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Variability in leaf size and shape in three natural populations of *Argania spinosa* (L.) Skeels

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A B S T R A C T

Shape of simple and grouped leaves in argan (*Argania spinosa* (L.) Skeels), based on visual characterization and principal component analysis was studied in three populations in south west Morocco: Ait Melloul (AM), Argana (AR) and Ait Baha (AB). Five leaf traits: length, width, petiole length, ratio (width / length) and area (length x width) were observed. In both type of leaves, four forms were defined: shape obovate with obtuse apex (OT), spatulate with obtuse apex (ST), lanceolate with acute apex (LA) and lanceolate shape with mucronate apex (LM). In all three localities, OT form was more common (78.9%) than ST form in which percentage was about 13.3%. LA and LM forms were lowly represented. Forms LA and LM were clearly distinguished from other shapes by blade apex. LA was characterized by acute apex, while LM was characterized by a mucronate apex. OT form was shorter, wider, with shorter petiole. ST form was longer and narrower, with longer petiole than OT. Morphometric traits explain most than 98.9% of the total variability in simple and grouped leaves. Leaf traits used seem to describe the variability observed for all shapes identified. The leaf can be considered as a microcopy of plant, variation in leaf morphology can reflect plant capacity to acquire, use and conserve resources since the argan tree is a source of food irremplacable in the southern regions.

Introduction

Leaves are important organs for photosynthesis and play an important role in survival and growth of a plant. Many previous studies have revealed that

variations in leaf traits are the result of adapting to growth habitats (Pandey and Nagar, 2002). Quantification and visualization of morphological variation of

leaves, flowers and other structures are essential for an overview of evolutionary and ecological processes of phenotypic diversification and is the fundamental basis from which to develop more complex studies to achieve new perspectives on the interaction of phenotype, genotype and environment (Jensen, 2003). Leaf traits are globally repeated despite large variations in the values of the traits across individual species with very diverse phylogenetic, biogeographical and environmental affinities. As well, leaf traits can thus provide a link between various environmental factors and leaf functions and they have been widely used in functional–structural plant models (Roche et al., 2004, Price and Enquist, 2007).

Shape, size and leaf morphology, whose interest in studies of biomass, and organic and mineral nutrition is well established. These knowledges are currently a component of increasing importance in studies of morphological and genetic diversity of many species, in order to improve their conservation and to base management efforts on sound scientific information on their biology (Dupouey et al., 1991, Harrison et al., 1997, Wu et al. 1997, Leite, 2002, Lutz Eckstein et al., 2006, Xu et al., 2009). In this context, several methods of describing the shape have been proposed in several species to establish a typology of leaves and to study leaf traits (Dupouey et al., 1991; Roudna, 1991, Cho, 2007, Dreyer et al., 2009). Various forms have been defined (ovate, lanceolate, elliptic, linear, oblong, sagittarius, ...) (Deysson, 1976; Tamin, 1991). In *Cydonia oblonga*, generally showed ovate leaves, cordate base, mucronated apex and obtuse apex angle (Guisado et al., 2009). ANOVA and principal component analysis (PCA) suggested a modest but nevertheless

appreciable variation in size and shape in two geographical populations of sessile oak leaves. Differences in leaf size and shape can occur at several levels such as geographical origin, population, tree and leaf (Viscosi and Cardini, 2011).

In Sapotaceae, Pennington (1990) reported in his monograph that the petiole is flattened laterally; the upper surface may be convex or plane. The central midrib can be plane, in relief or submerged at the upper surface of leaf. Three classes are recognized according venation pinnate secondary veins that end at the marginal areas of leaf (craspedodromous type) or decrease gradually towards the margin of leaf (eucamptodromous type), where they are joined by series of submarginal bukles (brochidodromous type).

In species *Synsepalum dulcificum* (Sapotaceae), leaves are entire (Edward and Ayensu, 1972), symmetrical, obovate, with a length of 4.3 to 7.5 cm, width ranged from 3.1 to 3.8 cm. The apex is obtuse but occasionally little acuminate. The base is cuneiform. Petiole is short, its length varied from 4 to 5 mm, flattened at the adaxial surface but circular at the abaxial surface. Venation is pinnate type, eucamptodromous but brochidodromous at leaf base.

