# Biometry of Acorn of the Putative Oak from Akfadou Forest in North Africa (Algeria)

Amel Akli<sup>1</sup><sup>1</sup>, Khellaf Rabehi<sup>2</sup>, Enrique Torres Alvarez<sup>3</sup>, Ricardo Alia<sup>4</sup>, Lynda Kheloul<sup>5</sup>, Mahand Messaoudene<sup>6</sup>

<sup>1</sup>University Tizi Ouzou-Mouloud Mammeri, Algeria, INRF, Azazga and University de Huelva, Spain <sup>2</sup>University Tizi Ouzou-Mouloud Mammeri, Algeria, INRF, Azazga, Algeria <sup>3</sup>University de Huelva, Huelva, Spain <sup>4</sup>INIA-CSIC. Avda A Coruña sn., Madrid, Spain <sup>5</sup>University Mouloud Mammeri, Algeria <sup>6</sup>INRF-Institute National of Research Forestery, Azazga, Algeria

#### ABSTRACT

In this study, we are interested in the acorn biometry of the putative oak of Akfadou forest in Algeria. We measured the size of acorns of 34 putative species' trees collected from 9 stands in the Akfadou forest massif (Algeria) and then we evaluated the growth of the seedlings by sowing the acorns in a nursery. We found that the performance of oak seedlings is strongly influenced by a "stand effect" and a "close pure species effect." Indeed, the position and the ecological region influence acorn morphology. The growth of putative oaks in the nursery is remarkably homogeneous in terms of seedling height and diameter. However, the "close pure species" effect appears in the case of the high altitude stand where holm oak is present. This diversity is probably the consequence of genetic variations and/or environmental conditions of the stands. With climate change and decreasing precipitation, the frequency of dry years will only increase. These putative hybrids trees could constitute a future alternative for reforestation, improvement, and diversification of species in Algeria.

Keywords: Akfadou massif, biometric parameters, Hybrid species, morphological parameters, Pure species, Quercus.

#### Introduction

The Mediterranean basin is considered an undisputed hotspot for global biodiversity, due to the diversity and richness of its flora. Indeed, it shelters approximately 10% of the flora known in the world on an area representing less than 2% of the surface of the earth, and about half of these species are found nowhere else in the world (Radford et al., 2011).

*Quercus* genus L. (oaks) is one of the most important economical and ecological tree genera, widespread in the Northern Hemisphere (Kubitzki, 1993; Messaoudene et al., 2007; Nixon, 1993). Oaks are cultivated in the Northern Hemisphere, but ornamental or forestry is also cultivated in the Southern Hemisphere (Vázquez et al., 2018). It is one of the richest in species, which includes about 500 forest species accompanied by many hybrids and is well represented in the spontaneous state throughout the Mediterranean basin (Bonfils et al., 2005).

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Corresponding Author: Amel Akli e-mail: amel.akli@alu.uhu.es

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Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International Licence *Quercus* L. is one of the most species-rich forest genera with up to 400 woody species of temperate and Mediterranean zones, including America, Europe, and Asia, among which some species are of high socio-economic and ecological importance (Nsibi et al., 2003; Sarir & Benmahioul, 2017).

Algeria has six species of oaks: *Quercus canariensis* Willd, *Quercus afares* Pomel, *Quercus suber* L, *Quercus coccifera* L, *Qfaginea*, and *Quercus ilex* L (Aissi et al., 2019; Cuénod et al., 1954; Hasnaoui, 1992). These species cover nearly 40% of the Algerian forest and play an undeniable role on the ecological, economical, and social levels (Alatou, 1994; Sarir et Benmahioul, 2017). Most oak species have a very complex evolutionary history, related to the existence of interspecific hybridization (Müller, 1952) and introgressions (Leroy et al., 2017 in Aissi et al., 2019; Hardin, 1975; Van Valen, 1976). Interspecific hybridization is a common phenomenon in oaks and can have important evolutionary consequences on taxonomy (Arnold, 1997 and Rieseberg, 1997; Matthew et al., 2004). The latter is complex due to the high variability and gene flow between these species (Mir et al., 2006); hence, the current representation of the range of these taxa needs to be reviewed and explored (Aissi et al., 2021).

The putative hybrids are detected from their morphological traits or using molecular markers (Cottam et al., 1982; Rusthon, 1993 in Mhamdi, 2013). Among the criteria for the infrageneric classification of the

genus *Quercus*, the morpho-anatomy of the floral organs, acorns, and their cups are mostly used to recognize different levels of taxa within the genus, especially they help to delineate species complexes (Maire, 1961).

