

## Phenotypic diversity analysis of red juniper (*Juniperus turbinata* Guss.) populations in Morocco

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### ABSTRACT

The aim of this study was to characterize and evaluate the phenotypic variation of *Juniperus turbinata* Guss. in its Moroccan range. Twenty-five quantitative and qualitative morphological characters related to the plant, branches, branchlets, leaves and galbules were analyzed across fourteen natural populations collected throughout their geographical range. Plant height varied considerably, reaching over 7 m in the Essaouira population and dropping to around 1 m in the Sakka population. The number of trunks per plant was particularly high in the Sakka and Martil populations, with over 10 trunks per plant. The galbule weight was around 0.63 g in the Demnate population, compared to only 0.11 g in the Sakka population. Similarly, the number of seeds per galbule (NSG) ranged from a maximum of around seven seeds in the Amesguen population to a minimum of three seeds in the Saïdia population. Analysis of variance revealed highly significant differences among populations for all measured traits. What's more, 80% of quantitative traits (16 out of 20) had a coefficient of variation greater than 15%, testifying to high morphological diversity. A significant positive correlation was observed between the number of seeds per galbule and galbule dimensional traits including GWe, GWi, GLe, GC and GD. Principal coordinate analysis (PCoA) and hierarchical clustering analyses classified the populations into three distinct groups, independently of their geographical origins and bioclimatic zones. The results of this study provide valuable information for developing an effective genetic improvement program, as well as managing and conserving the genetic resources of red juniper in Morocco.

**Keywords:** *Juniperus turbinata*, morphological traits, phenotypic variability, Morocco.

### INTRODUCTION

*Juniperus phoenicea* commonly known as red juniper, is an endemic plant of the Mediterranean region (Ferrer-Gallego et al., 2017, El-Barougy et al., 2023). Its geographical range extends from the Atlantic coasts of Portugal and Morocco in the West to the eastern Mediterranean and the Middle East (Jordan and Saudi Arabia) in the East, as well as the large Mediterranean islands (Farjon & Filer, 2013, Pavon et al., 2020). Morphological, biochemical and genetic studies have revealed that *Juniperus phoenicea* is a complex containing three species (Mazur et al., 2018), *Juniperus phoenicea* L. found in the Iberian Peninsula, southern France and northwestern

Italy; *Juniperus turbinata* Guss. located in coastal regions around the Mediterranean Sea (Sánchez-Gómez et al., 2018; Salvà-Catarineu et al., 2021) and in the mountains of northwest Africa (Adams, 2014), and *Juniperus canariensis* Guyot & Mathou found in the Canary Islands.

In Morocco, *Juniperus turbinata* Guss. is distributed across various habitats from the coastal Atlantic dunes (from Essaouira to Mehdyia city) and Mediterranean (from Tangier to Saïdia city) to the Saharan limits (Quezel & Gast, 1998; Benabid, 2000). It also thrives in the Atlas mountains at altitudes ranging from 1000 m to 2150 m (Farjon & Filer, 2013). Together with *Juniperus thurifera*, it forms a Juniperaie with a surface area of 240 000 ha (Benabid, 1985).

*Juniperus turbinata* Guss is a monoecious species or rarely dioecious (Farjon, 2017), a shrub of 3 to 5 meters of height or small tree reaching 8 to 10 meters, with a trunk 0.60 m in diameter and very dense branching. The young, cylindrical branchlets have cinnamon-brown bark, while the older branchlets and trunk are straight with a scaly grayish-brown rhytidome; the branches are grayish-brown and the slender branchlets have a diameter of 1 mm (Quezel & Gast, 1998; Benabid, 2000).

The red juniper is recognized for its economic and medicinal importance. It is used as lumber, firewood, charcoal and in the production of vegetable tar. Its leaves are also used in tanning processes (Benabid, 2000), as they are used to make smoking tobacco by the Saharan inhabitants (Quezel & Gast, 1998). The branches, leaves and galbules of the red juniper are often used in traditional medicine. Their beneficial properties, including antioxidant effects against certain bacteria and fungi (Mazari et al., 2010; Derwich et al., 2010), are due to the presence of phenolic compounds and essential oils (Stassi et al., 1996; Medini et al., 2009, Al Khlifeh et al., 2021). Biochemical analysis of the essential oil has shown that it is very rich in  $\alpha$ -pinene and  $\delta$ -3-carene (Achak et al., 2009). This is why it is also used as a traditional remedy for many illnesses in both humans and animals (Boratyński et al., 2024). The products used for medicinal purposes in Moroccan Berber populations are derived from the distillation of their wood to obtain oil and tar (Quezel & Gast, 1998, Terfaya et al., 2021).

According to the consulted literature, genetic research on Moroccan *Juniperus turbinata* Guss. has received little attention. To best of our knowledge, only one study of phenotypic variability of few Eastern populations was carried out by Mazur et al. (2010). Consequently, it would be very interesting to find more discriminating criteria that could provide additional information on genetic diversity of Moroccan red juniper populations. Despite the fact that the expression of morphological markers is strongly affected by environmental conditions of the species, they are highly recommended as a first step that should be achieved before biochemical and molecular analysis (Hoffman et al., 1995). Therefore, juniper (*Juniperus* spp.) assessment using morphological traits is of great importance for the assessment, identification, efficient management, preservation and breeding programs of genetic

resources (Ghorbanzadeh et al., 2021). As *Juniperus turbinata* Guss. an woody tree species with large geographic range, outcrossing breeding system, wind or animal-ingested seed dispersal, and both sexual and asexual modes of reproduction (Farjon, 2017), it's expected to have more genetic diversity than woody species with other combinations of traits (Hamrick et al., 1992). Accordingly, the present study was conducted to characterize and evaluate the genetic variability of red juniper populations using some morphological traits and to look for any space and bioclimatic structuring of such populations according to their entire distribution in Morocco.

## MATERIALS AND METHODS

### Plant material

During the summer months of 2023 (June and July), 14 populations of *Juniperus turbinata* were sampled throughout its Moroccan geographical range (Fig. 1). The locations of these populations were chosen according to their geographical coordinates (latitude, longitude and altitude) and ecological conditions (annual rainfall, mean temperature). Table 1 summarizes these geographical and ecological characteristics. In each population, 13 trees were randomly sampled. From each individual tree, 10 second-order branches and 10 galbules were collected for recording measurements and observations (Fig. 2).

### Morphological traits

In order to determine morphological variations, measurements and observations were made on 25 quantitative and qualitative traits (Table 2), related to the tree, branches, branchlets, leaves and galbules (Fig. 2), based on the published descriptor of International Union for the Protection of New Varieties of Plants (UPOV, 1986). Ten replicates were performed for each trait.

