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Characteristics of Argan tree (*Argania spinosa* L. (Skeel.)) introduced in Chlef plantation (North-west of Algeria): Seeds, plants growth and physicochemical parameters of the oil

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Abstract

Argan tree (*Argania spinosa* L. (Skeels)) is an endemic species in southwest of Algeria (Tindouf) and Marocco. It is rare and highly appreciated for its various qualities as fodder, oil, wood and its ability to resist drought, but threatened.

Seeds used in our studies are collected in Chlef plantation where this specie was introduced. Seed and almond morphological studies (length, diameter, weight and diameter/length ratio) revealed dimensional changes and three seed shapes were identified (fusiform, ellipsoidal and oval). Weight and seed shape are more dependent on diameter. The parameters of regeneration through the germination of Argan seeds (55.10%) and the height of seedlings after 8 weeks (15.35cm) gave satisfactory results. The physicochemical parameters of the oil analyzed, revealed that this oil is "extra virgin", rich in antioxidants, pure and linoleic oleic type. Argan tree introduced in Chlef is well adapted to local conditions, which are favorable to its conservation and extension of its range, where it would constitute an ecotype of the Argan tree.

Keywords: seeds, almonds, oil, seedling, Argan, adaptation.

1-Introduction

The Mediterranean region is home to a remarkable biological diversity, particularly in terms of rare and endemic species, among which some species are remarkably resistant and well adapted to drought. In North Africa, this flora constitutes a very important part of local genetic resources in terms of forestry, pastoral, medicinal and aromatic value (Amirouche, 2008). Unfortunately, most of the rare species are among the categories of endangered species that should receive special attention and need to be prioritized in terms conservation.

As part of the Mediterranean region, Algeria contains a high floristic diversity, with a high proportion of endemism. In this context, the species *Argania Spinosa* (L) (Argan), is one of the most marked endemic species of both Algeria and Morocco (Charrouf, 2002), and represents the only species of the Sapotaceae family (Kechairi, 2009).

In Algeria, its geographical range is mainly restricted to the region of Tindouf in the extreme west of Algeria, where it represents the second most important forest species after *Acacia radianna* (Benkheira, 2009), and grouped in the form of scattered populations along river banks in search of water (Benkheira, 2009). It's a hardy xerophilous species resistant to lengthy dry periods in arid climates which characterizes the sub-Saharan regions of Algeria and Morocco (Kechairi, 2009; Nouaim et al., 2007; Belacadi, 2017; Labaica-Rojas et al., 2022). Due to these severe climatic conditions, Argan tree stands in Algeria and Morrocco are reduced into a state of mediocre overgrazed bushes (Benkheira, 2009; Ouallal and al., 2013; Benkheira, 2009).

It is an oleaginous species (Charrouf, 2002), its oil constitutes an excellent nutritional and cosmetic value, due to its unique chemical properties, rich in antioxidants, polyphenols and sterols (Adlouni, 2010). It is also characterized by a significant genetic diversity (El Bahloul et al., 2014; El Moussadik and Petit, 1996; Metougu et al., 2017) and a significant morphological polymorphism (Berka et al.,2019; Belcadi et al.,2017; Bani Aameur et al., 2001; Bani Aameur, 2004; Zahidi et al.,2015; Metougui et al.,2017; Ferradous et al., 2021; Bendella et al., 2023) allowing it to adapt to different ecological habitats (Labaica-Rojas et al., 2022; Debbouri et al., 2024). It is a hardy forest species, resistant to the vagaries of the climate and prolonged periods of drought and it helps to prevent erosion and desertification through its highly diversified taproot system (Kechairi, 2009,). The pulp produced by pulping the nuts, the cake left over from pressing the kernels and the foliage are highly valued animal feedstuffs (Radi 2003; Cherrouf, 2002).

As part of the initiative to preserve this rare and endemic species, a number of experimental planting trials were carried out by the Algerian government in different climates and ecosystems across the country, as a result this species showed different levels of acclimatization. According to Djebbouri et al., (2024), this xerophilic species showed a good adaptation in the eastern and central coasts with a level of success greater than 75%, and a poor adaptation in the steppe areas with a level of success not exceeding 25%. This species has also been introduced and naturalized in much Mediterranean country, notably in Spain (Labaica-Rojas et al., 2022).