Many authors in the world have analyzed the effects of certain techniques (culture substrates, volume and shape of containers, quality of plants, conservation of acorns, etc.) on the recovery and growth of oak, for example, Abourouh et al. (1995); Aissi et al.(2021); Aussenac et al.(1988); Beissalah et al.(1987); Belghazi et al.(2011); EL Abou (2009); EL Ghazi (2005); Favre (1970, 1977); Jarvis (1986); Josiah and Jones (1992); Lamhamedi et al.(2000); Merouani et al.(2001); Nahidi (2006); Riedacker (1986).

In Algeria, Vázquez et al. (2018) reported the presence of two hybrids in Akfadou (AK) which are *Q.numidica* and *Q.kabylica*, but Algerian experts observed the existence of several putative species that mix with the pure oaks in AK alongside the four pure species of the genus *Quercus (Q. canariensis, Q.afares, Q.ilex,* and *Q. suber*), making taxonomic delimitation delicate and unstable (Amaral, 1990; Bussotti & Grossoni, 1998; Maire, 1961; Trabut, 1892). Without knowing the parents with certainty, these hybrids would result from the crossing of these pure species (Vázquez et al., 2018).

We think approaching the hybridization phenomenon by the means of a biometric analysis of the acorns produced and morphological analysis of the plants results from breeding in the nursery. Acorn is very important because it constitutes the primordial organ of regeneration which strongly depends on the regularity and abundance of acorns, the size (energy value), and the phytosanitary state (Merouani et al., 2001; Suszka et al., 1994).

The objectives of this work were to study the acorn biometry of putative species in AK, to follow the growth and the behavior of plants in the nursery, and to demonstrate the existence of the stand and near-pure species effects.

This study constitutes an easy management tool for the forester. It allows describing the forest population and its evolution, re-evaluating their taxonomic definition.

#### Methods

#### **Study Area**

The AK forest is located approximately 160 km east of Algiers and 20 km from the sea. The forest massif covers an area of approximately 11,000 ha or 18% of the deciduous oak grove of Algeria. Its orography is quite complicated; it revolves around a succession of ridgelines oriented to the northeast and southwest. Generally, the relief is quite rugged (slopes of 15%–45%), especially in its south-eastern part, the altitude of AK varies from 800 to 1646 m. The climate is humid to temperate variant (Messaoudene et al., 2007).

Sampling was carried out in trees located in nine stands (plots) in the forest massif of AK (Figure 1). Putative hybrid trees were identified in these plots by local experts, and trees from the species close to the hybrids were also sampled and located using global positioning system.

The fall of acorns often occurs in the first week of October and is still carried out in November and even December. Approximately, five suspected hybrid trees per stand were sampled and 100 acorns/trees were

collected. Between the stands, a distance of 60 m was maintained to sample a maximum genetic variability. The presence of close pure species is also mentioned (see Table 1) (*Q.suber, Q.canariensis, Q.afares,* and *Q.ilex*).

#### **Biometry of Acorns**

In the laboratory, the acorns were first separated by tree and stand of origin. They were cleaned and sorted by the ordinary water flotation test, and the non-viable (floating) acorns were removed (Dupouey & le Bouler, 1989). Their morphological parameters were measured: weight (Pg), length (Log), width (Lar), and length to width ratio (Lar/Log) using a precision scale and digital caliper (Tilki & Alpetkin, 2005). Mean values of phenotypic traits were examined by the analysis of variance at the inter-stand level.

#### Seedlings Growth

To examine the variability in seedling growth and assess overall seedling development at inter-stands. The experiment launched in December 2015 was conducted in a nursery at the regional forestry research station of Azazga, (Figure 2a, b). once measured, the acorns are put to soak for a few minutes in water, to let them germinate in vermiculite saturated with water at an ambient temperature of 16°C in the dark for 20–30 days (to ensure good conservation) (Guibert & Pichon, 2001). The germinated acorns were sown in polyethylene bags (35 cm × 25 cm). The pots containing the seeds were arranged in a completely randomized, prevalent-growth design. The substrate on which the acorns were sown consisted of potting soil taken from the same sampling site of origin (forest soil). The pots were regularly weeded and watered, three times a week depending on the temperature of the day and variations in the water content of the substrate.

The pots are placed according to a randomized experimental design, taking into account the stand effect and nursery with regular watering.

The measurement of height and diameter are the variables mostly used in the morphological characterization of plants. Neck diameter is generally correlated with several morphological variables (height, total dry weight, and dry weight of the aerial part) because height is a good indicator of photosynthetic capacity and transpiration area which are closely correlated with leaf area. It is a variable that integrates the morphological response to environmental factors (Razika Sarir & Benamar benmahioul, 2017).

