for the description of the taxonomic and genetic variability of Arbutus. The known variability of this species is expressed in the characteristics of plant growth and fruit traits. The main objective of this work is the analysis of the variation in morphological traits within and among natural populations of Arbutus in their natural distribution, and to contribute to the programs of conservation and improvement of this species in Morocco.

Material and Methods

Plant Material and Sampling

The sampling areas were located in natural forests dominated by *Quercus ilex* L. or *Pinus halepensis* Mill., and permanent rivers characterized by a soil lithology of clay-silt, clay-limestone, limestone-marl, marl and clay. Twelve natural populations were selected in different biogeographical regions, across its natural range in Morocco, namely: the High Atlas, the Middle Atlas, the Central Plateau, the Pre-Rif, the Central and Occidental Rif, and the North-West (Fig. 1; Table 1).

For each population, 10-33 trees were randomly selected for leaf and fruit collection (Table 1). The difference in the number of trees collected is related to the density of the population. To avoid sampling of trees that are close to each other, the distance between trees was kept at about 15 m.

The collection, observation and measurement of the material used for this work were carried out during the fruiting periods of the species, in November 2016 and February 2017. Three to four branches (Fig. 2B) were randomly taken from a mid-branch stage of each tree (Fig. 2A) to collect leaves. Twenty leaves per tree were taken from the branches and placed in paper bags with their identification (Fig. 2C). Also, from the same tree, ten fruits (Fig. 2D) were collected from different parts of the tree. The fruits were pitted (Fig. 2E) to separate the pulp from the seeds. Seeds extracted (Fig. 2F), were stored in paper envelopes at a laboratory room temperature of about 18 ± 1 °C until measurement (Table 2). A total of 3220 leaves and 1610 fruits from 161 trees were sampled. Measurements of trees were

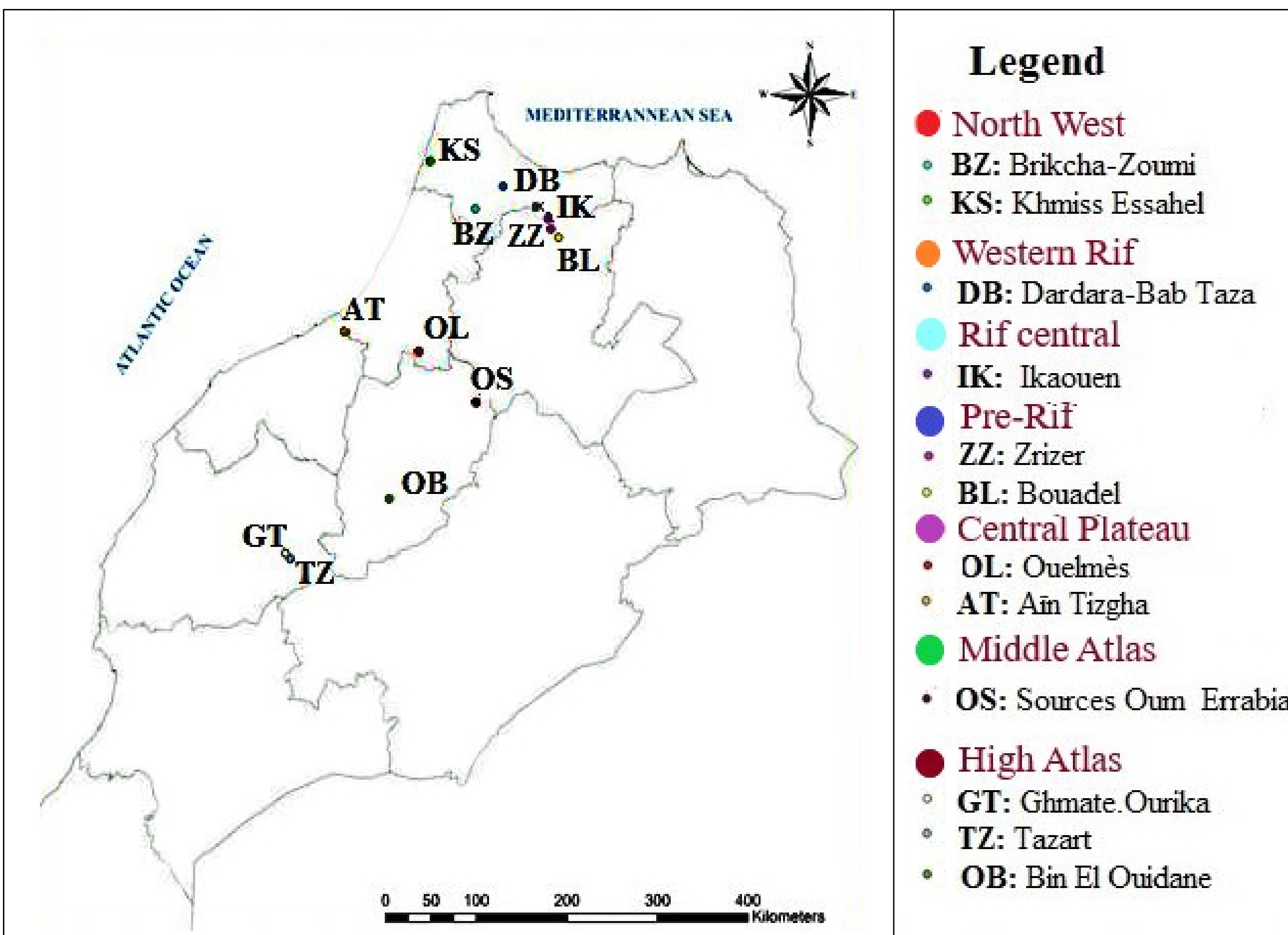

Figure 1. Location of the *Arbutus unedo* L. studied populations in Morocco.

Table 1. Name, code, number of sampled and measured individuals (N), geographic region and location and bioclimate types of the sampled populations. T(ºC) = Average annual temperature in ºC; Pr (mm) = Average annual precipitation in mm.