### Data analysis

The mean of the studied traits was calculated and used for statistical analysis. The minimum value, maximum value, standard deviation (SD), coefficient of variations ( $CV\% = \sigma/\mu \times 100$ ), and frequency of qualitative traits, were calculated for the measured traits as follows:

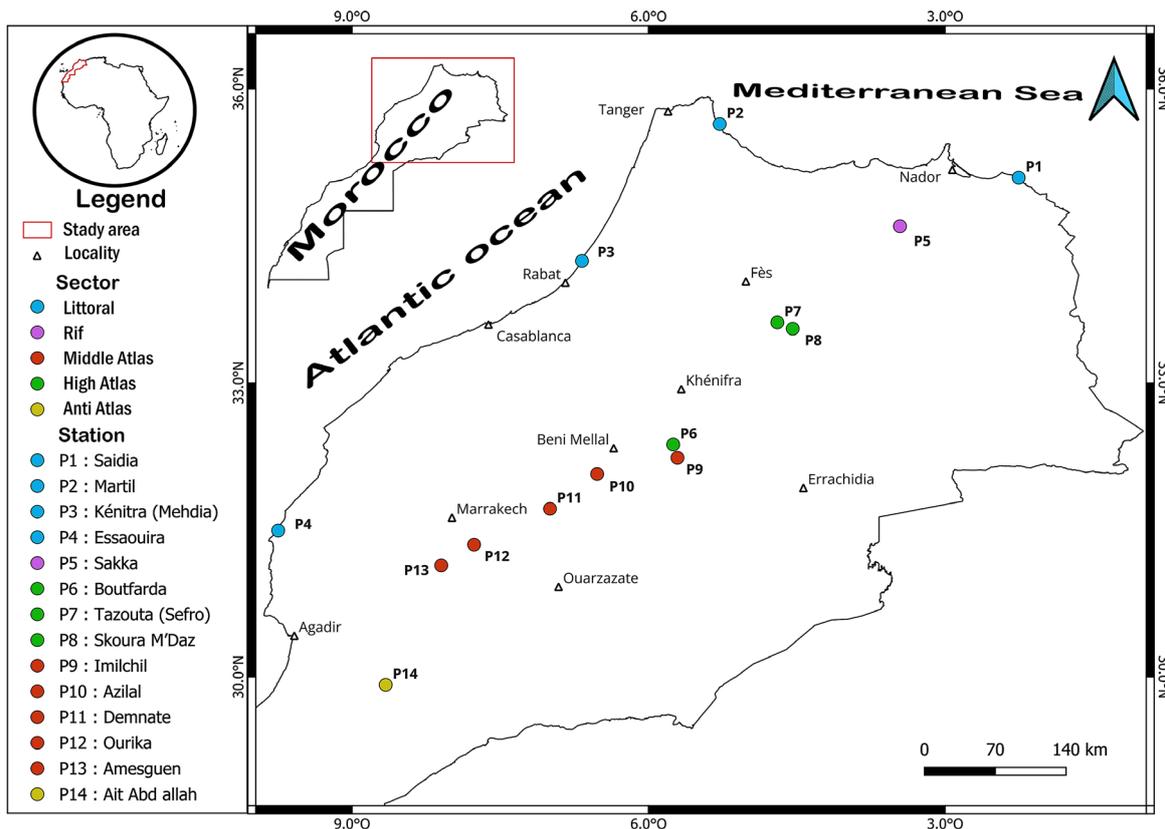
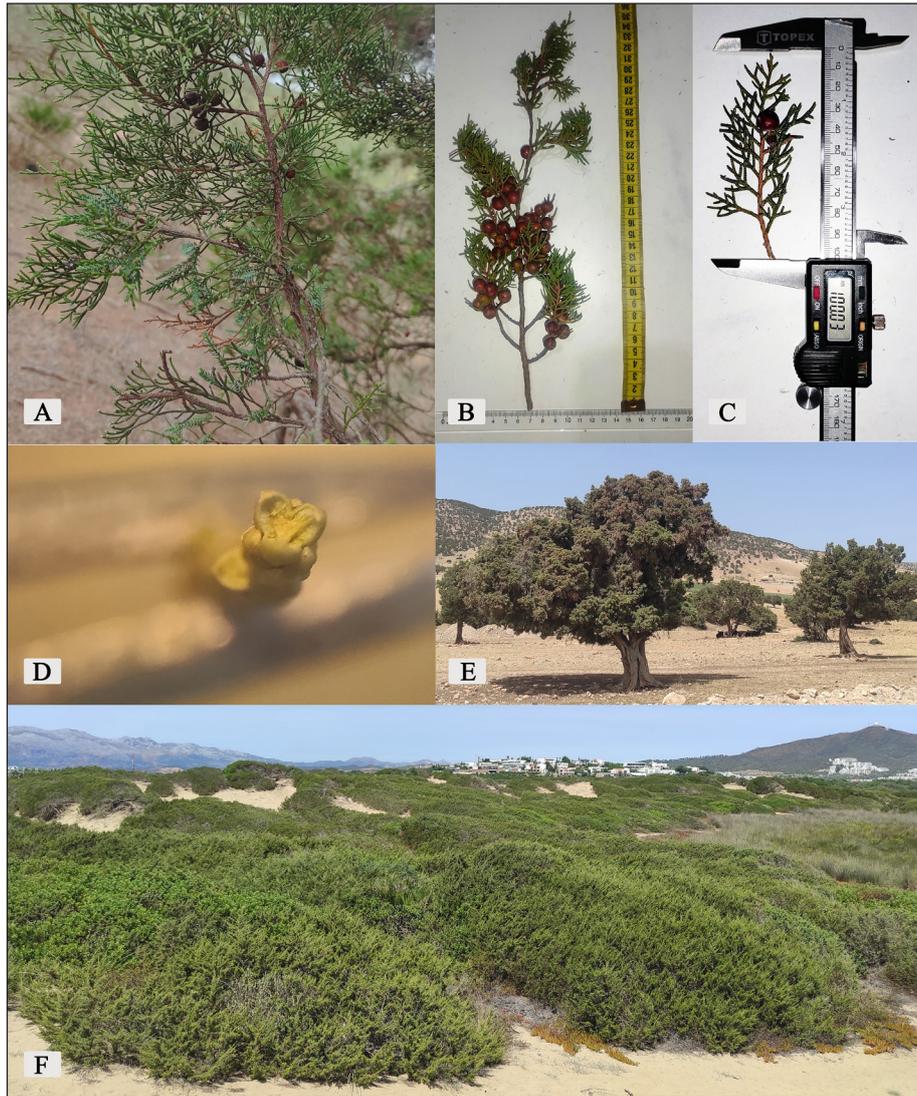


Figure 1. Map of Morocco showing the location of the studied populations of *Juniperus turbinata* Guss

Table 1. Geographical and meteorological conditions of *Juniperus turbinata* natural populations used in the study