The experimental setup remained in place during the 2 years of cultivation. The measurements were made at the end of the second year. The following morphological parameters were measured to assess the vigor of the putative oak seedlings studied. Growth in languor: the languor of the stems was measured with a ruler graduated to the millimeter.

Growth in diameter: seedling diameter at the collar is measured with a digital caliper.

- Leaf production: the number of leaves per plant was monitored and recorded weekly. Indeed, the estimation of the number of leaves is a good indicator of the assimilative capacities of the plant and its production of biomass (Abdenbi et al., 2016).
- Root growth: it is important to focus on root growth, especially when it is known that some oaks favor root growth at the expense of aerial growth early in their development (Abdenbi et al., 2016).
- Lar/Log: ratio between the width and length of the acorns in % (lar=width; log=length).



#### Figure 1.

Map of Akfadou Showing the Position of All Inventoried Plots. Map Produced Using Mapinfo V 7.5 Software, Resized with CSE Extebted Photoshop Software.

#### Table 1.

Description of the Inventoried Stand of Akfadou (Hybrid/Stands)

				Number of Trees		Presence of Pure Species and Putativ Hybrids Trees			Putative	
Plots	Geographical Coordinates	Altitude (m)	Slope (%)	Sampled	Exposition	S	Z	А	V	Н
LN	36°41′47.74″N 4°35′56.99″E	1280	8	5	Ν	Х		XX		XX
AS	36°36′54.35″N 4°30′50.58″E	1220	20	3	S		XX	Х	XXX	XXX
CA	36°37′4.58″N 4°31′30.67″E	1400	43	2	S		XX	Х		XXX
VM	36°40′59.68″N 4°33″45.12″E	1160	15	4	SE		Х	XX		XX
тк	36°40′28.36″N 4°33′35.81″E	1092	20	3	NE		XX	Х	Х	XX
AK	36°37′31.29″N 4°31′52.69″E	1327	15	6	E		Х	XX	XX	XX
но	36°35′20.93″N 4°31′1.99″E	1031	12	4	S			Х	XX	XX
YA	36°44′56.88″N 4°26′9.10″E	1280	8	5	N	Х	Х	XX		XXX
СҮ	36°41′14.91″N 4°35′43.57″E	1166	20	6	E		Х	XX		XXX

Note: AS = Ait Salah; LN = Lac Noir; CY = Cynegetic Center; TK = Tala Kitten; AK = Akfadou; VM = Vide Mehaga; CA = Col Ait Salah; HO = Houra; YA = Yakouren; (S = Q.suber; Z = Q.canariensis; A = Q.afares; I = Q.ilex; X = presence.



#### Figure 2.

View of the Experimental Device Used for the Culture of Akfadou Oak Hybrids in the Nursery, Where Each Box Represents a Stand at the Start and After Germination.

#### Statistical Analyses

Using different stands as classification variables, the following analyses were performed. The main goal is to distribute the different stands (populations) examined in homogeneous classes of different hybrids from the point of view of the most characteristic biometric and morphological traits. Descriptive statistical analyses (min, max, X, CV,  $\delta$ ) were used to assess the inter-population (between stands) biometric and morphological variability of traits (**Pg**: acorn weight in grams; **Log**: acorn length in millimeter; **Lar**: acorn width in millimeter and **La**:height, **D**::diameter of the crown and collar **Nr**: number of branches, **Nf**: number of leaves); it has been expressed by the coefficients of variation (CV) and by a graphical representation in mustache boxes which describe the dimensional characteristics of these organs and differences between stands (5%) threshold.

Pearson correlation tests were performed between phenotypic and germinative traits of acorns and between phenotypic traits of acorns and growth traits of seedlings. The significance thresholds of these multiple tests were corrected by the Bonferroni method (Bollen, 1989) (Supplementary Tables S1 and S2).

Principal component analysis (PCA) was used to transform the qualitative variables into quantitative variables that were later used to perform classification. This analysis allowed us to explore the relationships between the studied traits, identify morphological similarities between the sampled populations (stands/plots) and map the produced samples using Mapinfo V 7.5 software resized with CSE Extebded photoshop software of 297  $\times$  210 mm (300  $\times$  300 jpg [DPI]).

Statistical descriptive processing of the data has been carried out using S DESPCRIPTIVE STATISTICS BY EXCEL (XLSTAT). The software R (boxplots) Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/. This test identifies possible homogeneous groups to evaluate the stand or preventive effect. The choice of R is motivated by the innumerable facilities offered by this program; it offers rich documentation and allows collaborations on an international scale. The creation of scripts for various analyses allows sharing and exchanging within the scientific community (Rabehi, 2021).