Populations	Code	N	Geographic region	Latitude N	Longitude W	Altitude (m)	T (°C)	Pr (mm)	Bioclimate type
Brikcha-Zoumi (Ouezzane)	BZ	20	North West	34°48’	5°29’	422	17.90	889	Sub-humid
Khmiss Essahel (Laarache)	KS	10	North West	35°15’	6°02’	150	17.90	700	Sub-humid
Dardara-Bab Taza (Chefchaouen)	DB	21	Western Rif	35°01’	5°09’	745	15.30	1135	Humid
Ikaouen. Ketama (El Hoceima)	IK	11	Central Rif	34°43’	4°37’	654	12.40	831	Sub-humid
Zrizer (Taounate)	ZZ	33	Pre-Rif	34°37’	4°35’	645	18.20	613	Sub-humid
Bouadel (Taounate)	BL	11	Pre-Rif	34°32’	4°29’	709	16.60	612	Sub-humid
Ouelmès (El khmissat)	OL	11	Central Plateau	33°28’	6°09’	983	13.60	756	Sub-humid
Aïn Tizgha (Benslimane)	AT	10	Central Plateau	33°39’	7°02’	275	17.40	463	Semi-arid
Sources Oum Errabia (Khénifra)	OS	10	Middle Atlas	33°00’	5°29’	1613	13.20	702	Sub-humid
Ghmate.Ourika (Marrakech)	GT	12	High Atlas	31°33’	7°42’	967	17.70	337	Semi-arid
Tazart (Marrakech)	TZ	11	High Atlas	31°29’	7°24’	867	18.00	360	Semi-arid
Bin El Ouidane	OB	11	High Atlas	32°05’	6°29’	1313	17.60	490	Semi-arid

made in the same individuals sampled for leaves and the fruits. For morphological traits, we used methods published in the descriptors (IPGRI, 1997; UPOV, 2011).

A total of 27 morphological traits of tree, leaf, and fruit were considered for each sampled individual, and the number of individuals sampled per population is given in Table 1. Twelve qualitative and fifteen quantitative characters were studied (Table 2).

Data Analysis

Descriptive statistics of the qualitative and quantitative data were estimated using SPSS software, version 20. The variance assumptions were verified by testing homogeneity of the variance and data normality. The one-way analysis of variance (ANOVA) was done to evaluate the variability existing within and among the different populations. The ANOVA and the Pearson correlation test were applied to quantitative variables. The overall structure of the morphological characteristics of the populations studied was described by principal components analysis (PCA) jointly for all the characters measured using the software XLSTAT, version 2019. In addition, discriminant hierarchical classification by the Unweighted Pair Group Method with Arithmetical Average (UPGMA) aggregation method using Euclidean distances was done using the software STATISTICA STAT SOF, version 1997.

Results

Quantitative Characteristics

Descriptive statistics for the quantitative traits measured are presented in Tables 3 and 4. The mean values of traits and CV shows high levels of variation among populations. ANOVA showed a significant difference between populations for most of the quantitative traits ($P < 0.001$).

Coefficient of variation (CV) values showed differences among traits and populations (Table 3, 4). Overall, plant height and width (LGA, LRA), number of hooks per branch (NCR) and number of seeds per fruit (NGF) showed high variation (CV>20%). Branch diameter (DR), leaf length (LGL), leaf length to width ratio (LGL/LRL), fruit length to diameter ratio (LGF/DF), fruit weight (PF) and seed weight (PG) showed relatively low variation (CV<20%). Trait variation

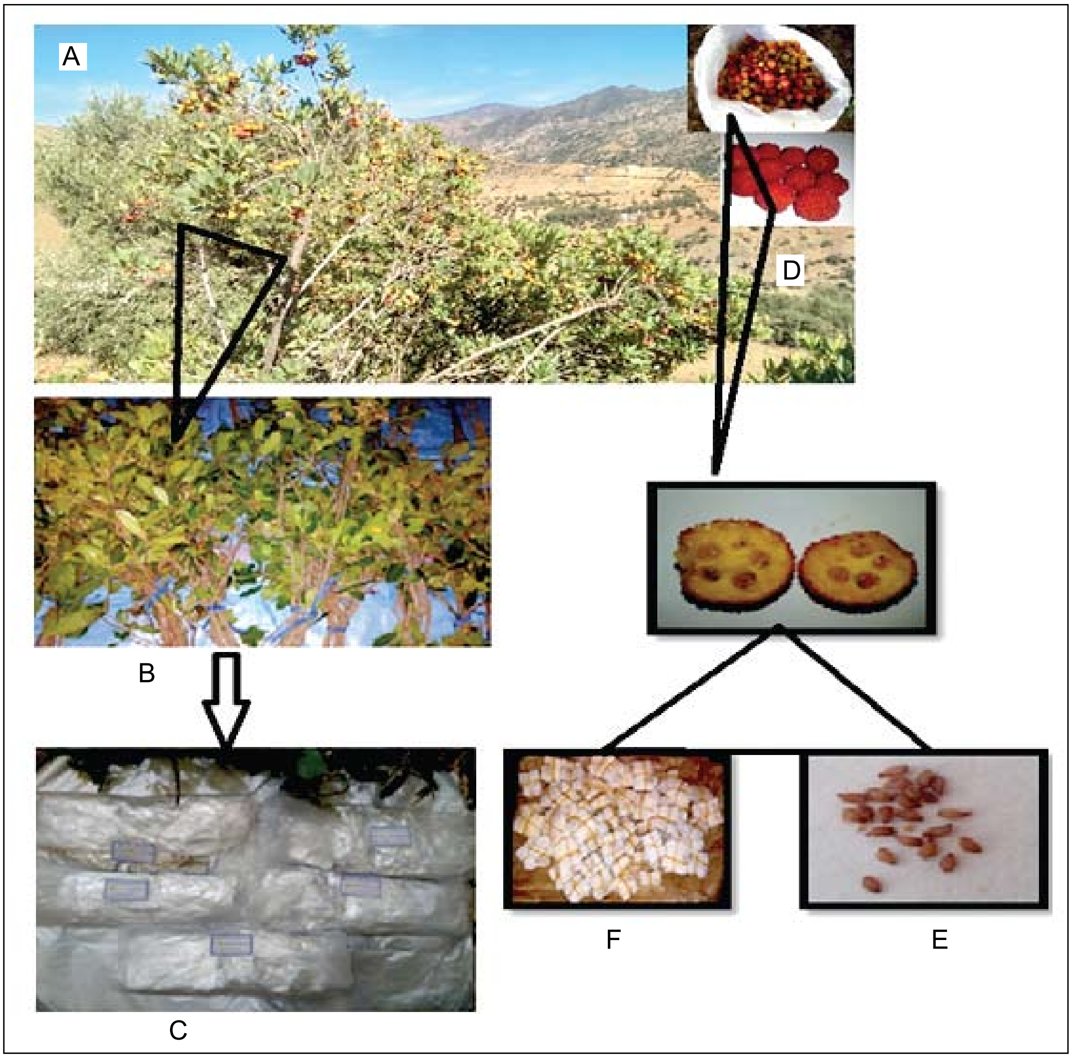

Figure 2. Plant material used for the measurement of *Arbutus unedo* L. morphological traits (A) Tree, (B) Branches bearing leaves, (C) Leaves measured and stored, (D) Fruit, (E) Seeds extracted from fruit, (F) Seeds measured and stored.

differed between populations, as seen in ANOVA results.