Through a study of seeds morphological, kernels, seed germination, plant growth and physic-chemical characteristics of the oil in Chlef plantation, our study aims to evaluate its capacity to adapt ex situ and the quality of oil with a view to its preservation and extension of its distribution area.

2-Materials and methods

The geographical area of this study is Chlef that is located in north-western Algeria between 36°09'54" N and 1°20'04" E, at 116 m above sea level. The climate is semi-arid with temperate winters (Q_2 = 36.26), the hottest month being August (38°C) and the coldest February (10.6°C). Argan tree was introduced in Chlef in 2002 from seeds originating from the natural settlement of Tindouf. The Seeds used in this study were harvested in November 2022.

2-1-Seed and kermel morphology

The seeds and almond morphological characteristics measured are length and diameter. Weight ratio of diameter to length is calculated. 200 seeds were measured. Electronic caliper and a precision electronic balance were used to carry out all measurements.

To assess the proportion of seed coat to total seed weight, we calculated the difference between average seed weight and average kernel weight, and the ratio of kernel weight to seed weight.

2-2-Seed germination and seedling growth

To carry out the germination test, seeds were first treated with boiling water, then for a week in ordinary water, and finally cold-wet for 48 h at 4°C to lift embryonic dormancy. This treatment produces a thermal shock that softens the seed coat, which is very hard and can be an obstacle to germination. Pre-treated seeds were placed individually in perforated plastic bags, about 5cm in diameter and 15cm deep, filled with a substrate composed of 1/3 potting soil and 2/3 forest substrate. Three (03) replicates of 50 seeds were adopted. The germination test was carried out in the laboratory at room temperature (24°C). The following parameters were calculated:

Germination capacity (GC %) expresses the rate of seeds capable of germinating under well-defined conditions.

-The mean germination time (MGT) is expressed as follows:

$$\mathrm{TMG} = \frac{\mathrm{N1T1} + \mathrm{N2T2} + \cdots \mathrm{NnTn}}{\mathrm{N1} + \mathrm{N2} + \cdots \mathrm{Nn}}$$

où N1T1 : number of germinated seeds per time T1 ; N : number of total seeds

To assess seedling growth, stem height from ground level to apex and collar diameter at the zone of separation between the root system and the aerial part were measured on 8 weeks-old seedlings, using an electronic caliper. The length/diameter ratio, known as the robustness ratio was calculated.

2-3-physicochemical characteristics of oil

Kermel oil yield is determined after extraction and is calculated using the following formula: **Rdt (%) = H/A×100** (H: Quantity in grams of oil obtained after extraction. A: Quantity in grams of kernel used) and **Relative density (D)** according to (LION, 1955) and (method ISO 6882 , 1987).

For oil extraction, fruits were first dried on a hot plate to facilitate pulping, which consists in separating the dry pulp from the fruit. Seeds are then crushed with a hammer and pestle to recover the kernels, this stage is extremely difficult, as the seed coats are hard and very thick, unlike the rather fragile almonds. The almonds were ground using a mortar and pestle. Extraction of the oil or lipid fraction from almonds using petroleum ether as an organic solvent was carried out using a 250 ml soxhlet. This step is followed by evaporation of the solvent in the open air (to avoid denaturing the oil with the heat of the oven, and to recover the extracted oil). In this way, we are able to extract the oil contained in the almonds. 20 g of ground almonds are introduced into the cellulose cartridge of the soxhlet and placed in the siphon extractor. The flask is filled 3/4 full with solvent. The soxhlet parts are assembled and the whole unit is placed in a water bath at solvent temperature. Heating then leads to boiling, and extraction is maintained continuously for 6 hours. The extracted oil is collected in a rotary evaporator and transferred to glass vials for analysis two weeks later.