In argan tree, first observations are of Cornu (1897), who found that leaves of young seedlings are elliptical. Wild and Vindt (1952) reported that leaves are entire, leathery, alternate, often united in fascicles. Their shape is lanceolate, oblong-lanceolate or spatulate. They are attenuated more or less stalked, hairless midrib clearly marked. Other authors found that leaves are lanceolate (Perrot, 1907; Ozenda, 1983, El Aboudi, 1990), spatulate (Perrot, 1907; Rieuf, 1962, El Aboudi, 1990), or

oblanceolate (Prendergast and Walker, 1992). Petiole is convex at the abaxial surface, but plan at the adaxial surface (El Aboudi, 1990).

Two leaf types coexist on the same tree. Simple leaves occur alternately on the stem of the year or on portion of growing shoot to unlimited growth, and grouped leaves on woody shoots (Rieuf, 1962; M'Hirit, 1989; Zahidi, 1997). Although the appearance of leaves was discussed, the literature is marked by an absence of detailed studies and none of these authors did not specified number of trees, or the type of leaves studied. Our objective is to establish typology of leaves in argan tree and determine their distribution in each of three populations: Ait Melloul, Ait Baha and Argana.

Materials and Methods

Thirty trees chosen at random from each of three geographic origins in south western Morocco: Ait Melloul (AM), Argana (AR) and Ait Baha (AB) described by Ferradous et al. 1996) were observed. According to the optimal sampling method (Zahidi and Bani-Aameur, 1998c), we sampled leaves among exposed to the north at the end of May, for three consecutive years:

Simple leaves (FS) on the branches of the Year (green branches) (RV).

Two great units of the first two grouped leaves (FG) from apex of branches in growth located on the main branches (Picture 1).

Twenty simple leaves of green shoots and twenty units of grouped leaves of each tree were visually distinguished. Leaf shapes were defined according to their botanical definition (Gatin, 1924; Metro and

Sauvage, 1955; Deysson, 1976). This distinction reflects leaf shape, apex form of leaf blade, shape of the base, blade cut and venation type. Leaves of different shapes have been designed and photographed (Picture 2 and figure 1).

Three characters were measured in centimeters: leaf length (LO), maximum width (LA), and petiole length (LOP). It was deduced the ratio ($RF = LA / LO$) and leaf surface as the product of the length and maximum width ($SURF = LA \times LO$) (Zahidi and Bani-Aameur, 1998c). All measurements were made using a caliper under binocular loupe (magnification x 20) for greater accuracy. Principal component analysis was performed using average of five characters of each tree (mean of three years), on the reduced centered data matrix (Frontier, 1981; Dagnelie, 1986; Godshalk and Timothy, 1987; Bernstein et al., 1988; Iezzoni and Pritts, 1991). Computer processing were performed using Statistix software, and NTSYS-pc STATITCF version 1.40 (Rohlf, 1988).

Result and Discussion

Leaf description

Simple and grouped leaves of argan are glabrous, entire, perfectly continuous margins and wiyh mitigated bases (picture and figure 1).

Blade apex can be obtuse, acute or mucronate. Four forms were distinguished: lanceolate acute apex (LA) or mucronate (LM), spatulate obtuse (ST) and obovate obtuse (OT). Leaf lamina is traversed by a prominent midrib extending petiole from which secondary veins spaced all along this vein. Venation is pinnate type eucamptodromous. Petiole is rounded at the abaxial surface but flattened at the adaxial

Picture.1 Simple leaves on green branch of the year (left) and grouped leaves on the shoots over a year and on the main branch (right).