Considering variation of trait values within populations, plant height (LGA) was lowest (1.91±0.57 m) in the population OB and highest (8.91±0.40 m) in the population OS, with an overall mean of 4.56±2.23 m and CV of 48.77%. Plant width (LRA) values was lowest in BZ (1.75±0.66 m) and highest in OS (4.95±0.55 m) with an overall mean of 2.99±0.90 m. The number of hooks per branch (NCR) varied from 15.00 ± 4.09 for the TZ population to 100.90±32.00 for the KS population, with an overall mean of 39.60±30.98 and a CV of 78%. The number of seeds per fruit (NGF) ranged from 8.79±3.74 for the GT population to 17.80±7.20 for the KS population with an overall mean of 11.26±2.51 (CV=22.31%). However, other measured traits showed more or less significant levels of variation both within and between populations (Table 3, 4).

Qualitative Characteristics

Tree traits such as vigour, branching density and apical dominance were highest in the three populations AT, GT and TZ (100%). Most plants had a spreading habit (57.50%), followed by the drooping type (39.40%), while the upright type was the least frequent (3.10%) (Table 5).

The results revealed that the majority of the studied populations had wider leaves in the middle (86.5%) compared to the tip (13.30), as well as 74.60% of these accessions have strongly toothed leaves at the margin. It is noted that the OS population records the highest values (100%) of strongly toothed margin while the lowest values are observed in the TZ population

Table 2. Morphological traits observed : «column 1 indicates plant part involved ;columns 2-5 relate to quantitative traits; columns 6-8 related to qualitative traits».

Plant part	Quantitative traits	Code	Units	Measurement method	Qualitative traits	Code	Value
Tree	Branch diameter	DR	cm	Folding ruler	Plant vigour	VP	3, weak; 5, average; 7, strong
Tree	Tree Height	LGA	m	Meter long	Plant branching density	DP	3, loose; 5, medium 7, dense
Tree	Tree Width	LRA	m	Calliper	Apical dominance	DA	3, weak; 5, medium ; 7, strong
Tree	Number of hooks per branch	NCR		Counting	Plant habit	PP	1, upright; 2, spread out; 3, dropping
Leaf	Leaf Length	LGL	cm	Digital calliper	Position of the widest part of the leaf	PPPL	1, towards the base; 2, in the middle; 3, to the top
Leaf	Leaf Width	LRL	cm	Digital calliper	Leaf margin	FB	1, entirely; 3, slightly toothed; 5, strongly toothed
Leaf	LGL/LRL	LGL/ LRL			Leaf tip	FS	1, acute; 2, obtuse; 3, rounded
Fruit	Fruit length	LGF	mm	Digital calliper	Leaf color	CL	1, light green, 2, green; 3, dark green
Fruit	Fruit diameter	DF	mm	Digital calliper	Fruit form	FF	1, oblong; 2, round; 3, flattened; 4, oval
Fruit	LGF/DF	LGF/ DF			Fruit color	CF	1, yellow; 2, light red; 3, medium red; 4, dark red
Fruit	Fruit weight	PF	g	Balance	Color of pulp	CCh	1, yellow; 2, orange; 3, pink; 4, red
Seed	Number of seeds per fruit	NGF		Counting	Color of seeds	CG	1, light brown; 2, medium brown; 3, dark brown
Seed	Seed width	LRG	mm	Digital calliper			
Seed	Seed length	LGG	mm	Digital calliper			
Seed	Seed weight	PG	g	Precision balance			
	15				12		

(9.10%). Leaf tip shape is overall acute (60.90%) in all populations studied, but more frequent in the BL population (92.30%) and less frequent in the OL population (36.40). Leaves are mostly dark green (41.30%), the KS population is distinguished from the other populations by the highest values of this character (90%).

The fruits were mainly dark red (42.20%). DB and IK populations have overall deep dark red berries with percentages of 60% and 59.1%, respectively. The pulp of the studied fruits is mainly yellow (51.60%), followed by orange (46.80%), while pink is less frequent (1.60%) and red was absent in all populations. As regards the form of the fruits (round, oval, oblong and flattened), the study revealed four forms of fruit from different Moroccan regions: the flattened form is the most observed (33.10%) followed by the oval form (24.40%). Round (33% in KS) and oblong (33.30% in GT, 32.40% in OL) forms were also recorded. The most common seed color was brown (43.10%) with a high percentage observed in the OB population (57.30%).

Phenotypic correlations among traits

Phenotypic correlations among the quantitative morphological characteristics of natural populations of Moroccan *A. unedo* are presented in Table 6. There are both positive and negative correlations; a few of these correlations are significant. For instance, branch diameter (DR) is positively correlated with fruit and

Table 3. Quantitative features measured on trees and leaves.