#### Results

#### **Biometry of Acorns**

Our results show that the phenotypic traits (biometric parameters) do not show significant differences (p > .05) in the nine stations for the width (Lar) and the width/length ratio (Lar/Log) of acorns.

The statistical results obtained indicate that the average width (24.6 mm; 20.66 mm) and the Lar/Log ratio (.83; .64) of the acorn oak are significantly more marked at the Ait Salah (AS) and AK stands. We note that the width (Lar) (14.49 mm) and the Lar/Log ratio (.47) of the acorns of the stand cynegetic center (CY) are the least marked, but this is compensated by the average width length (log = 30.61 cm) of its acorn (Table 2). Acorns from Houra stand (HO) are distinguished by a high length (Log) and weight which are 38.33 cm and 6.78 g, respectively, and a lower width/ length ratio (Lar/Log) than those from other stands (Figure 3).

Overall, in the stands of AK, Tala kitten (TK), and Vide Mehaga (VM), the parameters weight, width, and length of the acorns do not show significant differences between trees or the average weight (Pg) of acorns ranging from (6.81–9.11 g, Table 2). These results relate acorn biometry to its environment/tree/population origin stand (humidity/exposure/a ltitude).



Variation of the Report (Lar/Log) of Acorns in the Inventoried Stands/Plots.

## Table 2.Descriptive Statistics of the Parameters Measured at the Surveyed Stands

			Biometric Para	meters	Morphologic Parameters					
		Log	Lar	Pg	Lar/	La	Dc	Qv =		
Plots	Descriptives Statistics	(mm)	(mm)	(g)	Log	(cm)	(mm)	La/Dc	Nf	Nr
LN	Min	13./3	11.03	.91	.80	12	3.14	3.82	8	0
	Max	35.89	21.98	9.02	.61	38	6.02	6.31	4/	8
	X	26.24	16.18	3.55	.61	24. /1	4.40	5.61	24.22	2.44
	CV	.20	.16	.48	_	.41	.24	_	.68	.29
	δ	5.24	2.58	1./0	-	10.13	1.05	-	16.46	./0
AS	Min	17.16	16.63	5.4/	.96	10.10	2.23	4.52	9	0
	Max	44.26	39.18	14.81	.88	32.5	5./	5.70	6/	9
	<u>X</u>	29.67	24.65	9.08	.83	18.86	4.45	4.23	28.04	2.48
		.28	.31	.23	_	.28	.17	_	.53	.91
CV		8.30	7.64	2.08	-	5.28	./5	-	14.86	2.25
Cr	Min	23.66	17.0	2.24	.50	15	2.64	5.68	19	0
	Max	39.16	17.8	6.91	.45	42.6	5.35	7.96	0/	3
	<u>x</u>	30.61	14.49	3.91	.47	27.84	4.11	6.//	37.47	./8
		.11	.	.29	_	.27	.10	_	.38	.38
TI	0	3.30	12.12	1.13	-	1.51	./3	4.1.2	14.23	.29
IK	Min	20.68	13.12	1.42	.03	20	2.00 E 76	4.13	12	12
	Widx	45.03	10.22	0.11	.53	39	5.70	5.22	78	12
	<u>x</u>	30.27	19.22	9.11	.52	22.07	4.22	5.22	53.00	2.9
	s	.12	.18	.37	_	.33	.10	_	.52	.15
۸L	0 Min	4.55	10.77	2.57	-	1.20	.07	= E 72	17.50	.45
АК	Min	20.45	24.65	15.47	.45	20	Z.44 E 1 E	5.75	2.0 E.4	
	Widx	22.12	24.05	0.20	.02	29	1.06	5.05	26.66	1.06
		11	11	9.52	.04	10	17	5.50	20.00	1.00
	\$	.11		.27	_	.19	.17	_	12.52	2.04
VM	0 Min	12.5	11.66	1.2	- 06	4.15	1.57	202	12.55	0
VIVI	Max	15.5	27.00	12.41	.00	0	6.62	5.62	60	11
	V	45.25	10.22	6.01	.01	22 12	4.20	5 1 5	26.01	2.66
		24	76	37	.50	22.15	73	5.15	50	71
	\$	7.82	.20	.57	_	7 30	.23	_	13.40	2.50
	0	7.02	т.уу	2.51		7.50	.50		15.40	2.37
CA	Min	23.93	14.12	2.49	.59	13.40	2.79	4.80	6	0
	Max	44.02	23.02	13.37	.52	35.40	6.65	5.23	49	5
	X	36.94	17.90	7.85	.48	22.30	4.18	5.33	21.36	2.72
	CV	.12	.08	.24	_	.27	.23	_	.48	.63
	δ	4.43	1.43	1.88	_	6.02	.96	_	10.25	1.71
но	Min	8.57	13.5	3.25	1.57	13.7	3.15	4.34	7	0
	Max	44.5	20.69	12.10	.46	35.4	5.48	6.45	39	6
	X	38.33	16.25	6.78	.42	22.35	4.38	5.10	23.80	2.71
	CV	.14	.08	.22	_	.28	.15	_	.34	.62
	δ	5.36	1.3	1.49	_	6.25	.65	_	8.09	1.68