Population	Code	Geographical zone	Bioclimate type	Altitude (m)	Latitude N	Longitude W	Rainfall average (mm/yr)	Temperature average (°C/yr)
Saïdia	SAD	Littoral	Semi- arid	5 m ± 1	35.0937594 N	-02.2567308 W ± 4	477	20.65
Martil	MAR	Littoral	Semi- arid	10 m ± 2	35.639963 N	-05.2806902 W ± 4	504	20.2
Kénitra (Mehdia)	MEH	Littoral	Semi- arid	10 m ± 1	34.2442628 N	-06.6740797 W ± 4	476	22.4
Essaouira	ESS	Littoral	Arid	20 m ± 3	31.4949082 N	-09.7467906 W ± 4	241	21.05
Sakka	SAK	Rif	humid	915 m ± 3	34.5980876 N	-03.4577033 W ± 4	610	18.8
Boufarda	BTD	Middle Atlas	Semi- arid	1473 m ± 5	32.3712812 N	-05.7512730 W ± 4	563	15.3
Tazouta (Sefro)	TZO	Middle Atlas	humid	1542 m ± 3	33.6168113 N	-04.697909 W ± 4	700	16.6
Skoura M'Daz	SKR	Middle Atlas	Semi- arid	898 m ± 3	33.5525789 N	-04.5421938 W ± 4	599	15.25
Imilchil	IML	High Atlas	Semi- arid	1914 m ± 3	32.2363032 N	-05.70824 W ± 4	402	12.5
Azilal	AZI	High Atlas	Semi- arid	1403 m ± 3	32.0707107 N	-06.5205208 W ± 4	456	15
Demnate	DEM	High Atlas	Semi- arid	1080 m ± 3	31.715903 N	-06.997494 W ± 4	330	15.5
Ourika	ORK	High Atlas	Arid	921 m ± 10	31.350392 N	-07.7652159 W ± 4	283	18.2
AmesgueuN	ASG	High Atlas	Semi- arid	927 m ± 4	31.1385538 N	-08.09772 W ± 4	328	17.05
Ait Abd allah	AAL	Anti-Atlas	Arid	1718 m ± 3	29.9208963 N	-08.6593779 W ± 4	213	19.35



**Figure 2.** (A) last-order branchlets with both scales and needles leaves from the Saidia population (SAD); (B) second-order branch bearing first-order branchlets, which in turn bear galbules; (C) first-order branchlets bearing penultimate-order branchlets with galbules and leaves; (D) leaves arranged in whorls of 3 scales; (E) *Juniperus turbinata* plants with spreading habit and a single trunk from the Amesguen population (ASG); (F) *J. turbinata* plants showing a creeping habit with several trunks from the Martil population (MAR)

$$fr = \frac{f}{N} \quad (1)$$

where:  $fr$  is the relative frequency of a category,  $f$  the number of occurrences of the category, and  $N$  is the total number of observations in the variable being studied).

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \mu)^2} \quad (2)$$

where:  $\sigma$  is the standard deviation,  $N$  is the number of observations,  $x_i$  is each individual data point, and  $\mu$  is the mean of the data.

Requirements of ANOVA are checked by normality of data distribution according to Shapiro-Wilk test (1965), and by testing the

homogeneity of variance of residual means using Levene’s test of equality of the error variances (Levene, 1960) respectively at significance value of 0.05. Analysis of variance for all the traits was performed using one-way ANOVA to determine the significant differences among populations. In addition, post-hoc comparisons (Tukey’s test) means was done to compare populations means of each trait at  $p$ -value < 0.05. The coefficient of variation (CV%) was determined as a variability index. The interaction between the traits was evaluated by carrying out correlation between population means for each morphological trait using bivariate Pearson correlation coefficient as follows:

**Table 2.** Morphological traits analyzed of Moroccan natural populations of *Juniperus turbinata* Guss

Traits	Label
Plant traits	
Plant height	PHe
Plant habit	PHa
Trunk circumference	TC
Number of trunks per plant	NTP
Type of leaves	TL
Traits of second-order branches	
Branch length	BLe
Branch width	BWi
Number of first order branchlets per branch	NFOB
Traits of the branchlets	
Length of the first order branchlet	LeFOB
Width of the first order branchlet	WiFOB
Number of penultimate-order branchlets per first-order branch	NPOB
Number of last-order branchlets per penultimate-order branch	NLOB
Number of galbules per first-order branchlet	NGFOB
Traits of leaves	
Color of leaves	CL
Number of leaves per whorl	NLW
Traits of galbules	
Galbule weight	GWe
Galbule width	GWi
Galbule length	GLe
Galbule circumference	GC
Galbule diameter (average of 2 independent measures of angle of 90°)	GD
Ratio: length/diameter	RLeD
Ratio: length/width	RLeWi
Shape of the galbule (lateral view)	SG
Color of galbules	CG
Number of seeds per galbule	NSG

$$r_{xy} = \frac{cov(x,y)}{\sqrt{var(x)} \cdot \sqrt{var(y)}} \quad (3)$$

where:  $cov(x, y)$  is the sample covariance of  $x$  and  $y$ ;  $var(x)$  is the sample variance of  $x$ ; and  $var(y)$  is the sample variance of  $y$ .

All these analyses were carried out by using IBM SPSS software version 25.0.0.

The dissimilarity between populations was measured using Euclidean distance according to the following formula (Anderson, 1984) and using XLSTAT® software version 2014.1.

$$D(x, y) = [\sum_i (xi - yi)^2]^{1/2} \quad (4)$$

where:  $D$  is the Euclidean distance, and  $(xi, yi)$  are the Cartesian coordinates of two points.

The generated triangular distance matrix was used as input data for principal coordinate analysis (PCoA, Legendre and Legendre, 1998) using XLSTAT® software version 2014.1, while cluster analysis was carried on rectangular matrix of mean values of measured characters for populations using Euclidean distance and UPGMA method (Sneath and Sokal, 1973; STATISTICA® version 5 software). Then, scatter plots was created according to the two first components (F1 and F2) to ordinate and classify populations.

## RESULTS

### Morphological description

The analysis of variance showed highly significant differences between all the populations studied for all traits measured. This result was confirmed by Tukey’s honest significant difference (HSD) test, which measures the smallest significant difference between populations.

Plant height (PHe) varied considerably between populations, with a maximum mean of  $7.15 \pm 1.60$  m recorded in the ESS population and a minimum mean of  $1.13 \pm 0.23$  m recorded in the SAK population (Figure 2-E, F). The two traits, trunk circumference (TC) and total number of trunks per plant (NTP), had obtained the highest coefficients of variation, 83.33% and 71.91% respectively. Trunk circumference (TC) ranged considerably from  $126.08 \pm 56.13$  cm in the ESS population to  $11.46 \pm 2.83$  cm in the SAK population. The total number of trunks (NTP) varied in the range of 10 for both the MAR and SAK populations and  $1.23 \pm 0.80$  for the ESS population.