Traits	Branch diameter (DR) (cm)	Tree height (LGA) (m)	Tree width (LRA) (m)	Number of hooks per branch (NCR)	Length of leaf (LGL) (cm)	leaf width (LRL) (cm)	LGL/LRL
BZ	2.93±0.45 1.90-3.63 (15 .66)	2.58±1.04 0.95-5.10 (40.36)	1.75±0.66 0.80-3.19 (37.86)	66.7±44.6 10-150 (66.94)	5.49±0.94 3.09-8.66 (17.21)	2.06±0.41 1.08-3.92 (20.06)	2.71±0.41 1.70-4.83 (16.49)
KS	3.24±0.29 2.93-3.9 3 (8.98)	2.80±0.42 2.00-3.50 (15.06)	1.80±0.58 1.0-3.0 (32.61)	100.9±32.0 66.0-152.0 (31.37)	5.43±0.79 3.20-7.27 (14.72)	2.08±0.48 1.03-3.95 (23.21)	2.70±0.58 1.39-4.55 (21.42)
DB	2.90±0.31 2.46-3.50 (10.60)	3.47±1.13 1.50-5.50 (32.42)	2.55±1.12 0.80-5.40 (43.95)	74.2±53.30 12.0-210.0 (71.85)	5.47±2.52 2.51-5.20 (46.05)	2.22±0.49 1.21-3.87 (21.85)	2.47±0.47 1.29-3.95 (19.19)
IK	3.65±0.25 3.23-4.23 (6.92)	5.43±1.53 2.50-7.50 (28.29)	4.07±0.92 2.50-5.50 (22.61)	18.9±4.90 10.0-25.0 (25.96)	5.76±0.77 3.70-8.22 (13.48)	2.28±0.48 1.25-3.60 (21.17)	2.61±0.53 1.40-4.68 (20.31)
ZZ	3.39±0.32 2.97-4.20 (9.62)	2.16±0.77 1.0-4.0 (35.87)	2.59±0.56 1.50-3.50 (21.82)	22.27±7.13 11.0-35.0 (32.05)	5.52±0.79 3.01-7.89 (14.40)	2.24±0.50 1.02-3.98 (22.64)	2.57±0.63 1.04-5.39 (24.41)
BL	3.49±0.32 2.96-4.13 (9.40)	4.17±1.01 2.50-5.50 (24.21)	3.04±0.94 1.60-4.30 (31.09)	15.73±2.41 12.0-20.0 (15.34)	6.53±0.95 4.43-8.88 (14.53)	2.48±0.55 1.50-4.50 (22.26)	2.72±0.53 1.54-4.49 (19.69)
OL	3.09±0.22 2.46-3.36 (7.19)	7.79±0.91 6.40-9.20 (11.77)	3.45±0.65 2.10-4.20 (19.01)	77.9±42.20 42.0-183.0 (54.24)	4.44±0.75 2.07-6.88 (17.03)	1.89±0.37 1.10-2.78 (19.85)	2.40±0.44 1.05-3.58 (18.37)
AT	2.99±0.26 2.53-3.26 (8.77)	4.92±0.62 3.80-5.80 (12.70)	3.62±0.83 2.50-5.50 (22.96)	15.3±2.66 12.0-20.0 (17.44)	5.47±0.88 4.02-7.83 (16.13)	2.0±0.39 1.02-2.83 (19.58)	2.81±0.58 1.51-5.51 (20.67)
OS	3.23±0.41 2.83-4.16 (12.82)	8.91±0.40 8.50-9.50 (4.53)	4.95±0.55 4.0-5.50 (11.12)	19.9±5.06 12.0-30.0 (25.45)	6.62±0.79 5.04-8.41 (12.67)	2.74±0.70 1.47-6.62 (25.55)	2.37±0.44 0.94-3.51 (18.63)
GT	3.61±0.62 2.88-4.46 (17.23)	4.25±1.15 2.50-6.0 (27.04)	2.55±0.52 1.50-3.20 (20.51)	19.25±4.4 10.0-25.0 (22.82)	4.88±0.87 3.19-8.81 (17.85)	2.13±0.37 1.30-3.18 (17.67)	2.33±0.41 1.52-3.54 (17.47)
TZ	4.37±0.57 3.36-5.20 (13.23)	6.32±1.47 3.50-8.29 (23.31)	3.32±0.69 2.50-4.50 (21.0)	15.0±4.09 10.0-20.0 (27.33)	4.58±0.82 3.55-8.86 (18.08)	1.83±0.41 1.05-4.16 (22.56)	2.57±0.53 1.78-4.19 (20.62)
OB	2.99±0.12 2.80-3.20 (4.10)	1.91±0.57 1.50-3.50 (29.75)	2.10±0.60 1.0-3.0 (29.04)	29.2 ±4.91 22.0-40.0 (16.84)	4.30±0.65 1.55-6.48 (15.22)	1.81±0.32 1.19-2.99 (17.87)	2.43±0.46 1.00-3.88 (19.04)
Mean	3.33±0.42 1.90-5.20 (12.68)	4.56±2.23 0.95-9.50 (48.77)	2.99±0.9 0.80-5.50 (31.97)	39.60±30.98 10.0-210.0 (78.0)	5.35±0.69 1.55-8.88 (12.88)	2.15±0.27 1.02-6.62 (2.60)	2.56±0.50 0.94-5.51 (19.69)
F value	15.90***	66.50***	19.40***	15.80***	13.70***	7.18***	21.60***

Mean ± standard deviation; minimum-maximum; CV: coefficient of variation in parentheses (%). Separation of the groups within and among-population indicated as follows: ***, p <0.001.

Table 4. Quantitative traits measured on fruits and seeds of *Arbutus unedo* L.