(Continued)

Table 2.
Descriptive Statistics of the Parameters Measured at the Surveyed Stands (Continued)

			Biometric Para	meters		Morphologic Parameters						
Plots	Descriptives Statistics	Log (mm)	Lar (mm)	Pg (g)	Lar/ Log	La (cm)	Dc (mm)	Qv = La/Dc	Nf	Nr		
YA	Min	29.13	14.36	4.08	.49	15	3.18	4.71	14	0		
	Max	45	20.09	12.31	.44	38.9	5.98	6.50	55	11		
	Х	38.88	17.29	7.80	.44	28.27	4.65	6.07	31.60	3.5		
	CV	.08	.07	.22	-	.19	.16	-	.38	.72		
	δ	3.11	1.21	1.71	-	5.37	.74	-	12	2.52		

*Note:* Lh = Length in Height; Dc = Diameter at the Neck; Nf = Number of Leaves; Nr = Number of Branches; Log = Length of the Acorn; Lar = Width of the Acorn; Pg = Weight of the Acorn.

From the results in Figure 5, we can see that the rate of acorn germination in the sampled stands is appreciable where it reached its ceiling in the stands of village Mehaga "VM,""AK,"Yakouren "YA," and "AS" with a rate of 100% except the case of the stands of the Lac noir (LN) with a low rate of 30% (Figure 5) (noticeable low production for LN).

The detailed descriptive analysis of the growth of the plants involving several parameters (aerial height, diameter at the collar, and number of leaves and branches) measured in the nursery show that overall, the measurements of the morphometric variables (La, Dc, Log, Lar, and Pg) are not widely dispersed within each stand (Table 2) since the CV does not exceed .20 except for the stands LN, AS, YA, HO, and VM where the CV is greater than .50 for the number of leaves and branches. However, the stands AS and HO stand out as showing the lowest values for growth in crown diameter.

The graphical approach allows for comparing the morphological and biometric parameters according to the stands (in graphical form (box plot) Figures 4a-f and 6). This shows the morphological variability of the hybrid oak acorns and the comparative growth of the resulting plants. Figures 4 and 5a-h show that, for the width of the acorns, the dispersion appears weak through all the stands except for the AS stand, which is characterized by the presence of clumps of holm oak and is also at high altitude. The value of the width of the acorns is the highest in the stand "TK," and the lowest is noted between CY; however, the weight of acorns is more homogeneous (low dispersion). With regard to crown diameter, this parameter appears stable across all stands. The degree of variability is low with all values between 4.1 and 4.5 cm. The mean of the parameter "La" appears almost identical on all the stands (AK-CA-HO-TK-VM-YA) where it reaches its maximum value at the LN stand and minimum at AS. The interguartile range is more spread out at stand CY. The diameter at the neck varies from 4.06 to 4.65 mm and is more marked at the two stands CA and YA. The interguartile is more spread out at the CA stand, which tells us about its high variability (characterized by favorable stand conditions and located near holm oak stands).

The results related to the parameter "Qv" ratio of the height of the stem to the diameter of the neck are visualized in Figure 7, which allows recognizing the plants with ideal size in height, diameter, and good robustness (EL Boukhari et al., 2013).

### **Discussion and Conclusion**

Discussion in this study, we have shown that the biometric and morphometric parameters of the acorns of putative species that mix with the pure species give us information on the origin of the acorns, the place of origin of the trees (stand effect), and the surrounding pure species. It emerges from the biometric study that the acorns of putative oaks from the stands AK, AS, and Tk are distinguished from the other stands by relatively longer (Log) or wider (Lar) and heavier (Pg) acorns. This difference would be the result of wetter stand conditions in altitude and the effect of the nearby pure species which are the *Q.afares* oak and O.canariensis as the case of the stand TK where we found the most marked weight. The influence of environmental and micro-environmental characteristics and genetic variation may be related to the nearby pure species, and as in the case of the stand AS which is fresh, the close pure species is the holm oak. A combination of these two factors could be the origin of this morphological variability (Aissi et al., 2019). Mercier, 1996 study indicates that the morphology of Quebec oak acorns is not influenced by field conditions but rather is controlled by genotype. In addition, the morphological variability of the acorns could have an origin related to the stand close pure species; on the germination potential and the subsequent development of seedlings and plants. Climatic conditions on the stands can also generate variations in acorn size and morphology (Baquedano et al., 2008; Bonito et al., 2011).