Figure.1 Forms of simple and grouped leaves in argan tree: OT: obovate obtuse, ST: Spatulate obtuse, LA: Lanceolate acute apex and LM: Lanceolate mucronate apex

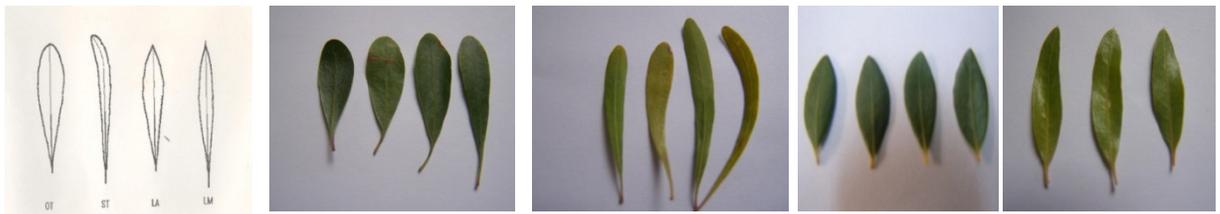


Figure.2 Frequency of forms obovate obtuse (OT), spatulate obtuse apex (ST), lanceolate acute apex (LA) and lanceolate mucronate apex (LM) of simple (A) and grouped (B) leaves in argan trees.

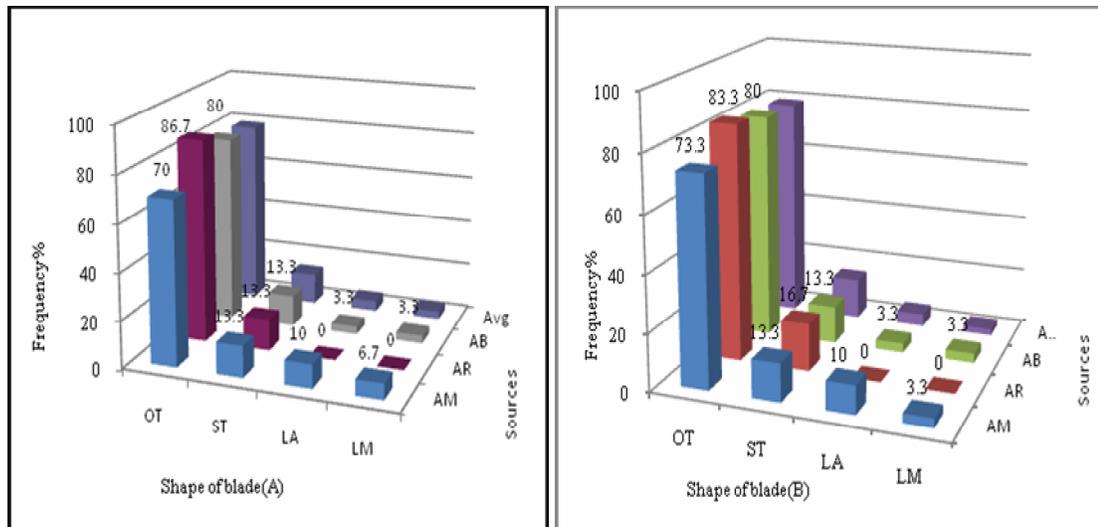


Table.1 Maximum (Max), minimum (Min), average (Avg) and coefficient of variation (CV) of length (LO), width (LA), ratio (RF), petiole length (LOP) and surface (SURF) in leaf forms.