Traits	Fruit Length (LGF) (mm)	Fruit Diameter (DF) (mm)	LGF/DF	Fruit weight (PF) (g)	Number of seeds per fruit (NGF)	Seed width (LRG) (mm)	Seed length (LGG)(mm)	Seed weight (PG) (g)
BZ	13.18±2.06 7.50-19.40 (15.64)	15.0±2.28 10.10-23.10 (15.25)	0.89±0.13 0.50-1.20 (14.54)	2.04±1.02 0.70-6.50 (50.08)	11.4±7.28 3.0-35.0 (63.88)	1.30±0.10 0.70-1.53 (7.97)	3.09±0.27 1.39-3.75 (8.85)	0.003±0.0006 0.002-0.005 (21.57)
KS	15.37±2.13 11.20-21.30 (13.92)	17.12±2.80 12.60-22.80 (11.71)	0.90±0.09 0.60-1.18 (9.91)	2.61±0.98 1.10-5.90 (37.68)	17.80±7.20 4.0-40.0 (40.42)	1.33±0.90 1.12-1.93 (6.78)	3.12±0.18 2.61-3.58 (5.99)	0.003±0.0005 0.002-0.005 (16.86)
DB	17.0±2.01 10.10-22.80 (11.84)	17.40±2.80 10.60-27.10 (16.56)	0.93±0.12 0.70-1.35 (12.56)	2.81±1.36 0.80-7.60 (48.55)	11.7±5.06 4.0 -30.0 (43.21)	1.35±0.07 1.12-1.58 (4.99)	3.13±0.22 2.33-3.83 (19.20)	0.003±0.0006 0.002-0.005 (7.04)
IK	16.40±3.0 10.10-24.30 (8.30)	18.0±3.60 10.60-27.10 (20.0)	0.92±0.12 0.71-1.35 (12.67)	3.18±1.70 0.80-7.60 (53.49)	12.1±5.77 4.0-30.0 (47.56)	1.41±0.04 1.21-1.53 (3.38)	3.58±0.13 3.33-4.01 (3.74)	0.003±0.0005 0.003±0.005 (12.97)
ZZ	13.60±2.20 11.0-18.0 (16.33)	13.80±1.60 12.0-18.0 (12.03)	0.96±0.10 0.60-1.28 (10.02)	2.22±1.00 0.70-6.50 (45.85)	13.24±7.12 3.0-40.0 (53.85)	1.42±0.53 1.21-1.67 (3.75)	3.59±0.19 2.44-4.19 (5.30)	0.003±0.0005 0.003±0.006 (14.55)
BL	15.70±2.0 12.10-21.50 (12.57)	16.20±2.30 12.0-21.90 (14.46)	0.94±0.10 0.73-1.24 (10.95)	2.41±0.67 1.10-4.90 (28.01)	11.63±5.15 4.0-30.0 (44.30)	1.43±0.04 1.31-1.57 (3.79)	3.60±0.13 3.34-4.07 (3.77)	0.003±0.0004 0.003±0.005 (11.54)
OL	15.0±2.50 5.0-21.10 (16.91)	15.60±2.20 9.80-21.20 (14.42)	0.96±0.12 0.42-1.27 (12.15)	2.07±0.88 0.30-4.50 (42.56)	9.77±5.07 1.0 -25.0 (51.96)	1.38±0.07 1.23-1.69 (5.74)	3.13±0.20 2.65-3.76 (6.63)	0.003±0.0008 0.002-0.007 (25.96)
AT	15.60±2.0 12.10-21.50 (13.41)	16.20±2.80 12.0-21.80 (17.74)	0.95±0.11 0.71-1.24 (11.51)	2.33±0.71 1.10-4.90 (30.51)	10.20±3.38 4.0-20.0 (33.13)	1.58±0.14 1.34-2.11 (9.42)	3.57±0.29 3.21-4.62 (8.22)	0.003±0.0006 0.002±0.004 (19.25)
OS	15.0±2.40 10.0-21.1 (15.98)	15.60±2.20 9.80-20.80 (14.25)	0.97±0.11 0.76-1.27 (11.08)	2.01±0.86 0.30-4.50 (42.79)	8.89±3.3 4.0-18.0 (37.08)	1.27±0.83 1.02-1.43 (6.52)	3.14±0.25 2.52-3.81 (8.25)	0.003±0.0006 0.002-0.005 (19.95)
GT	15.15±2.08 10.90-22.60 (16.19)	15.41±2.08 9.8-20.10 (13.54)	0.98±0.10 0.80-1.27 (9.75)	2.47±0.69 1.10-4.30 (28.0)	8.79±3.74 3.0-24.0 (42.58)	1.42±0.69 1.30-1.66 (4.89)	3.63±0.24 2.68-4.21 (6.73)	0.004±0.0007 0.003±0.006 (18.82)
TZ	14.60±2.0 10.50-21.10 (13.45)	15.2±2.0 9.8-20.20 (12.74)	0.97±0.08 0.73-1.23 (8.77)	1.96±0.69 0.60-4.50 (35.17)	10.70±5.66 3.0-29.0 (52.84)	1.43±0.05 1.23-1.56 (4.02)	3.63±0.21 2.68-4.19 (6.03)	0.004±0.0007 0.003±0.006 (17.06)
OB	12.50±1.37 11.0-15.0 (11.03)	13.80±1.30 2.0-15.0 (9.45)	0.98±0.08 0.80-1.23 (8.23)	2.0±0.57 1.0-3.0 (25.87)	8.80±4.40 3.0-31.0 (50.18)	1.31±0.07 1.08-1.43 (5.36)	3.18±0.27 2.68-4.19 (8.71)	0.003±0.0006 0.002-0.005 (22.68)
Mean	14.90±1.30 5.0-24.30 (8.71)	15.8±1.30 9.80-27.10 (8.29)	0.95±0.10 0.42-1.35 (11.01)	2.35±0.38 0.30-7.60 (16.09)	11.26±2.51 1.0-40.0 (22.31)	1.39±0.08 0.70-2.11 (5.96)	3.37±0.25 1.39-4.62 (7.30)	0.003±0.0005 0.002-0.007 (12.96)
F value	20.50***	24.40***	12.10***	4.63***	21.70***	13.20***	17.20***	7.34***

Mean ± standard deviation; minimum-maximum; CV: coefficient of variation in parentheses (%). Separation of the groups within and among-population indicated as follows: ***, $p < 0.001$.

Table 5. Frequency of qualitative traits (%), in three or four classes, measured in the *Arbutus unedo* L. populations.

Characteristics	Plant Vigour (VP)			Plant branching density (DP)			Apical dominance (DA)			Plant habit (PP)			Position of the widest part (PPPL)			Leaf margin (FB)		
Level	3	5	7	3	5	7	3	5	7	1	2	3	1	2	3	1	3	5
BZ	0	35	65	5	25	70	0	30	70	5	50	45	0.50	67.30	32.30	3.30	13.30	83.50
KS	0	10	90	0	20	80	0	10	90	0	40	60	0.50	89	10.50	0.50	16	83.50
DB	19	23.80	57.10	19	28.60	52.40	19	28.60	52.40	14.30	52.40	33.30	0	73.10	26.90	1.20	13.60	85.20
IK	0	45.50	54.50	0	45.50	54.50	0	45.50	54.50	0	63.60	36.40	0	92.70	7.30	0.90	12.70	86.40
ZZ	0	45.50	54.50	0	33.30	66.70	0	45.50	54.50	0	75.80	24.20	0	91.40	8.60	0	5.30	94.70
BL	0	36.40	63.60	0	36.40	63.60	0	36.40	63.60	0	36.40	63.60	0	100	0	15.90	5.50	78.60
OL	9.10	36.40	54.50	9.10	45.50	45.50	9.1	45.50	45.50	18.20	54.50	27.30	0	81.40	18.60	19.10	15.90	65
AT	0	0	100	0	0	100	0	0	100	0	70	30	0	88	12	0	20	80
OS	0	70	30	0	70	30	0	70	30	0	60	40	0	92	8	0	0	100
GT	0	0	100	0	0	100	0	0	100	0	41.70	58.30	0	90	10	17.10	40.40	42.50
TZ	0	0	100	0	0	100	0	0	100	0	100	0	0	100	0	68.20	22.70	9.10
OB	0	45.50	54.50	0	45.50	54.50	0	36.40	63.60	0	45.50	54.50	0	73.40	25.70	0.9	11.30	86.90
Mean	2. 30	29	68.60	2.80	29.20	68.10	2.30	29	68.70	3.10	57.50	39.40	0.10	86.50	13.30	10.60	14.70	74.60