The number of first order branchlets per branch (NFOB, Figure 2-C) changed considerably between populations, the lowest  $21.85 \pm 5.60$  is registered in the BTD population and the highest  $38.69 \pm 6.68$  is recorded in the AAL population with an overall mean of  $29.09 \pm 7.41$ . For the number of penultimate-order branchlets per first-order branch (NPOB), the overall mean recorded is  $18.25 \pm 6.44$ , with the maximum mean of  $24.13 \pm 6.93$  is observed in the MAR population, while the minimum mean of  $12.54 \pm 4.29$  is observed in the BTD population. The number of last-order branchlets per penultimate-order branch (NLOB, Figure 2-A) ranged also considerably between populations, with a maximum mean of

6.18±1.11 recorded in the SKG population and a minimum mean of 3.10±0.74 in the MEH population. The overall mean is 4.72±1.51.

The number of galbules per first-order branchlet (NGFOB, Figure 2-B) recorded an important range of variation with a coefficient of variation of 67.54%. Hence, the lowest number (1.55±0.62) was registered by the SAK population and the highest by (7.77±3.05) by the SKR population, with an overall mean of 4.16±2.81. For the number of leaves per whorl (NLW), the mean ranged from 2.28±0.45 in the SKR population to 2.78±0.41 in the SAK population, with a mean of 2.47±0.50 (Table 3).

The mean galbule weight (GWe) showed also an important variability between populations with a coefficient of variation of 42.10%. The lowest value 0.11±0.03 g was recorded in the SAK population, while the highest 0.63±0.17 g was obtained by the DEM population, with a mean of 0.43±0.18 g. Fruit sizes, also recorded an important range of variation. Mean galbule width (GWi) ranged from 6.25±0.57

mm in the SAK population to 10.93±1.01 g in the DEM population, with a mean of 9.35±1.45 mm. Mean galbule length (GLe) varied from 6.08±0.70 mm in the SAK population to 10.96±0.99 g in the DEM population, with a mean of 9.72±1.52 mm. Galbule diameter (GD) changed considerably between populations, with the minimum mean 6.16±0.57 mm observed in the SAK population, while the maximum mean 10.60±0.96 mm is observed in both the AZI and ASG populations, with an overall mean of 9.53±1.41 and a CV of 14.86%.

The galbule length/diameter ratio (RLeD) varied narrowly between populations as revealed by a weak value of CV (4.55%). So, the minimum was observed in the AAL population (0.99±0.04) and the maximum 1.06±0.05 in the ESS population, with an overall mean of 1.02±0.05. Galbule length/width ratio (RLeWi) ranged from 0.97±0.09 in the SAK population to 1.13±0.11 in the ESS population, with an overall mean of 1.04±0.10. The number of seeds per galbule (NSG) showed

**Table 3.** Mean values and ‘F’ value from one-way ANOVA of morphological quantitative traits analyzed in red juniper natural populations