The final germination of acorns in pots (bags) can be extended to provenances (stands) as well as to families of the same provenance. Although it is not currently possible to identify whether the phenomenon is influenced by the weight of the acorns, there appears to be a close relationship between germination and the weight of the acorns and some authors note that the variation between different seedlings only becomes clear after a few years of development (Houston, 1987; Kremer, 1994), as in the case of the stands (AS-AK-VM-YA) where the weight of the acorns is more marked, respectively (9.08 g; 9.32 g; 6.81 g; 7.80 g) (Figure 4(a-b-c-d-e-f)) (see Table 2). The difference would be the result of wetter stand conditions in altitude and the effect of the close pure species which are the Afares oak as in the case of the stand TK in length of the acorns (Log) or the holm oak which is the pure species close to the AK and AS stands, where we found the most marked acorn weights (Pg) and width/length ratios (Lar/Log), which reflects the richness of the favorable conditions of the harvesting stands (origin) (see Table 2).

The results obtained showed that acorn size has no influence on acorn germination, as well as on seedling development, which is consistent with the observations and results of researchers; Ke and Werger (1999); Houston (1987) and Thompson and Rabinowitz (1989); Tilki and Alpetkin (2005) and Navarro et al. (2006) who also noted the lack of a correlation between acorn size and oak seedling size, which they interpreted as



Figure 4.

(a-f). Box Plots of Biometric Parameters of Putative Oak Acorns in the Nine Stand Studied in Algeria (Akfadou) (Pg=Weight of Acorns in Grams; Log=Length of Acorns in Millimeter; Lar=Width of Acorns in Millimeter).

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Acorn Germination Rate in the Inventoried Stands After 2 Months of Cultivation in 30 Seed Pots in Each Stand.

the result of high variation between stands. In contrast, other authors suggested that the height of red oak (*Quercus rubra* L.) seedlings after 2 years of cultivation correlates positively with acorn weight (Kolb & Steiner, 1989; Kremer, 1994).

In addition, Long and Jones (1996) and Quero et al. (2007, 2008) report that plant size is strongly dependent on acorn size during the early years of development. This effect fades progressively until it disappears after a few years, (Lamhamedi et al., 1997, 2000), and has shown that collar diameter can explain more than 97% of the observed variation in total plant mass. Plants with a large diameter generally have well-developed lateral roots while giving the plants a better survival rate.

The analysis of the morphological traits of the seedlings shows that the stands AS, CY, and Tk are distinguished by large dimensions in height (La) and diameter at the collar (DC). The decrease in seedling size presents a form of adaptation to climatic figures 4 conditions for Mediterranean oaks, generating a better resistance to water deficit (Baldocchi & Xu, 2007; Gil-Pelegrín et al., 2017; Peguero-Pina et al., 2014). Given that the growth conditions of the seedlings were homogenous, it can be suggested that the observed differences are possibly related to genetic (population) variation that would originate from adaptation to environmental (stand) conditions.

The study of seedling growth shows no differences within and between the different study stands. Indeed, some authors note that variation between different seedlings only becomes clear after a few years of development (Houston, 1987; Kremer, 1994).

The comparison of the average aerial heights and diameters at the collar shows a strong growth at the stand AK where the growth in height is about 29 cm. While the diameter at the collar is almost the same for all plants, rangin from 4.06 to 4.65 mm, also for the parameter number of leaves which is a good indicator of assimilative capacities of the plant and is production in biomass. The histogram of the number of leaves per plant parameter shows directly that it follows the aerial height. The longer the plants are, the more leaves they have (Figure 6). However, height does not show a systematic correlation with survival but a good relationship with growth (Lamhamedi et al., 2000).