Characters	Form	Simple leaves				Grouped leaves			
		obovate obtuse (OT)	Spatulate obtuse (ST)	lanceolate acute apex (LA)	lanceolate mucronate apex (LM)	obovate obtuse (OT)	Spatulate obtuse (ST)	lanceolate acute apex (LA)	lanceolate mucronate apex (LM)
LO (cm)	Max	4.12	4.86	3.47	3.52	3.62	4.46	3.33	3.08
	Min	1.44	1.81	2.95	3.14	1.88	1.92	2.89	2.93
	Avg	2.65	3.08	3.32	3.33	2.76	3.01	3.11	3.01
	CV	20.33	31.28	6.63	8.06	15.19	26.11	6.52	3.53
	Max	0.81	0.58	0.79	0.57	0.7	0.54	0.66	0.52
LA (cm)	Min	0.37	0.37	0.55	0.52	0.33	0.33	0.45	0.45
	Avg	0.56	0.48	0.63	0.54	0.52	0.44	0.55	0.485
	CV	16.12	16.87	14.76	6.48	14.77	17.21	16.72	10.21
	Max	0.32	0.24	0.23	0.2	0.25	0.18	0.22	0.17
RF	Min	0.15	0.12	0.16	0.16	0.14	0.1	0.14	0.15
	Avg	0.22	0.16	0.19	0.18	0.19	0.15	0.18	0.16
	CV	14.5	17.88	14.45	15.71	13.66	18.05	18.61	8.84
	Max	0.16	0.17	0.15	0.13	0.16	0.16	0.15	0.14
LOP (cm)	Min	0.06	0.08	0.1	0.08	0.06	0.06	0.08	0.07
	Avg	0.09	0.11	0.12	0.11	0.09	0.10	0.11	0.11
	CV	25.12	27.51	17.67	33.67	27.28	27.11	25.53	47.14
	Max	3.41	2.8	2.76	2.04	2.26	1.99	1.96	1.61
SURF (cm ²)	Min	0.66	0.77	1.83	1.64	0.68	0.7	1.43	1.33
	Avg	1.54	1.58	2.11	1.84	1.47	1.36	1.69	1.47
	CV	33.2	43.46	17.72	15.37	26.16	36.05	15.96	13.47

Table.2 Correlation matrix of simple and grouped leaves traits observed in three geographical origins.

Characters	LO	Simple leaves				Grouped leaves				
		LA	RF	LOP	SURF	LO	LA	RF	LOP	SURF
LO	1.0					1.0				
LA	0.64	1.0				0.47	1.0			
RF	-0.68	0.06	1.0			-0.55	0.44	1.0		
LOP	0.59	0.3	-0.5	1.0		0.57	0.22	-0.38	1.0	
SURF	0.92	0.87	-0.38	0.5	1.0	0.85	0.85	0.46	-0.07	1.0

Table.3 Correlation between principal components and traits of simple and grouped leaves in three geographical origins.

Characters	PC1	Simple leaves		PC1	Grouped leaves	
		PC2	PC3		PC2	PC3
LO	0.97	-0.07	0.19	0.93	-0.25	-0.24
LA	0.73	0.67	-0.03	0.7	0.71	-0.01
RF	0.71	-0.35	-0.62	-0.29	0.92	0.23
LOP	-0.6	0.74	-0.28	0.68	-0.35	0.64
SURF	0.95	0.29	0.1	0.95	0.26	-0.14
Eigenvalues	3.23	1.21	0.51	2.81	1.61	0.54
Percentages explained (%)	64.5	24.2	10.2	56.2	32.3	10.8
Cumulative percentages (%)	64.5	88.7	98.9	56.2	88.5	99.3

CP1: First principal component, PC2: Second principal component, PC3: Third principal component.

Figure.3 Projection of four forms of simple leaves of the plane defined by the first two principal components. (T: OT, S: ST, M: LM, L: LA)

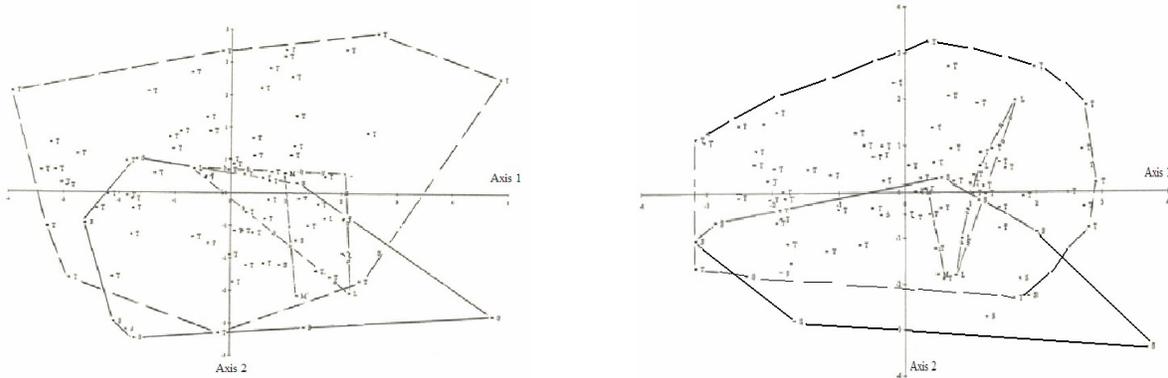


Figure.4 Projection of four forms of simple and grouped leaves in space defined by first three principal components.