Physical and chemical parameters studied are: **specific extinction (E270 and E232)** (method described by Harhar et al., (2010) ; **acidity (a)** ; **acidity index (Ia)** (Lion, 1955) ; **peroxide index (Ip)** (Chimi, 2005) ; **saponification index (Is)** (ISO 3657 method, 1977) ; **iodine index (Ii)** (method standard ISO 3961, 1979). Seed oil yield was also determined.

To explain and interpret our results, we used statistical and graphical analyses as arithmetic average, One-factor analysis of variance, Histograms and Correlation matrix (Dagnellie, 2013). Data processing was carried out using Excel (2007) and Excel Stat software.

3-Results

Table1: Descriptive statistics for seed and almond dimensions

	Seed				almond			
	L (mm)	D(mm)	W(g)	Ratio D/L	L(mm)	D(mm)	P(g)	Ratio D/L
Mean	24.3 ±2.41	11.7 ±1.5	2.13 ±0.63	0.47 ±0.06	21.29 ±1.6	9.78 ±1.38	0.29±0.04	0.45 ±0.06
min.	14	7	1.03	0.37	17	7	0.19	0.33
Max.	29	15	4.5	0.63	26	13	0.38	0.58
CV. (%)	9.91	12.82	**29.57**	12.76	7.5	14.11	13.79	13.33

Legend : L: Length, D: Diameter, W: Weight, Max : Maximum, min : minimum, CV:coefficient of variation.

Almonds Length varies between 26 and 17 mm with an average of 21.29 mm and coefficient of variation (CV) of 7.5%, the diameter varies between 13 and 7 mm with an average of 9.78 mm and CV of 14.11%, their weight varies between 0.38 g and 0.19 g with an average of 0.29 g and CV of 13.79%. Diameter/length ratio is on average 0.45 varying between 0.33 and 0.58 with a CV of 13.38. According to the coefficients of variation, almond morphology variability is very low (Figure 2)

Seed length varies between 29 mm and 14 mm with an average of 24.32 mm and CV of 9.91%, diameter varies between 15 and 7 mm with an average of 11.68 mm and CV of 12.82, seed weight varies between 4.5g and 1.03 g with an average weight of 2.13g and CV of 29.57%. Average diameter/length ratio is 0.47, ranging from 0.37 to 0.63 with CV of 12.76.

The average tegument weight is 1.84 g or 86% of the total seed weight. The average kermel/seed weight ratio is 0.13. This result explained the high proportion of seed coat in Argan seeds.

Three types of seed were identified (Figure 1 and 2): oval seeds which are very rare, fusiform seeds and ellipsoid seeds which are dominant.

1: ellipsoid, 2: oval, 3: fusiform.

Figure 1 : Argan seed shape of Chlef

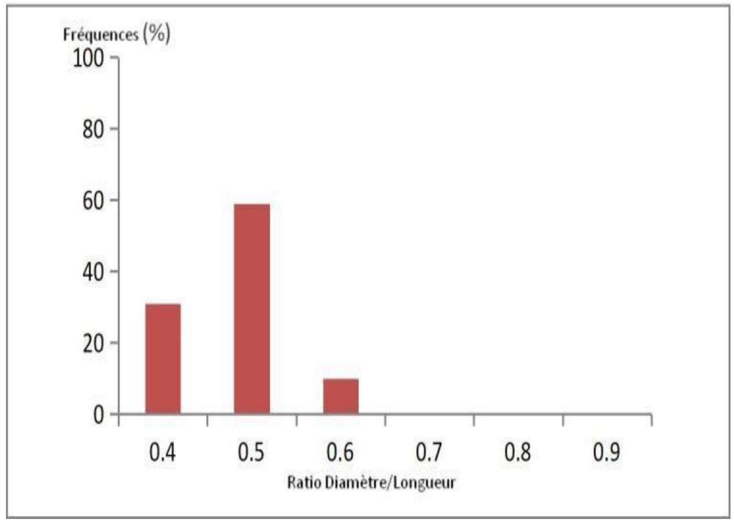

Figure 2: Distribution of average seeds ratios

Legend : fusiform seeds (0.4), ellipsoid seeds (0.5), oval seeds (0.6),

Table 2: Pearson correlations for morphological characteristics of seeds

	L.	D	P	R
L	1			
D	0.40	1		
P	0.56*	0.83***	1	
R	0.56*	0.70***	0.38	1

Legend : L : Lenght, D : Diameter, P : weight , R : Ratio

Seed length and seed shape are significantly correlated (r= 0.56 and r=0.56) but seed weight and seed shape are more dependent on diameter with very significant correlation (r=0.77 and r=0.83 and r=0.70, r=0.72) (Table2).