Popu- lation	BLe	BWi	NFOB	LeFOB	WIFOB	NPOB	NLOB	NGFOB	NLW	GWe	GWi	GLe	GC	GD	RLeD	RLeWi	NSG	PHe	TC	NTP
SDA	24.97± 5.04c	4.65± 0.69bc	31.82± 0.86e	7.04± 2.42a	1.84± 0.39b	18.83± 5.87e	4.82± 1.29d	4.18± 1.78e	2.38± 0.49ab	0.33± 0.09b	8.43± 0.96b	9.30± 0.86b	30.51± 2.75b	8.86± 0.82b	1.05± 0.05d	1.11± 0.10e	2.75± 0.87a	2.15± 0.75b	25.69± 7.58c	2.92± 1.50e
MAR	35.12± 6.91g	4.65± 0.62bc	33.94± 7.18f	12.21± 3.95h	1.61± 0.29a	24.13± 6.93h	4.87± 1.60d	4.69± 1.59f	2.39± 0.49abc	0.52± 0.14fg	9.34± 0.91de	10.40± 0.86e	33.50± 2.71d	9.87± 0.78ef	1.05± 0.04d	1.12± 0.10e	4.86± 1.21d	2.02± 0.69b	16.46± 4.77ab	10.00± 0.00j
MEH	25.38± 5.72c	3.88± 0.70a	27.37± 8.96bc	7.38± 2.67ab	1.64± 0.30a	20.33± 7.48fg	3.10± 0.74a	2.65± 1.29b	2.42± 0.49bcd	0.41± 0.10cd	9.56± 0.64e	9.75± 0.76cd	33.71± 2.31d	9.66± 0.63de	1.01± 0.03bc	1.02± 0.07bc	5.18± 1.02ef	6.62± 1.34g	93.31± 27.45i	1.54± 0.64b
ESS	28.64± 5.34e	4.64± 0.81bc	28.53± 5.76cd	11.00± 3.51g	1.98± 0.40c	20.76± 6.14fg	5.52± 1.47e	6.89± 2.92g	2.48± 0.50bcd	0.32± 0.13b	8.34± 1.01b	9.38± 3.67b	31.18± 3.67b	8.86± 1.02b	1.06± 0.05d	1.13± 0.11e	3.94± 0.97c	7.15± 1.60h	126.08± 56.13j	1.23± 0.80a
SAK	18.30± 3.86a	5.04± 0.97e	30.03± 8.23d	7.82± 2.19bc	2.06± 0.50cde	19.28± 5.44ef	5.37± 1.35e	1.55± 0.62a	2.78± 0.41e	0.11± 0.03a	6.25± 0.57a	6.08± 0.70a	23.01± 2.50a	6.16± 0.57a	0.99± 0.04a	0.97± 0.09a	3.78± 1.43c	1.13± 0.23a	11.46± 2.83a	10.00± 0.00j
BTD	25.86± 6.97cd	4.82± 0.86cde	21.85± 5.60a	8.06± 2.36bcd	2.13± 0.43def	12.54± 4.29a	4.52± 1.59d	3.19± 1.83cd	2.54± 0.50d	0.33± 0.10b	9.03± 0.97c	9.32± 0.86b	32.05± 2.61c	9.17± 0.82bc	1.02± 0.05bc	1.04± 0.10cd	5.33± 1.22f	4.83± 1.26f	56.15± 28.21g	3.00± 1.52e
TZO	20.62± 4.07b	4.89± 0.78de	27.41± 4.80bc	8.58± 2.05de	2.15± 0.40ef	15.68± 4.12b	4.61± 0.75d	6.61± 2.84g	2.40± 0.49abcd	0.43± 0.13d	9.45± 0.95de	9.95± 1.01d	32.49± 4.34c	9.70± 0.89de	1.03± 0.04c	1.06± 0.09d	4.91± 1.48de	2.68± 0.98c	33.15± 11.72de	4.23± 1.05gh
SKR	24.52± 3.83c	4.93± 0.59de	29.64± 4.19d	9.63± 1.68f	2.17± 0.33f	17.15± 4.08bcd	6.18± 1.11f	7.77± 3.05h	2.28± 0.45a	0.39± 0.10c	9.22± 0.78cd	9.68± 0.77c	32.70± 2.42c	9.45± 0.70cd	1.02± 0.04c	1.05± 0.08d	6.41± 1.40h	3.42± 0.65d	43.00± 28.51f	4.38± 1.78h
IML	26.13± 7.41cd	4.94± 0.98de	26.84± 6.35b	7.48± 2.51abc	2.03± 0.47cd	16.18± 5.80bc	4.12± 1.39c	3.40± 2.01d	2.42± 0.49bcd	0.44± 0.14d	9.83± 1.08f	10.20± 1.06e	35.39± 3.11e	10.02± 0.98ef	1.02± 0.04bc	1.04± 0.09cd	5.42± 1.19f	3.95± 1.84e	56.23± 38.96g	3.54± 1.74f
AZI	28.99± 8.03e	4.74± 0.93bcd	26.28± 6.61b	8.72± 3.09de	1.99± 0.47c	18.04± 6.65de	4.74± 1.44d	2.76± 1.08bc	2.53± 0.50cd	0.54± 0.12gh	10.46± 0.8h	10.74± 0.77fg	36.46± 2.47f	10.60± 0.74g	1.01± 0.03bc	1.03± 0.06bc	5.22± 1.08ef	3.41± 0.90c	42.31± 16.98f	1.92± 0.92c
DEM	25.06± 5.87c	4.58± 0.87b	26.39± 5.44b	7.48± 2.69abc	1.99± 0.44c	17.95± 6.37de	4.83± 1.47d	2.76± 1.22bc	2.41± 0.49abcd	0.63± 0.17i	10.93± 1.01i	10.96± 0.99g	37.76± 3.48g	10.95± 0.81g	1.00± 0.05ab	1.01± 0.11b	3.22± 1.23b	3.33± 1.32d	36.31± 13.00e	2.77± 1.19e
ORK	28.79± 6.78e	3.95± 0.81a	28.92± 5.33cd	8.19± 3.32cd	1.75± 0.38b	15.84± 5.75bc	3.71± 1.10b	2.45± 1.26b	2.47± 0.50bcd	0.47± 0.11e	10.11± 0.69g	10.33± 0.81e	35.38± 2.36e	10.22± 0.68f	1.01± 0.03bc	1.02± 0.06bc	5.98± 1.27g	2.81± 1.03c	29.62± 5.00cd	2.23± 0.89d
ASG	27.11± 5.42d	5.43± 1.00f	29.48± 5.56d	9.02± 2.94ef	2.24± 0.54f	17.28± 5.52cd	4.19± 1.49c	6.85± 3.06g	2.50± 0.50bcd	0.57± 0.15h	10.48± 0.8h	10.1± 1.23f	37.12± 2.92fg	10.60± 0.96g	1.01± 0.04bc	1.02± 0.07bc	6.93± 1.64i	5.00± 0.68f	66.23± 24.84h	4.00± 1.36g
AAL	31.04± 6.65f	5.37± 1.03f	38.69± 6.6g	8.79± 2.69de	2.14± 0.45def	21.45± 5.92g	5.57± 1.08e	2.47± 1.38b	2.54± 0.50d	0.50± 0.21ef	9.49± 1.43e	9.26± 1.46b	34.28± 4.07d	9.37± 1.40cd	0.99± 0.04a	0.98± 0.07a	5.42± 1.35f	1.96± 0.75b	19.69± 6.50b	4.69± 1.49i
Mean	26.47± 7.18	4.75± 0.94	29.09± 7.41	8.67± 3.10	1.98± 0.46	18.25± 6.44	4.72± 1.51	4.16± 2.81	2.47± 0.50	0.43± 0.18	9.35± 1.45	9.72± 1.52	33.25± 4.66	9.53± 1.41	1.02± 0.05	1.04± 0.10	4.95± 1.69	3.60± 2.01	46.84± 39.03	4.03± 2.90
CV%	27.14	19.79	25.47	35.80	23.31	35.27	32.00	67.54	20.23	42.10	15.51	15.52	14.02	14.86	4.55	9.49	34.14	55.82	83.33	71.91
F value	62.18*	31.15*	49.96*	35.61*	22.77*	31.99*	48.69*	134.47*	7.49*	131.11*	203.98*	195.14*	188.55*	233.12*	38.6*	38.38*	114.31*	324.01*	216.34*	680.42*

**Note:** \*Means are significantly different at 0.1% for all traits. Bold are minimum and maximum values.

considerable variability between populations, with CV of 34.14%. The lowest value  $2.75 \pm 0.87$  was recorded in the SDA population, whereas the highest  $6.93 \pm 1.64$  was obtained in the ASG population, with an overall mean of  $4.95 \pm 1.69$ .

High amount of variability was observed among evaluated populations based on qualitative traits of phenotypic evaluation (Table 4). The majority of the populations showed a predominance of “scale” type leaves (71.4%), while the remaining bore both scales and needles (28.6%), with a total absence of trees with needles only (data not shown). The most frequent color of leaves was green (42.7%), followed by light green (33%), while the other colors (yellow-green and dark-green) were less frequent (16.8% and 7.5%, respectively). Among the three shapes of galbule, the ‘elongate’ shape was the most dominant (55.2%), followed by ‘flat’ shape (27.4%) and ‘round’ (17.4%). In terms of galbule color, around half the populations had a red-brown tint (48.4%), followed by brick-red (34.7%) and orange-brown (16.9%).

### Correlations between traits

To evaluate the force and direction of associations between morphological traits measured and calculated in the 14 *Juniperus turbinata* populations, a Pearson correlation analysis was performed, as shown in Table 5.

Several significant correlations, both positive and negative, were observed between these morphological traits. Galbule weight (GWe) was positively and significantly correlated with galbule width (GWi) ( $r = 0.88$ ), galbule length (GLE) ( $r = 0.82$ ), galbule circumference (GC) ( $r = 0.85$ ) and galbule diameter ( $r = 0.89$ ). Galbule circumference (GC), besides its positive and significant correlation with galbule weight, was also positively and significantly correlated with galbule width (GWi) ( $r = 0.88$ ), galbule length (GLE) ( $r = 0.84$ ) and galbule diameter ( $r = 0.89$ ). Galbule

length (GLE) showed a significant positive correlation with galbule width (GWi) ( $r = 0.82$ ) and galbule diameter (GD) ( $r = 0.95$ ). The length of first-order branchlets (LeFOB) was positively and significantly associated with the number of penultimate-order branchlets per first-order branch (NPOB) ( $r = 0.51$ ) and the number of last-order branchlets per penultimate-order branch (NLOB) ( $r = 0.36$ ). There was also a significant positive correlation between the number of seeds per galbule (NSG) and galbule dimensional traits [GWe ( $r = 0.36$ ), GWi ( $r = 0.38$ ), GLE ( $r = 0.29$ ), GCi ( $r = 0.36$ ) and GD ( $r = 0.35$ )].