In most of the plots, the inferred relationships show highly significant positive correlations at  $\alpha$  = .05 threshold; the correlations obtained are between .39 and .91. The strongest relationships are between the diameter at the crown (Dc) and the width of the tassel (Dc and Lar) and between the aerial length and the number of leaves (La and Nf). This could be explained by changes in the physiological state of the acorns reflected by a decrease in fresh weight, accompanied by an increase in moisture content (Branco et al., 2002). In fact, acorn mass was used as a covariate in a family study by Kolb and Steiner (1989) on 1- and 2-year-old seedlings. This could be explained by changes in the physiological state of the acorns resulting in a decrease in fresh weight, accompanied by an increase in moisture content (Branco et al., 2002).

Although these two plots, CA and AK, are similar, they differ in terms of their ecological conditions: low slope, high density, and mechanized land for the first. It should also be noted that the highest aerial lengths are accompanied by the largest collar diameters. The aerial length is remarkably higher in the two stands VM and TK; this may be related to the variety and size of the acorns. Some studies have already high-lighted a significant positive correlation between acorn mass and seed-ling growth (Gall & Taft, 1973; Kolb, 1988; Kriebel, 1964). The AS stand is characterized by a larger diameter at the collar. This criterion is rather sought for a better quality of the seedling (Mercier et al., 1996).

The observed diameter of hybrids plants varies from 4.1 to 4.5 cm and the aerial length ranges from 32.4 to 44 cm, while Lamhamedi et al. (2000) report that a good forest seedling should reach a target of 28–40 cm when the diameter at the crown varies between 4 and 5 cm. Examination of the eigenvalues from the PCA allows us to retain the 1  $\times$  2 factorial design, which explains 78.91% of the total variance, that is 56.01% for axis 1 and 22.90% for axis 2. The projection of the variables on the factorial plane and the examination of the correlation matrix (Table.1S and Table.2S IN SUPPLEMENTARY) show a positive relationship





(a-h). Box Plots of Morphometric Parameters of Putative Oak Acorns in the Nine Stands Studied in Algeria (Akfadou) (La=Height; Dc=Diameter at the Collar; Nr=Number of Branches; Nf=Number of Leaves at the 5% Threshold).



Variation of the Robustness Quotient (Lh/Dc) of Oak Seedlings Vigor Quotient (Qv) in the Inventoried Stand. AS=Ait Salah; LN=Lac Noir; CY=cynegetic center; TK=Tala Kitten; AK=Akfadou; VM=Vide Mehaga; CA=Col Ait Salah; HO=Houra; YA=Yakouren.

between the aerial length and the length of the acorn, between the number of leaves and the number of branches of the young plants. Finally, the positive influence of the weight does not appear in the morphological parameters studied.

The results obtained did show that acorn size has no influence on acorn germination, as well as on seedling development. In contrast, other authors suggested that the height of red oak (*Quercus rubra* L.) seedlings after 2 years of cultivation is positively correlated with acorn weight (Kolb & Steiner, 1989; Kremer, 1994). Furthermore, according to Long & Jones (1996) and Quero et al. (2007, 2008), the size of the

seedlings depends strongly on the size of the acorns during the first years of their development (Quero et al., 2007) (Figure 7). It is a variable that integrates the morphological response to environmental factors (close stand — species effect).

The projection of the variables on the factorial plane and the analysis of the contributions of the individuals allow us to identify divergences and analogies (Figure 8). In relation to axis 1, the aerial length and the diameter at the neck increase in one direction and the width and length of the acorn increase in an opposite direction, and we notice a relevant link between the mass of the acorns, the growth in height, and the



#### Figure 8.

Projection of the Variables on the Selected Factorial Design. DC=Diameter at the Neck; La=Aerial Height; Lar=Width of the Tassel; Log=Length of the Tassel; Pg=Weight of the Tassel; Nf=Number of Leaves; Nr=Number of Branches( at the 78.91%).



Projection of Individuals (Stand-Plots) on the Selected Factorial Plane, Mentioning the Dominant Close Pure Species.

diameter of the seedlings after 2 years of cultivation in the nursery having statistically shown (boxplots).

Through the projection of individuals on the factorial plane, we notice that the stations that present the same ecological conditions such as the slope and altitude are grouped as the stands AK and AS (see Figure 8). Indeed, the stands AK and AS share the same stationary conditions, being located at high altitude with the presence of holm oak.

For the projection of individuals on the factorial plane (Figure 9), we deduce the grouping of stands according to their geographical position and presence of the pure species nearby. As in the case of the stands AS and AK, geographically close, the pure close species is the holm oak. The same situation is observed for the stands black lake (LN) and CY with the *Q.afares* oak as a close species. Regarding the other stands together(VM;TK;YA;HO;CA) where we find a mixture of pure oaks Q.afares and Q.canariensis(zeen).