The average seed germination (Figure 3) rate (GT) is 55.10% and the germination time (TMG) is 19.14 days. Dynamics of seed germination is low during the first days and the accelerate from the 10^{th} day to reach a maximum of 55.10% at the 36^{th} day (Figure 4).

Figure 3: Seed germination

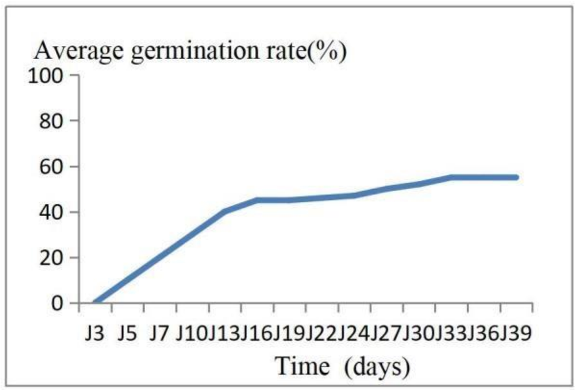

Figure 4: Dynamics of seed germination

Table 3: Average height and diameter of seedlings

Average seedling height (cm)				Average seedling diameter (cm)			
Maximum	Minimum	mean	VC%	Maximum	Minimum	Mean	VC%
22	4,5	**15,37**±3,70	24	0.43	0.17	**0.31**±0,59	19

Figure 5: Seedling growth

Seedling length (Figure 5) ranged from 4.5 cm to 22 cm, with an average of 15.37 cm and a CV of 24% (Table 3), revealing fairly marked heterogeneity between plants Neck diameter ranged from 0.175 cm to 0.43 cm, with an average of 0.31 cm; the CV is 19% (Table 3). The robustness coefficient is 4.95.

Table 4: Oil yield in relation to quantity of kernel

	H (g)	A(g)	R%
Chlef	30,24	103,4116	29,24

Legend: H: oil Quantity obtained by extraction. A: Test sample of kernel used.

Table 5: physical parameters of the oil

Parameters		Chlef	Maroccan standards
Density à 20 °C		0,918	0,906-0,919
Extinction Spécifique	**270nm**	0,027 ±0.01	0,35-0,45
	232nm	0,012 ±0.01	
	ΔK 270nm	0.002	≤ 0.01

The yield is 29.24% (Table 4). Oil density is 0.918. Absorbance values obtained at 270 nm and 232 nm are respectively 0.027 and 0.012 (Table 5).

Table 6: Chemical parameters of the oil

The acidity values is around 0.34%, while the acidity index is 0.68, Peroxyd index values obtained is 1meq O2/kg oil. The saponification index values obtained is 112.2. The iodine indices are 91.36 (Table 6).

Parameters	Chlef	Maroccan standards
Acidity (% d'acide oléique)	0.34% ±0.005	0,8 - 2,5
Acidity Index (mg de kOH/goil)	0,68 ±0.005	
Peroxyd Indec de (Meq d'O2/Kgoil)	1 ±0.01	15-20
Saponification Index (mg de KOH/g oil)	112,2 ± 0.1	189 – 199,1
Iodine Index (g iodine/100g oil)	91,36 ±0.005	91 - 110

4-Discussion

Three seed shapes were found, where ellipsoid shape is dominant, fusiform phenotype is intermediate and oval one is very rare, different results were found by Belcadi Haloui et al. (2017) and Berka et al. (2019) for Tindouf argan tree, this authors indicate the presence of spherical, rounded and ovoid shapes, they did not report ellipsoid and fusiform shapes in Tindouf populations, but it was found by Bani Aameur et al. (2004) in Moroccan populations, where the oval shape is dominant and spherical and round shapes are very rare. This character is very discriminating and allows us to suggest that Chlef population is an intermediate ecotype between Morocco and Tindouf Argan trees.