The plant height (PHe) was positively and significantly correlated with trunk circumference (TC) ( $r = 0.80$ ), while it was negatively and significantly correlated with number of trunks per plant (NTP) ( $r = -0.47$ ). The number of trunks per plant (NTP) was negatively and significantly correlated with galbule width (GWi) ( $r = -0.33$ ), galbule length (GLE) ( $r = -0.32$ ) and galbule circumference (GC) ( $r = -0.33$ ).

### Populations’s classification

To evaluate the genetic distances between the populations studied, a dissimilarity matrix was drawn up (Table 6). The smallest distance was recorded between the BTD and IML populations ( $D = 7.24$ ), AZI and DEM ( $D = 7.73$ ), SKR and AZI ( $D = 9.21$ ) and, between TZO and DEM ( $D = 9.408$ ). The largest distances were observed between ESS and SAK ( $D = 116.11$ ), ESS and MAR ( $D = 110.53$ ), ESS and AAL ( $D = 107.26$ ) and ESS and SDA ( $D = 100.79$ ).

The diagram constructed from the two components (F1 and F2) of PCoA showed a wide dispersion of the populations studied. Three groups of populations could be distinguished (Fig. 3).

The first group is composed of two populations, Essaouira (ESS) and Mehdiya (MEH) originating from Littoral and are distinguished by their

**Table 4.** Dominant frequencies of qualitative traits of Moroccan natural populations of *Juniperus turbinata* Guss

Trait	Label	Evaluation scale	Dominant character	Frequency %	ANOVA signification
Plant habit	PHa	Conical; spread; columnar; creeping	Columnar (3)	58.2	***
Type of leaves	TL	Scales; needles; scales and needles	scales (1)	71.4	***
Color of leaves	CL	Light-green; yellow-green; green; dark-green	green (3)	42.7	***
Shape of the galbule (lateral view)	SG	Flat; round; elongate	Elongate (3)	55.2	***
Color of galbules	CG	Orange-brown; brick-red; red-brown	red brown (3)	48.4	***

**Table 5.** Correlation matrix among some important morphological traits in the evaluated Moroccan *Juniperus turbinata* populations

	BLE	BWi	NFOB	LeFOB	WiFOB	NPOB	NLOB	NGFOB	NLW	GWe	GWi	GLe	GCI	GD	RLeD	RLeWi	SG	NSG	Phe	PHa	TC	NTP
BWi	.33***																					
NFOB	.42***	.27***																				
LeFOB	.33***	.21***	.11***																			
WiFOB	-.03	.29***	-.03	.33***																		
NPOB	.24***	.14***	.33***	.51***	.11***																	
NLOB	.05*	.24***	.10***	.36***	.22***	.26***																
NGFOB	-.007	.09***	.01	.16***	.04	.01	.14***															
NLW	-.05*	.01	.006	-.04	.01	-.006	-.01	-.08***														
GWe	.22***	.03	.06**	.01	.005	.02	-.10***	-.001	-.09***													
GWi	.21***	-.004	-.038	-.05*	.008	-.07***	-.19***	-.001	-.11***	.88***												
GLe	.23***	-.04	-.04*	.034	-.04	-.05*	-.15***	.10***	-.13***	.82***	.82***											
GC	.23***	.006	-.02	-.01	.02	-.05*	-.16***	.01	-.10***	.85***	.88***	.84***										
GD	.23***	-.03	-.04	-.008	-.02	-.06**	-.17***	.05*	-.12***	.89***	.95***	.95***	.89***									
RLeD	.07**	-.06**	-.01	.14***	-.05*	.03	.06**	.19***	-.05*	-.06**	-.24***	.33***	-.02	.05*								
RLeWi	.06**	-.06**	-.01	.14***	-.08***	.04	.07**	.19***	-.05*	-.07***	-.25***	.32***	-.03	.04	.99***							
SG	.06**	-.04*	-.025	.10***	-.05*	.03	-.003	.16***	-.09***	-.01	-.02	.19***	.023	.10***	.80***	.38***						
NSG	.12***	.07**	.04	.05*	.04	-.06**	-.07***	.12***	-.008	.34***	.38***	.29***	.36***	.35***	-.15***	-.17***	-.03					
PHe	.006	-.12***	-.21***	.05*	-.008	-.02	-.14***	.17***	-.04*	.02	.11***	.19***	.15***	.17***	.14***	.15***	.11***					
PHa	-.01	.12***	-.03	.03	.11***	-.05*	.12***	-.06**	.04	-.10***	-.13***	-.14***	-.12***	-.14***	-.03	-.03	-.04	-.01	-.22***			
TC	.003	-.11***	-.17***	.08***	.01	.02	-.07***	.16***	-.04*	0	.06**	.12***	.09***	.09***	.12***	.11***	.14***	.04	.80***	-.19***		
NTP	-.006	.15***	.24***	.14***	-.02	.16***	.15***	-.04	.07**	-.17***	-.33***	-.32***	-.33***	-.34***	-.02	-.01	-.06**	-.03	.47***	.35***	.47***	
TL	.18***	-.19***	.11***	.15***	-.26***	.27***	-.06**	.10***	-.06**	-.11***	-.18***	-.005	-.14***	-.10***	.32***	.33***	.26***	-.28***	.26***	-.29***	.30***	-.02

**Table 6.** Matrix of the estimated Euclidean genetic distance between studied *Juniperus turbinata* populations

	SDA	MAR	MEH	ESS	SAK	BTD	TZO	SKR	IML	AZI	DEM	ORK	ASG
MAR	17.70												
MEH	68.11	78.63											
ESS	100.79	110.53	33.67										
SAK	19.55	22.74	83.72	116.11									
BTD	32.96	44.97	38.53	71.02	48.33								
TZO	11.16	25.60	60.89	93.63	25.98	24.83							
SKR	18.75	30.64	51.09	83.48	35.22	16.99	11.28						
IML	31.70	43.02	37.55	70.52	48.23	7.24	24.21	14.98					
AZI	19.46	29.91	51.44	84.28	37.11	16.70	14.11	9.21	14.59				
DEM	14.44	26.35	57.41	90.47	31.65	22.15	9.41	11.44	20.39	7.73			
ORK	9.62	19.91	64.12	96.99	26.69	28.18	10.73	15.84	26.94	13.32	9.32		
ASG	41.77	51.85	28.27	60.55	58.42	15.23	34.30	24.02	11.44	24.79	30.80	37.16	
AAL	12.64	10.26	74.99	107.26	22.16	41.69	21.76	26.86	39.24	26.46	22.49	15.70	48.25

large, columnar trees and high height over 9 m (Figure 2-E). Besides, the trees of this group had a large trunk circumference, varying between 95 cm and 125 cm, and large-elongated galbules.