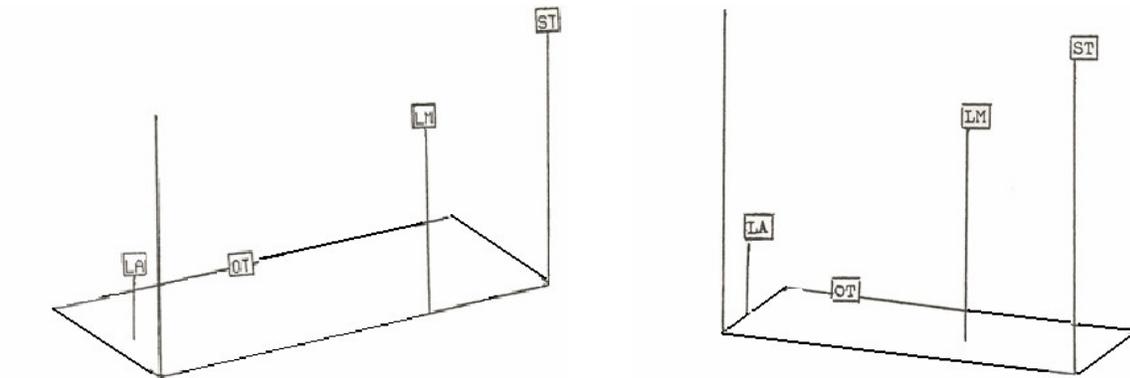


Table.4 Characterization of forms for simple and grouped leaves of trees from Ait Melloul, Argana and Ait Baha.

Form	Shape of apex	Length	Width	Petiole	Ratio	Surface
Obovate obtuse (OT)	Obtuse	Short	Moderately wide	Short	High	Low
Spatulate obtuse (ST)	Obtuse	Long	Narrow	Long	Low	Low
Lanceolate acute apex (LA)	Acute	Longer	wider	Long	Medium	Important
Lanceolate mucronate apex (LM)	Mucronate	Long	Moderately wide	Long	Medium	Medium

surface. Number of secondary veins varied according to leaves forms. It is between 12.2 and 13.2 in OT form, between 13.2 and 15.4 in ST form, from 13.1 to 14.7 in LA form and from 12.2 to 15.1 in LM form. Between the secondary veins, fine tertiary nerves forming an array of ribs inside lamina.

Simple leaves and grouped leaves

In all three geographic origins, shape obovate obtuse (OT) was the most abundant with an average frequency of 78.9% (Figure 2).

The other three forms were more rare (21.1%). Spatulate obtuse shape (ST) was more frequent (13.3%) than lanceolate acute apex (LA) (4.4%), and lanceolate mucronate apex (LM) with an average frequency about 3.3%. OT form was more abundant in Argana (86.7%) than in Ait Baha (80%) than at Ait Melloul when its frequency is 70%. Forms LM and LA, not listed in Argana. Each of these two forms represented only 3.3% at Ait Baha, whereas their frequencies were respectively 6.7% and 10% at Ait Melloul. Frequency of ST form was the same in all three stations, about 13.3%. Similar results were observed in leaves grouped although fréquences however very variable.

Metric characterization of forms + Obovate obtuse

OT form was shorter and moderately large in both simple and grouped leaves; lengths vary from 1.44 to 4.12 cm (Table 1). Petiole was short; ratio varied from 0.06 to 0.32. Leaf surface is low; it varied from 0.66 to 3.41 cm², with an average of about 1.54 cm². Overall, a middle change in OT form was observed for all five traits. Surface shows more variation than petiole

length, leaf length and width. Coefficients of variation were respectively 27.3% and 26.2%.