Characteristics	Leaf tip (FS)			Leaf color (CL)			Fruit form (FF)				Fruit Color (CF)				Color of the pulp (CCh)				Color of seeds (CG)		
Level	1	2	3	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
BZ	40.50	45	14.50	20.50	34.80	44.80	27.10	23.10	27.60	22.10	1.50	13.60	39.70	5.20	54.80	43.70	1.50	0	27.10	39.20	33.70
KS	51	37	12	1.50	8.50	90	5	33	44	18	7	19	33	41	59	38	3	0	33	40	27
DB	53.30	35	11.70	18.30	17.60	46	14.30	22.40	32.40	31	1.40	9.50	29	60	39.50	57.60	2.90	0	21.40	48.10	30.50
IK	65.50	29.50	5	8.20	64.10	27.70	12.70	25.50	30	31.80	0.90	14.50	25.50	59.10	36.40	62.70	0.90	0	20.90	47.30	31.80
ZZ	76.80	15.50	7.70	12.10	48.50	39.40	17.90	27.30	34.80	20	3	13.90	41.20	41.80	52.70	45.50	1.80	0	20	45.80	34.20
BL	92.30	7.70	0	9.10	27.30	63.60	13.60	28.20	30	28.20	2.70	8.20	48.20	40.90	50.90	46.40	2.70	0	27.30	33.60	39.10
OL	36.40	47.30	16.40	32.70	19.10	48.20	32.40	18.90	31.50	17.10	2.7	10.80	36.90	49.50	50.50	47.70	1.80	0	27	45.90	27
AT	67.50	28.50	4	60	20	20	15	25	28	32	2	9	36	53	47	49	4	0	9	33	58
OS	72.50	25	2.50	0	30	70	32	15	36	17	3	11	44	42	56	44	0	0	5	36	59
GT	42.10	32.90	25	26.30	73.80	0	33.30	10	33.30	23.30	0	19.20	46.70	34.20	60	40	0	0	26.70	48.30	25
TZ	70	30	0	90.90	0	9.10	20	18.20	37.30	24.50	0	13.60	43.60	42.70	49.10	50.90	0	0	18.20	42.70	29.10
OB	63.20	21.40	15.50	27.70	35.90	36.40	30.90	9.10	31.80	28.20	0	19.10	44.50	36.40	62.70	36.40	0.90	0	20	57.30	22.70
Mean	60.90	29.60	9.50	25.60	31.60	41.30	21.20	21.30	33.10	24.40	2	13.50	39	42.20	51.60	46.80	1.60	0	21.30	43.10	34.80

Table 6. Correlation between quantitative traits measured on trees, leaves, fruits and seeds. *, significant at p<0.05; **, significant at p<0.01.

	DR	LGA	LRA	NCR	LGL	LRL	LGL/ LRL	LGF	DF	LGF/ DF	PF	NG	LRG	LGG	PG
DR	1														
LGA	-0.022	1													
LRA	-0.479	0.725**	1												
NCR	-0.031	-0.183	-0.526	1											
LGL	0.329	0.001	-0.112	-0.019	1										
LRL	-0.189	0.316	0.479	-0.269	0.681*	1									
LGL/ LRL	-0.197	-0.197	0.168	-0.147	0.180	0.023	1								
LGF	0.724**	-0.158	-0.654*	0.355	0.606*	-0.059	-0.214	1							
DF	0.720**	-0.191	-0.675*	0.390	0.598*	-0.082	-0.185	0.994**	1						
LGF/DF	0.523	0.056	-0.325	-0.240	-0.025	-0.245	-0.636*	0.414	0.371	1					
PF	-0.477	-0.077	0.386	-0.038	-0.018	0.293	0.392	-0.395	-0.375	-0.830**	1				
NG	0.576	-0.430	-0.748**	0590*	0.515	-0.122	0.120	0.786**	0.827**	-0.010	-0.206	1			
LRG	0.382	-0.136	-0.201	-0.260	0.187	-0.310	0.438	0.398	0.365	0.199	-0.109	0.222	1		
LGG	0.667*	-0.174	-0.191	-0.542	0.298	-0.073	0.215	0.383	0.354	0.343	-0.113	0.205	0.778**	1	
PG	0.686*	-0.162	-0.170	-0.449	0.264	0.072	0.014	0.284	0.266	0.238	0.004	0.206	0.405	0.861**	1

seed traits (LGF, DF, LGG, PG). On the other hand, tree width (LRA) is positively correlated with leaf width (LGA) but negatively correlated with fruit and seed traits (LGF, DF, NG). As well, the length of fruit (LGF) is positively correlated with the diameter of fruit (DF) and number of seeds per fruit (NG) which are positively correlated with each other. Finally, the weight of seed is correlated positively with its dimensions (LRG, LGG).

Morphological Variation within and Among Populations

Phenotypic variability between populations for almost all measured morphological traits (except for a few traits related to leaves, fruits and seeds) was highly significant as shown by the one-way ANOVA test (Tables 3, 4). Principal component analysis (PCA) shows that 71.39% of the total variation between natural populations of *Arbutus* in Morocco was explained by the first three components (Fig. 3, Table 7). The first component (PC 1), which explains 36.43% of the total variation, has high factor loadings of variables related to branch diameter (DR), fruit length and diameter (LGF, DF), LGF/DF ratio, number of seeds per fruit (NG), seed length and width (LGG, LRG) and seed weight (PG) (Table 8). The second component (PC 2), which explained 18.95% of the total variation, was strongly associated with seed traits including: Length (LGG) and seed weight (PG). The third component (PC 3) which explained 16.01% of the total variation was associated with leaf and fruit traits: leaf length (LGL) and leaf length to width ratio (LGL/LRL) and fruit weight (PF). These results show the sources of phenotypic variability, especially fruit and seed, within and between populations.

The dendrogram resulting from UPGMA analysis reveals two dissimilar groups (I, II). Group I consists of nine accessions, one from the Central Rif (IK), the Middle Atlas (OS), Central Plateau (AT), the northwest (BZ), two from the Pre-Rif (ZZ, BL) and three from the High Atlas (GT, TZ, OB). Group II consists of three populations, namely KS from the Northwest, DB from the Central Rif and OL from the Central Plateau. Two populations in the northwestern regions BZ (fire affected) and KS (different vegetative traits) appear to be anomalous.