The second group comprised three populations: Boutfarda (BTD) and Imilchil (IML) coming from Middle Atlas, and Amesguen (ASG)

sampling from High Atlas. The trees of these populations are shrubs (Figure 2-F) measuring 4 to 5 m in height with a high number of seeds per galbule (5 to 7).

The third group is subdivided into two subgroups. The first subgroup is composed of three populations: Martil (MAR, Littoral). Sakka (SAK,

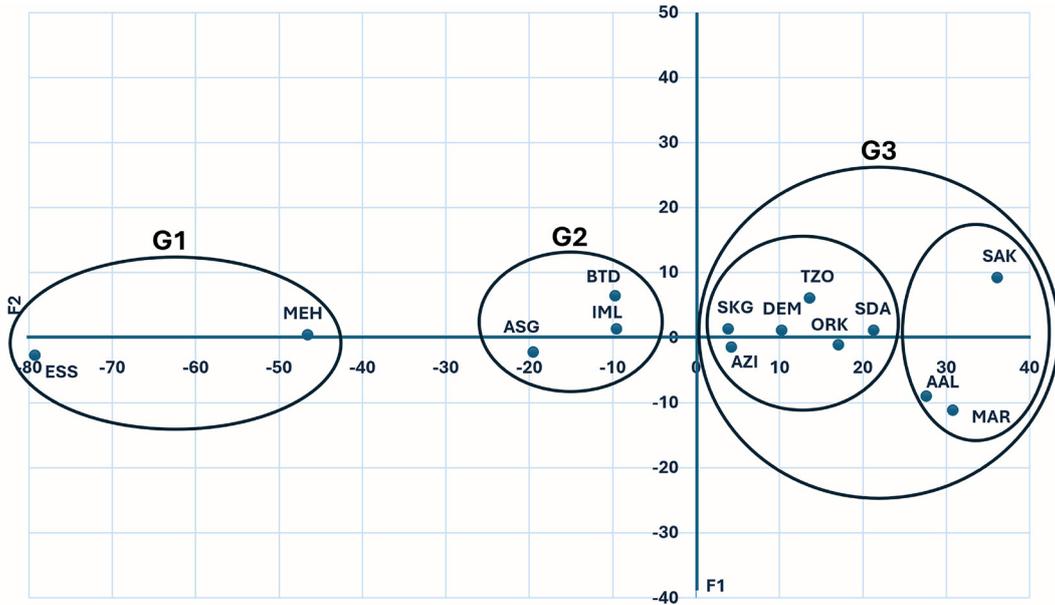


Figure 3. Scatter plot of the studied *Juniperus turbinata* populations, based on the two principal components F1 and F2

Rif) and Aït Abdellah (AAL, Anti-Atlas). These populations are characterized by small shrubs, 1 to 2 m in height, with a creeping habit (Fig. 2-F), a very high number of trunks per shrub exceeding 10, and a very small trunk circumference. The galbules are small and rounded and the number of galbules per branchlet (NGFOB) is low, varying between 2 and 4. Branches and branchlets, though short and narrow, are grouped together in compact clumps.

The second subgroup is composed of six populations: Saïdia (SDA), Demnate (DEM), Azilal (AZI), Ourika (ORK), Tazouta (TZA) and Skoura M'Daz (SKR). These populations are characterized by short shrubs (Fig. 2-F), varying in height

from 2 m to 3.5 m, low number of trunks per plant ranging from two to four, very elongated galbules, highest galbule weight reaching 0.63 g and leaves arranged in whorls in groups of two (Fig. 2-D).

The agglomerative hierarchical clustering of the 14 populations studied based on 25 measured traits, permitted to identify 3 groups (Fig. 4).

The first group, distinctly different from the others, included two populations from the Atlantic coast, Essaouira and Mehdia that belong respectively to arid and semi-arid zones.

The second group included three populations, one from the Middle Atlas (Boutfarda BTD) and two from the High Atlas (Imilchil IML and

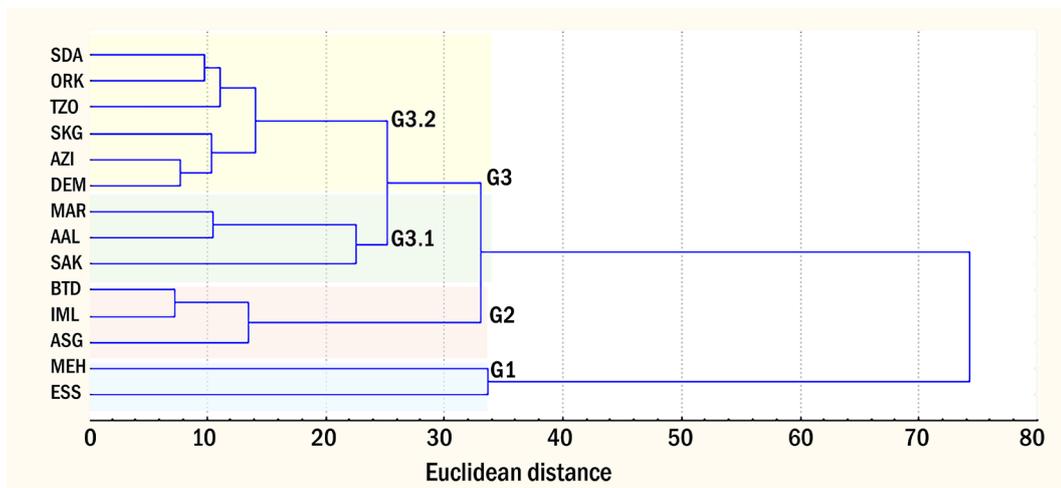


Figure 4. Dendrogram of *Juniperus turbinata* Guss populations based on morphological traits and constructed according to UPGMA method (Abbreviations as in Table 1)

Amesguen ASG). These three populations come from the semi-arid zones.

The third group bifurcated in two subgroups: the first subgroup is formed by three populations of different origins: Martil (MAR) from the Mediterranean littoral, Sakka (SAK) from the Pre-RIF and Ait Abdellah (AAL) from the Anti-Atlas. These three populations, Martil, Sakka and Ait Abdellah, were sampled respectively from semi-arid, humid and arid zones.

The second sub-group included six populations, one from the Mediterranean Littoral (Saïdia SDA), three from the High Atlas (Demnate DEM, Azilal AZI and Ourika ORK) and two from the Middle Atlas (Tazouta TZA and Skoura M'Daz SKR). These populations originating from three different bioclimatic zones (respectively arid, semi-arid, and humid bioclimate).