+ Spatulate obtuse

ST leaves were longer but narrower than OT in simple and grouped leaves. Lengths vary between 1.81 and 4.86 cm (Table 1). Leaf width was ranged from 0.33 to 0.58 cm. Petiole was long, and ranged between 0.06 to 0.17 cm. Leaf ratio was lower compared to OT form between 0.10 and 0.24. Surface was higher in simple but less important compared to OT. All measured variables showed more variation. In ST form, coefficients of variation four five traits were ranged from 17.2% to 36.1%.

+ Lanceolate acute apex shape

LA leaves are longer and wider than ST and OT. Length varied between 2.89 and 3.47 cm (Table 1). Leaf width is ranged from 0.45 and 0.79. Petiole is longer and ranged from 0.08 and 0.15 cm. Ratio is higher than in ST and varied between 0.22 and 0.16. Leaf area was higher than OT and ST. For all variables, coefficients of variation are lower in LA than in ST. Coefficients of variation ranged from 6.5% to 25.5%.

+ Lanceolate mucronate apex shape

LM leaves were relatively longer but narrower than LA in both simple and grouped leaves (Table 1). Petiole was longer; ratio was relatively less important. Surface was lower than LA, but higher than in OT and ST. Excepted for petiole length, generally in LM, a small variation is observed for all characters unlike ST and OT. Surface and ratio showed more variation than leaf and petiole lengths.

Principal component analysis

The relationship between measured traits was illustrated by differences of correlation coefficients (Table 2). Leaf length was highly correlated to leaf area and moderately correlated to leaf width, petiole length and ratio. Leaf width was highly correlated to surface. Ratio was low correlated to petiole length which was low correlated to leaf area. The first three axes explained 98.9% in simple and 99.3% in grouped leaves of the total variability of which more than 88% is absorbed by the first two axes. Characters used seem to describe the variability observed for all leaf shapes (Table 3). In simple leaves, the first axis in relation to an eigenvalue of 3.23, explains 64.5% of total variation. It is highly correlated to length and leaf surface, but moderately correlated to leaf width, petiole length and leaf ratio.

Leaves which were longest, widest, and with larger area (LA and LM), were projected on the positive side, while most of OT and ST shapes which have lengths, widths and surfaces very lowest were situated on the negative side of this axis.

Second axis in relation to an eigenvalue of 1.21, explains 24.2% of total variation. It is correlated to ratio and leaf width. Leaves with short petiole (OT) are projected on positive side of the axis, while leaves with petiole longer (ST, LA and LM) are situated on negative side of this axis.

Third axis, related to an eigenvalue of 0.51, explains 10.2% of total variability. Majority of this variation is attributed to petiole length, with a negative correlation coefficient of about -0.62. Overall, distribution of leaves forms relative to this latter main component is secondary for distinction between shapes.

The screening of individuals in the plane defined by the first two principal axes (Figure 3), reveals a dispersion of OT form more spread out along the first two axes. This distribution is mainly due to variation of dimensions of leaf form (Table 1). ST form is distributed along first axis but remains relatively limited to negative side of second axis. If ST is represented in all leaf lengths, it is characterized by low widths (Table 1). Both LA and LM forms were mostly projected on positive side of the first axis. They are among leaves which are longest and wider. Overlapping polygons observed of four forms was only apparent. In fact, their projection in space defined by three main components, allows a better distinction. Thus, LM and ST were projected on positive side of first principal component (Figure 4).

LA was on positive side of second principal component, whereas OT is on negative side of first and third principal component, but on positive side of second principal component of PCA. Four forms of simple leaves are visually distinct. LA form is characterized by an acute apex, with longer petiole, leaves are wider and with great area. Form LM was characterized by a mucronate apex, with longer petiole, leaves are narrower and with surfaces less important compared to LA form. LA and LM are clearly separate from OT and ST which have obtuse apex. Generally, OT forms are characterized by larger widths near tip of blade, and by shorter petioles unlike ST forms that are narrower at the same region and whose petioles are longer. Similar distribution is observed in grouped leaves (Figure 3, 4).