These results and this interpretation are perfectly compatible with the concept of distribution of pure species proposed by Messaoudene (2007), where the *Q.canariensis* species is the dominant species (1646 m altitude) and Q.afares oak, located at 1250m above sea level, abounds on some ridgelines, the south and south-west slopes (more or less clayey soils). Mixed stands of *Q.canariensis* and *Q. afares* are found throughout the transition zones. The same is true for mixed stands of *Q.canariensis* and *Q.suber*, limited to an altitude of 1100 m. (Messaoudene, 1989). Through this reference, we can superimpose and deduce the position of pure species through our sampling site (inventoried stand. Table 1). This is, for example, the case of the putative species of the "AK" stand which are individualized on the two axes of our PCA (Figure 9)/compared to the other seedlings (hybrids) since the close pure species is

either *Q.suber* or *Q.afares*; but we clearly notice the putative species of the stands (YA, HO, TK, VM, CA) are found together (Figure 8) because the common pure species *Q.canariensis* or *Q.afares* are present (stand). Through the results obtained, this research highlights "the stand effect" and "the effect of the close neighboring species" on the distribution of hybrid seedlings. Our results are the same as those of Aissi (2021) showing a link between the taxa and environmental factors of the stands.

Finally, we will be able to affirm that all the species of the same stand lead to more homogeneous results taking into account the close species (pure) that are in the surroundings of the inventoried putative oaks (the putative species of oak inventoried follow the neighboring pure species).

Through this research, we attempted to study the variability of acorn biometry of putatives oaks to evaluate the growth (vigor) of seedlings from the harvested acorns.

The main results show that the growth and development of the seedlings of supposedly hybrid oaks (putative), all origins combined, are influenced by the altitude effect and the effect of the nearby species, as in the case of the Col AS stand (CA) where the holm oak (Ilex) is close to the putative oaks inventoried. After 2 years of nursery culture, the results obtained show that the germination rate, height, and diameter of the putative oak seedlings (hybrids) inventoried are strongly influenced by a few site factors related to the origin of the acorns (harvesting site).

The position, as well as the ecological region, influences the morphology of the acorns. The growth of putative oak in the nursery is homogeneous in terms of the height and diameter of the plants. However, the "close species" effect appears in the case of the high altitude stand where holm oak is present (cool stand).

Studies are underway to complete this contribution with genetic and morphometric analysis of adult tree leaves for specific morphological identification and to better explain species diversity and distribution in Algeria (AK).

With climate change and decreasing rainfall, the frequency of dry years will only increase. To this end, in the case of countries that will be affected by the aridity of the future climate, such as North Africa, it is necessary to rethink the development of new technical itineraries adapted to the reforestation sector of tomorrow.

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Supplementary Table.1S. Contributions of individuals, based on correlations											
	Fact. 1	Fact. 2	Fact. 3	Fact. 4	Fact. 5	Fact. 6	Fact. 7				
LN	5,44311	1,46486	66,55698	0,00761	11,66201	0,01628	0,61195				
AS	11,07287	37,15181	0,61759	2,41698	9,77222	0,53227	25,83695				
СҮ	70,41593	1,91006	9,92195	1,3798	3,5829	1,64735	0,03091				
тк	7,23558	1,18256	2,82374	0,85996	3,45107	58,13394	3,17473				
AK	2,17075	9,4898	5,71809	3,30597	28,66747	6,24304	14,3822				
VM	2,46883	0,21702	9,29246	1,60196	39,74698	0,66349	22,07602				
CA	0,39135	1,46445	3,87617	17,74153	0,06887	24,61407	5,99489				
НО	0,02403	7,23019	1,17105	36,85151	2,61526	1,41501	22,79917				
YA	0,77753	39,88924	0,02197	35,8347	0,43322	6,73455	5,09319				

Supplementary Table.2S. Contribution of variables based on correlations

	Fact. 1	Fact. 2	Fact. 3	Fact. 4	Fact. 5	Fact. 6	Fact. 7
La	0,123712	0,206358	0,003873	0,53391	0,004577	0,126648	0,000922
Dc	0,202458	0,012335	0,120464	0,052477	0,228378	0,379827	0,004061
Nf	0,210148	0,027262	0,050496	0,13721	0,117127	0,410952	0,046806
Nr	0,131792	0,168509	0,135876	0,001471	0,478106	0,000028	0,084217
Log	0,018217	0,36919	0,341138	0,078802	0,002234	0,000065	0,190354
Lar	0,142695	0,213408	0,022114	0,131565	0,151146	0,075286	0,263785
Pg	0,170979	0,002938	0,326038	0,064564	0,018432	0,007194	0,409855