Consequently, the genetic structure of the 14 populations studied within three main groups was not correlated with their geographical origins and bioclimatic zones.

## DISCUSSION

The results of the morphological variability in Moroccan *Juniperus turbinata* populations showed great diversity. Thus, 80% (16 out of 20) of the quantitative characters measured had a CV greater than 15%, and over 45% (9 out of 20) had a CV greater than 30%.

For plant height (PHe), our measurements align with the range of heights reported in the literature, from 1 m to 7 mm (Maire, 1952; Quezel & Gast, 1998; Benabid, 2000; Adams, 2008). Concerned, trunk circumference (TC), the obtained values are lower than the averages of 60 cm reported by Maire (1952) but higher than 100 cm value mentioned by Farjon (2017).

Regarding the number of first-order branchlets per branch (NFOB), our results indicate significantly higher values than those reported by Medini, who found a range of 0.23–9.00. Our data for first-order branchlet length (LeFOB) were lower than those of Medini, who observed a wider range (9.02–19.40 cm, (Medini et al., 2016).

Our results for the number of seeds per galbule (NSG) varying in a range of 3–7 are comparable with the values reported by Romo et al., (2019), and also in line with those of Mazur et al. (2003), where the coefficient of variation was

over 30% in all three populations studied, although individual populations showed a slightly lower CV, varying between 20% and 24%, with a higher mean of seeds per galbule (6.39). Mazur and their coauthor's study underlines that seed numbers are a crucial trait for distinguishing subspecies, particularly in the Iberian Peninsula. In a study carried out in eastern Morocco, Sahib et al. (2022) reported an average of seeds per galbule (4.84) very close to ours. The Moroccan populations studied by Mazur et al. (2010) showed averages of 6.09 to 7.52 seeds for *Juniperus turbinata*, a slightly higher than ours. In another study, Mazur et al. (2016) reported averages for Sicilian populations (6), *Juniperus phoenicea* (7.77) and *Juniperus turbinata* (6.20), values generally higher than our results.

Varying in a range of 0.97–1.13, our results on galbule length/width ratio (RLeWi) are similar with those found in Algerian populations (Elmir et al., 2024). For maritime populations, the mean ratio was slightly higher (1.12) with a CV of 11.07%, while for mountainous populations, the mean was 1 with a CV of 9.52%, which is very similar to our coefficient of variation of 9.49% (Elmir et al., 2024).

Our finding on galbule length (GLe) showed an average of 9.72 mm, and a CV of 15.52%. These results are very close to those obtained by Mazur et al. (2018) for *Juniperus turbinata* populations in Morocco, which showed an average of 9.85 mm, but with a lower CV (13.3%). Whereas, the authors reported a shorter mean length of 7.88 mm and a CV of 11.84% for *Juniperus phoenicea* populations. Other studies, such as that of Sahib et al. (2022) in eastern Morocco, reported an even shorter mean length of 7.82 mm.

Regarding galbule width (GWi), the data obtained were very similar to those reported by Mazur et al. (2003), who found an average of 9.29 mm with a lower CV of 10.93%. Our results also showed an average galbule weight of 0.43 g with a CV of 30.59%, which is slightly higher than the data of Lebreton and Louis Perez (2001), who reported lower averages, notably 0.30 g for populations from Crete and Cyprus, and 0.243 g for those from the Arabic Maghreb. Populations from the Canary Islands were revealed with higher average weight of 0.440 g (Lebreton & Pérez De Paz, 2001).

For mean galbule diameter (GD), Mazur et al (2010) found slightly higher mean diameters for Moroccan populations of *Juniperus turbinata*,

varying between 9.68 mm and 10.62 mm (Mazur et al., 2010). Study of Algerian populations showed slightly smaller diameters, with an average of 8.38 mm for maritime populations and 8.78 mm for mountainous populations (Elmir et al., 2024).

Concerning the ratio of length to diameter (RLeD), our results were close to those reported by Mazur et al. (2010) for Moroccan populations, which ranged from 1.01 to 1.02 mm, with an average of 1.04. In contrast, Elmir's results for Algerian populations showed slightly higher values, particularly for maritime populations, with a mean of 1.19 and a CV of 11.24% (Elmir et al., 2024).

Our results on the number of leaves per whorl (NLW) showed an average of 2.47 with a CV of 20.23%. Furthermore, 53.30% of individuals were with two leaves per whorl, while 46.70% were with three leaves per whorl. These observations concurred with those of Adams (2008), Quezel & Gast (1998), Farjon (2017) and Pavon et al. (2020) who stated that juvenile *Juniperus* leaves are needle-shaped, whereas mature leaves are scale-shaped. They are arranged either in pairs, or in whorls of three.

The positive and significant correlations between the number of seeds per galbule (NSG) and galbule dimensional traits (GWe, GWi, GLe, GCi and GD) obtained in this study were strengthened by those found by Mazur et al. (2018) in a study carried out on *Juniperus phoenicea* s.l. populations sampled at various localities in the Mediterranean basin. Grouping populations of *Juniperus turbinata* revealed either by hierarchical clustering or PCoA showed their differentiation into three distinct groups. The population's distribution was operated according to their discriminating morphological traits, irrespective of their geographical origin (mountainous or coastal). This statement was found for several species in Morocco, namely *Euphorbia resinifera* (Abd-dada et al., 2023), *Malus x domestica* Borkh (Khachtib et al., 2020) and *Prunus domestica* L. (Ait Bella et al., 2021). These results are different from those obtained by Medini et al. (2016) in his study of *Juniperus phoenicea* populations in Tunisia. Indeed, these authors found a grouping of populations in two distinct groups according to their geographical origin: the first was composed of coastal populations and the second formed of continental populations (Medini et al., 2016).

## CONCLUSIONS

The morphological study of the fourteen populations of red juniper (*Juniperus turbinata* Guss.) collected from the species' Moroccan range, based on morphological traits, revealed great diversity among the populations studied across all measured characters. Similarly, significant phenotypic variability was observed in the genetic material from other Mediterranean regions (France, Spain, Tunisia, Algeria, Jordan, etc.). This high diversity is mainly due to the characteristics of the galbule and the plant, as well as to the peculiarities of the branches, branchlets and leaves. Principal coordinate analysis and hierarchical clustering classified these populations into three distinct groups, independently of their geographical origins (mountain or littoral) and bioclimatic zones.

The finding revealed that Moroccan *Juniperus turbinata* is characterized by creeping habit, high number of trunks per plant, as well as the tolerance of environmental constraints (extreme temperatures and low rainfall). These results are prompting researchers to explore its use in combating desertification and soil erosion in the most arid zones.

The present study has provided important results that can contribute to the implementation of an effective breeding program, as well as to the management and conservation of red juniper genetic resources in Morocco. Additional studies, currently underway, are needed to confirm this diversity by using biochemical and molecular markers.

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