Seed weight and shape are more dependent on diameter, with highly significant correlation. Variations in diameter lead to variations in seed shape: Larger seeds tend to be spherical or rounded.

The almonds from Chlef are longer than those from Mostaganem and Tindouf, as reported by Kouidri (2008) tha results are close to our own. Chlef almonds are heavier and therefore richer in nutrient reserves, which favor their germination capacity. Coefficient of variation obtained highlights a low variability in the sharpe of almonds.

Dimensions and shapes of fruits and seeds of Argan tree are the most discriminating characters in the differentiation of Argan genotypes (Metougui et al., 2017 and Berka et al., 2019). Variability of Argan tree explains an important degree of plasticity in this species, as it thrives in a highly variable arid Mediterranean climate, this plasticity within Argan populations plays a key role in its adaptation (Bani Aameur, 2004). Argan tree polymorphism gives it the ability to adapt to different habitats with different ecological optima, confirming its presence in different habitats (Labarca Rojas et al., 2022). Phenotypic differences in fruits and seeds can also be explained by different bioclimatic conditions (Bani Aameur and Ferradous, 2001), while significant variations between years can be attributed to climatic differences, especially rainfall (Metougui et al., 2017). Seed and fruit characters, especially shape and size, are highly heritable with very high heritability values
(Metougui et al., 2017 and Farradous, 2021). These traits can be effectively used to describe argan tree that can
be positive for selection (Metougui et al., 2017).

The average germination rate obtained for Chlef seeds is 55.10% this result is close to those of Hamel (2016) whose rates vary between 40% and 60% for seeds collected in Tindouf and those of Sid Ali et al. (2022), who obtained varying germination rates between 57% and 87% depending on seeds origin. Our results are lower than those found by Benaouf et al. (2014), whose germination rates are very high (80%, 85% and 95%) for seeds pre-soaked in water for 96h and 120 h at 27 to 30°C, with sterilization preventing microbial contamination and improving germination. The thickness of the integument of Argan tree seeds from chlef is very important, which could affect the germination process, which is highly dependent on it. It can slow down the process of water imbibitions (BERKA, 2019), according to SID ALI et al. (2022), the tegument one cracked facilitates the humification of the almond and starts the germination. Integumentary dormancy is due to the impermeability of the sclerotized integumentary envelopes to water, thus delaying their imbibitions and the physiological activity of the seed (Hartman et al.,1990). Seed size is directly related to tegument thickness and inversely related to water uptake (Beninger et al., 1998), so increasing seed size reduces water uptake capacity and slows germination, small seeds have a high potential for water uptake (Berka et al., 2019). Likewise composition and granulometry of the substrate may be responsible for variation in germination rates (El mandouri et al., 2020). According to Sid ali et al.

(2022) seed germination can be improved with the combination of freezing at -20°C and hot water (100°C) which have an optimal, uniform and fast germination of Argan seeds, unlike our method of breaking dormancy where the damp cold treatment lasts 48hours at 4°C. Phytophagous nematode larvae were also found in our seeds, which would explain the inability of many seeds to germinate, in the same way fungi also appeared towards the end of the test.

The average height of seedlings (15.37 cm) explains the good growth of Chlef Argan tree in similarity with other results, in fact, Kechairi (2013) found an average of 5.8 cm for Tindouf seedlings in the nursery after one quarter and after 12 months the average height was 32.47 cm. For Benaouf (2014), the average height of Mascara (north west of Algeria) seedlings after one month is 6.5 cm and 18 cm after two months. In Marroco, seedling height is between 15.80 cm and 37.80cm after 8 months and diameter between 2.23cm and 5.51 cm (Ferradous al.,2021). In our experiment, few seed germination is late that explain the slow seedling growth to compared to those that germinated first. The container could have an effect on plants growth; the depth of the bag has an advantage for tap rooted species such as Argan (Ferradous et al., 2017).