The populations of first group (except for BZ) are characterized by lower number of hooks per branch (NCR), longer trees, broader and heavier seeds than those in the second group. The BZ population is characterized by low values for most of the measured characters compared to the other populations. The accessions of second group are characterized by similar

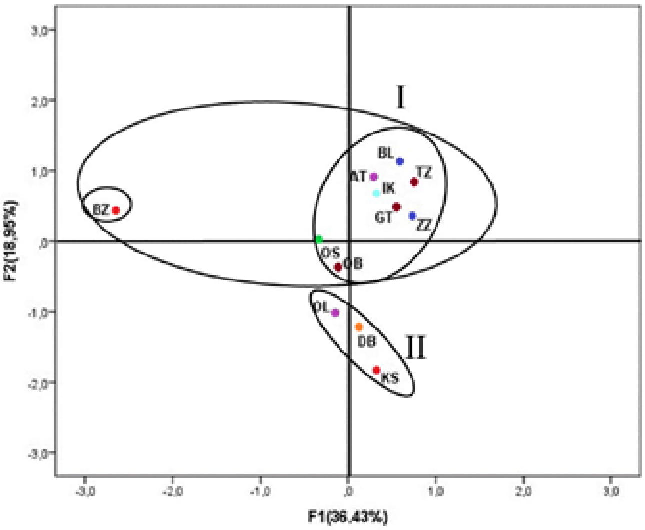

Figure 3. Principal Component Analysis based on the pairwise genetic matrix of the morphological traits measured in 12 *Arbutus unedo* L. populations in Morocco.

Table 7. Total variance of the measured morphological trait explained by the principal components.

Component	Extraction Totals of squares of selected factors		
	Total	% of variance	% cumulated
PC 1	5.465	36.434	36.434
PC 2	2.842	18.950	55.383
PC 3	2.402	16.013	71.396
PC 4	1.982	13.214	84.610
PC 5	0.971	6.475	91.084

values for most morphological traits, including the highest values for fruit length and diameter, number of hooks per branch and number of seeds per fruit.

The differences between the north-western populations are due to the branch diameter (DR) and fruit traits (LGF, DF, NG) related traits which are highly correlated with PC1 (0.858, 0.916, 0.913, 0.782 respectively), as well as plant height and width (LGA, LRA) and number of hooks per shoot (NCR) traits. Populations from the Rif regions differed in LGA, LRA and NCR. Similarly, the differences between the High Atlas populations are due to the traits DR, LGF and NG which are correlated with PC1 (0.858, 0.916, 0.782 respectively) and the plant traits LGA and NCR. The central plateau populations differ from each other in the LGA and NCR traits. (Fig. 3, Table 8).

The correlations of morphological characteristics and PC1-PC3 with altitude, latitude, longitude, temperature and precipitation are presented in Table 9. Variation in leaf traits was related to latitude and that in fruit traits to longitude. Leaf width, leaf length (LRL, LGL) and PC2 are positively correlated with latitude (r=0.630, r=0.530 and r=0.614 respectively). The fruit weight (PF), the ratio between the length and the diameter of the fruits (LGF/DF), as well as the two components PC1 and PC2 were correlated with the longitude (r=0.638, r=-0.824, r=-0.620, r=0.759 respectively). On the other hand, the ratio between leaf length and width (LGL/LRL), and between fruit length and diameter (LGF/DF), as well as PC3 are correlated with altitude (r= -0.819, r=0.717, r=0.760 respectively). Finally, the ratio (LGF/DF), seed length LGG, PC1 and fruit weight showed significant correlations with annual rainfall (r= -0.643, r=0.608, r=-0.601, r=-0.727 respectively).

Table 8. Loadings of morphological traits on the first three PCs (The highest ones, >0.5 threshold, are in bold and underlined.

	PC1	PC2	PC3
DR	**<u>0.858</u>**	0.222	-0.184
LGA	-0.336	0.236	-0.334
LRA	-0.764	0.477	-0.055
NCR	0.191	-0.884	0.266
LGL	0.469	0.230	**<u>0.500</u>**
LRL	-0.232	0.325	0.333
R(LGL/LRL)	-0.138	0.325	**<u>0.704</u>**
LGF	**<u>0.916</u>**	-0.147	0.097
DF	**<u>0.913</u>**	-0.187	0.135
R(LGF/DF)	**<u>0.521</u>**	0.077	-0.806
PF	-0.501	0.169	**<u>0.655</u>**
NG	**<u>0.782</u>**	-0.347	0.450
LRG	**<u>0.512</u>**	0.498	0.129
LGG	**<u>0.608</u>**	**<u>0.751</u>**	0.009
PG	**<u>0.517</u>**	**<u>0.664</u>**	0.007

Discussion

The results of the present study showed a high level of variation in morphological characters between natural populations of *A. unedo* in Morocco. The analysis of variance (ANOVA) and coefficients of variation (CV) showed differences within and between populations for the majority of the morphological traits and which are in good agreement with the results of previous studies reported in the literature (Ozcan & Haciseferogullari, 2006; Celikel et al, 2008; Takrouni & Boussaid, 2010; Molina et al., 2011; Bertsouklis & Papafotiou, 2016; Boussalah et al., 2018; Colak, 2019).

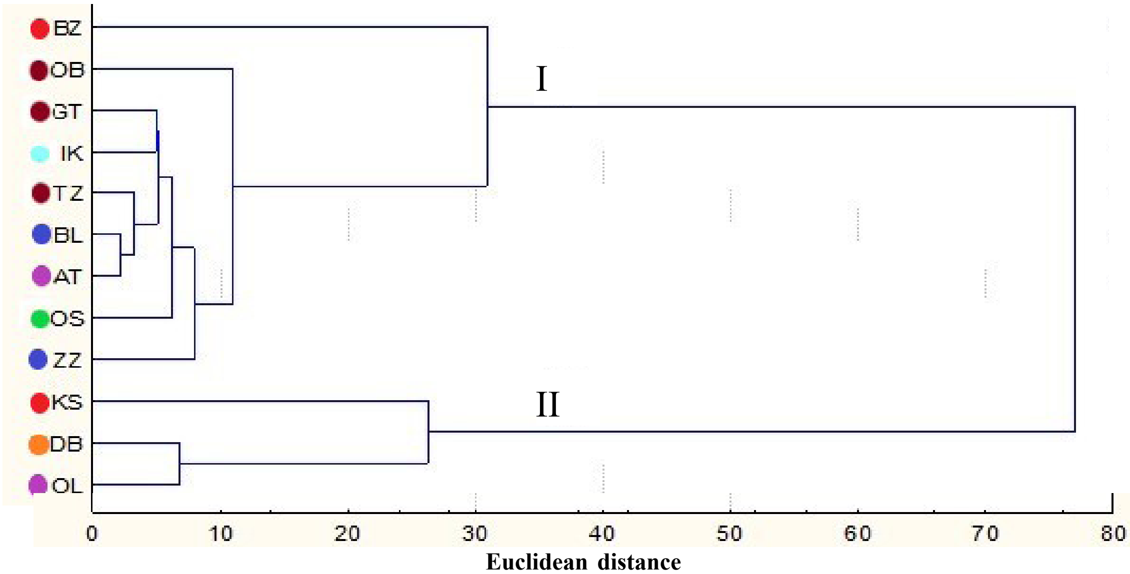

Figure 4. Population's dendrogram using the UPGMA clustering methods and the Euclidean distance based on the quantitative morphological characteristics data measured on trees, leaves, fruits and seeds.