Forms described in this work differ slightly from those cited in the literature. Lanceolate acute apex (LA) or mucronate (LM) approaches lanceolate form described

by (Sauvage and Vindt, 1952; Ozenda, 1983 and El Aboudi, 1990). Spatulate obtuse shape (ST) is identical to that described by (Perrot, 1907; Wild and Vindt, 1952; Rieuf, 1962; ElAboudi, 1990). Obovate obtuse (OT) observed in *Synsepalum dulcificum* species (Edward Ayensu, 1972) was not previously described in argan tree. Leaf lamina is traversed by a prominent midrib extending petiole from which secondary veins spaced all along this vein. Venation is pinnate type eucamptodromous. Petiole is rounded at the abaxial surface but flattened at the adaxial surface in accordance with observations of ElAboudi (1990).

Blade shapes and frequencies distribution of grouped leaves were relatively similar to those of simple leaves. But, in tree 26 at Ait Melloul, simple leaves are lanceolate with mucronate apex (LM), while the grouped leaves are obovate obtuse. The same finding was observed in Argana. For tree 12, simple leaves are obovate obtuse, but grouped leaves are spatulate with obtuse. We did not observe any similar cases at Ait Baha. LA and LM of simple leaves of green branches were longer than ST as OT. LA shape was wider than OT and LM, they even wider than ST. The petiole was longer in LA, LM and ST than in OT. The ratio was higher in leaves OT than in the other three forms. The surface of leaves was higher in leaves LA than LM, whereas in ST and OT, the surfaces are lowest. LA shape of grouped leaves was longer than LM, ST and OT. LA form was wider than OT. Shape LM was wider than ST form. Petiole was longer in LM, LA and ST form, whereas it was shorter in OT form. Ratio was higher in LA and OT forms, as in LM and ST forms. Surface was higher in LA than in OT and LM forms. In ST form, surface was the lowest. Although characterization of botanical leaf shape was

identical in both simple and grouped leaves, but measured variables change depending on type of leaves considered. Changing size of leaves observed in the argan tree has been noticed in other plant species. Several authors suggested that more or less the same trends in morphological variations may be an important explanation for coexisting species to adapt to their habitats (Cho and al., 2007; Egea-Gilabert, 2009; Xu and al., 2009). Correlations between leaf traits varied in large proportions. Therefore, intrinsic or extrinsic factors can influenced tree traits in the same or in opposite directions. These characters are "plastic" in the same way or because they were subject to one or more common sources of variation (Van Dijk, 1984). Leaf length, leaf width, petiole length, ratio and leaf area used in this work seem to describe the variability observed for all leaf shapes. Variables that measured overall leaf size such as length, width, petiole length and surface used in other plant species (Mosseddaq, 1988; Roudna, 1991; Hokanson et al., 1993; Xu and al., 2009), have allowed a better distinction of four forms. Description of patterns of leaves of argan tree contributed to development of a standard range of characters including the shape of fruit and stone (Bani Aameur and Ferradous, 2001; Bani Aameur, 2004), the phenological stages of the flower, pollen grains (BaniAameur, 2002) and morphological types of the tree (Zahidi, 1997). These characters are currently available for mapping morphological diversity of the argan forest. Quantification and visualization of morphological variation of leaves, flowers and other structures are essential for an overview of evolutionary and ecological processes of phenotypic diversification and is the fundamental basis from which to develop more complex studies to achieve "new

perspectives on the interaction of phenotype, genotype and environment as shown by Jensen (2003).

Visual distinction combined with principal component analysis has identified four shapes as well as in simple than in grouped leaves. Lanceolate acute apex (LA), lanceolate mucronate apex (LM), spatulate obtuse (ST) form and obovate obtuse (OT) described for first time in argan tree. Some of these forms have been described separately by several authors. But none has distinguished between simple and grouped leaves, and their samples were not large enough to detect different forms and their relative importance. LA form was characterized by an acute apex, while LM was characterized by a mucronate apex. They were clearly distinct from forms with obtuse apex (OT and ST) by apex shape of lamina. OT forms were shorter, and characterized by larger widths near the tip of blade, and with shorter petioles. Instead ST forms were longer, narrower at the tip of blade, their petioles were longer compared to OT leaves. Variables that measured overall leaf size such as length, width, petiole length and surface used in other plant species, have allowed a better distinction of the four forms.

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