The robustness coefficient (4.95) reflects a good balance between plant height and diameter, and should be less than 8 to avoid thread-like plants. It is a good indicator of the ability of plants to overcome the shocks of transplanting (Ferradous et al., 2017).

The oil yields obtained in this way are very interesting since the standards generally set them between 25 and 55. Mountasser (1999) states that the granulometry of the ground almond particles influences the oil yield, which varies between 27.9% at 320µm and an optimum of 35.5% at 800µm. Oil yield can also be influenced by extraction method, type of solvent used, geographical origin, and weight of kernels. The shape of seeds and kernels also affects the oil yield of the kernels (Belcadi Haloui et al., 2017).

Density of an oil provides information on its purity, which is a function of chemical composition of the oil and the temperature (Karleskind, 1992). Our values are in line with those described in the Moroccan standard, for which oil Argan density generally varies between 0.906-0.919 at 20°C. Similar results were found for oil from Mostaganem by Kouidri, (2008) and Bengendouz et al. (2017). Densitie of our oil is also similar to those of olive oil (0.910 to 0.916), indicating a high concentration of mineral elements and other non-lipid compounds, additionally they are of the oleic-linoleic type, as evidenced by the findings of Naudet (1992) and Karleskind (1992).

The extinction index of anoil is indicative of its oxidation state, and thus serves as a criterion for detection of the oxidation state of fats (Wolf, 1968). Hydroperoxides exhibit absorption at 232nm, while oxidation products display absorption at 270nm. An elevated index at wavelength 270 nm is indicative of a greater degree of peroxidation, which is associated with a reduced capacity for preservation (Gharby et al., 2014). The specific extinction index values obtained for Chlef are very low, indicating that this oils is very rich in antioxidants and its oxidation state is negligible. This result corroborate with Moroccan standards, as the values are below 0.35 which confirms that the oils are of the extra virgin variety (Rahmani, 2005). In their study, Benguendouz et al. (2017) reported a value of 0.3 at 270 and 1.23 at 232 for argan oil sourced from Mostaganem and Kechebar and al. (2017) raported values of 1.155 at 232 and 0.251 at 270 for oil derived from Algeria. According to ΔK values at 270nm, which are within Moroccan standards (≤ 0.01), since high ΔK values at 270nm indicate a high degree of oxidation and poor quality (Rahmani, 2005), oils from Chlef is pure and fresh, for this reason oils analyzed is very excellent quality.

The acidity of our oils is very low, this result is lower than Moroccan standard, which sets it between 0.8-2.5%. Benguendouz et al. (2017) also found a lower acidity (0.21%) for argan oil from Mostaganem. Low acidity gives the oil greater oxidative stability (Chimi, 2005). The oil analyzed has an acidity lower than 0.8% it is therefore considered "extra virgin". The peroxide index of Chlef, falling within the range set for Moroccan argan oils of 0.25 to 4.1 meq O_2/kg oil (Hilali, 2000) and 0.2 to 11 meq O_2/kg oil (AFSSA, 2002) and the Moroccan standard of less than 20 meq O*2*/kg oil (Rahmani, 2005). In addition, an Ip index < 3 explains the richness of oils in antioxidants according to Yaghmur et al., (1999).

The iodine index of Chlef oil obtained corroborates within the AFSSA (2002) range. The oils analyzed are therefore "edible oils" (Kouidri, 2008).

5-Conclusion

Our study has revealed three seed shapes. Seed weight and shape are more dependent on diameter. The results of seed germination and plant growth are very interesting.

All physico-chemical parameters analyzed showed that Argan oil from Chlef has excellent qualities, many of which meet Moroccan standards. It is rich in antioxidants and is also considered an "extra virgin" “oleic-linoleic oil”. The ecological factors of Chlef sem to be favorable habitat for the development of the argan tree and constitute an Chlef region ecotype. It is recommended to increase the area planted with argan trees in Chlef region, which seems to have a favorable ecosystem for this rare and endemic species. This project will make a definite ecological, forestry and economic contribution to this region.

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