Table 9. Correlation between morphological traits principal components and environmental characteristics (longitude, latitude, altitude, precipitation, and temperature). Significant values are represented by P<0.05*; P<0.01.**

	Latitude N	Longitude W	Altitude (m)	T (°C)	Pr (mm)
DR	-0.269	-0.447	0.208	-0.072	-0.536
LGA	-0.104	-0.200	0.358	-0.568	0.098
LRA	0.179	0.116	0.066	-0.322	0.281
NCR	0.045	0.342	-0.194	-0.122	0.399
LGL	0.530	0.362	0.019	-0.395	0.121
LRL	**0.630***	0.392	0.180	-0.399	0.379
R(LGL/LRL)	0.220	0.553	**-0.819****	0.285	0.046
LGF	0.001	-0.078	0.136	-0.346	-0.145
DF	0.023	-0.048	0.113	-0.351	-0.137
R(LGF/DF)	-0.463	**-0.824****	**0.717****	0.036	**-0,643**
PF	0.413	**0.638***	-0.550	-0.154	**0.608***
NG	0.147	0.266	-0.264	-0.054	-0.047
LRG	-0.273	-0.100	-0.435	0.150	-0.459
LGG	-0.160	-0.275	-0.155	0.173	**-0.601***
PG	0.061	-0.188	-0.022	0.106	-0.408
PC1	-0.466	**-0.620***	0.272	0.137	-0.727**
PC2	**0.614***	**0.759****	-0.503	-0.267	0.434
PC3	0.262	-0.088	**0.760****	**-0.740****	0.360
PC4	0.283	-0.014	0.106	-0.296	-0.123
PC5	-0.359	-0.031	-0.244	-0.390	0.026

The results of this variance between populations could be explained by the effect of provenance (Northwest, Rif regions (Pre-Rif, Central Rif and Western Rif), Central Plateau, Middle and High Atlas). Provenance is characterised by extreme variations in environmental conditions, particularly climatic, which affect plant phenotype (Nicotra et al., 2010; Wahid et al., 2016). The orographic variability from one region to another in Morocco contributes to a great variability in environmental and climatic that may partly explain the observed morphological variation. Populations BZ and KS from the North West show high values for fruit weight, while populations from the Rif (ZZ, BL, IK DB), Central Plateau (OL, AT and OS) and Middle Atlas (OS) regions present medium values for these traits, while populations from the High Atlas (OB, GT and TZ) showed the low values. High levels of association between the different morphological traits and climatic and geographic variables. This correlation could be explained by the effect of environmental factors and/or the interaction between genetics and environment on the expression of these traits.

This comparison showed that the Northwestern populations with heavy fruits grow at higher levels of humidity (rainfall) and medium annual temperature, compared to the High Atlas populations which are characterized by less heavy fruits and grow at high temperature levels and low humidity. Similarly, the other quantitative traits measured are closely related to the environmental conditions of the provenances of the sampled individuals, since they show variations notably according to geographical and ecological gradient as those noted for the traits: leaf width (LRL), ratio between leaf length and width (LGL/LRL), ratio between length and diameter of fruit (LGF/DF) and seed length (LGG). In fact, most of the quantitative traits measured record high values for the populations studied going from the High Atlas to the northern populations and the Rif region. Also, the tree and leaf traits increase with increasing altitude except for some populations (BZ, KS, AT, TZ) that showed tall tree and leaf dimensions despite their location in low altitudes. Also, with the exception of the populations BZ and KS (NW), these populations are characterised by lower precipitations and higher temperatures. These variations could be explained by the possibility that these traits are controlled by environmental climatic conditions and/or the interaction between environment and the genetic factors of the individuals constituting the populations as has been previously showed for other species in this area, such as *Myrtus communis* (Wahid et al., 2012; Wahid et al., 2016). For the BZ population, we noticed that most of its traits present low values especially those related to fruits which could be explained by the effect of a forest fire that occurred in the region coinciding with the period of our sampling which could be a source of stress for the plants. Indeed, the important role of provenance in determining the genetic diversity of this species has been demonstrated by previous studies using molecular markers (Lopes et al., 2012; Gomes et al., 2013; Ribeiro et al., 2017; Fazenda et al., 2019).

The correlations observed among different morphological traits may help to answer the hypothesis of a close relationship between provenance and phenotypic traits of the Arbutus tree, and to have clear ideas and set objectives for the selection of more performing and adaptive varieties.

Finally, the PCA results showed that the first three components explained 71.39% of the total variation between populations. Among the traits with high factor loadings that contribute to the formation of the principal components are: branch diameter, fruit length and diameter, number of seeds per fruit, seed length, seed weight, fruit weight. In general, it was observed that the IK populations from central Rif recorded the highest values in fruit weight (PF). As such, the fruit is highly valued for consumption and herbal medicine. And revealing the correlations between the traits of the fruit and those of the tree, leaf or seed will help to create bioindicators to select germplasm for genetic improvement.

In addition, fruit length and diameter are significantly correlated with branch diameter, tree width, leaf length, fruit diameter and number of seeds per fruit. These results and those of the correlation between morphological traits and geographical factors of the provenances, made it possible to classify the populations studied into two main groups independently of their geographical origin.

The results of the present study show that the bulk of variation overall is in fruit and seed traits; the bulk of the latitudinal variation is in leaf traits, of the longitude variation in fruit traits; differences between northwestern populations are due to plant size and number of hooks per branch; dendrogram shows that two of the three populations in group II anomalous in some ways.

In conclusion, the variability and morphological structuring of the natural populations of *Arbutus* in Morocco, demonstrated in the present study, could be used for conservation and genetic improvement programs and the implementation of another approach to evaluate the genetic diversity of this species in more profound ways using molecular markers.

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