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MORPHOLOGICAL DIVERSITY OF LEAF CHARACTERS IN THREE NATURAL POPULATIONS OF *Argania spinosa* L. SKEELS

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ABSTRACT

The genetic diversity within and between three natural populations of *Argania spinosa* in south west Morocco was investigated by using nine morphological characters of simple and grouped leaves for three consecutive seasons. Relative contribution of seasonal variations and seasonal variations x locality interaction on the phenotypic variance is low (0% to 17.1%) for all characters of simple and grouped leaves. Relative contribution related to genotype x environment interaction (season x tree / locality) on the phenotypic variance is greater (17% to 40.5%) for all traits except for leaf length, midrib length and dry matter content in simple leaves (0% to 14.9%). Argan tree shows a high degree of genetic diversity especially within populations than between populations. Leaf length, midrib length, leaf width, leaf area and stomata number are the most discriminating characters, being responsible for 54.6% of the total variation, but differentiation between the three populations was not established. This substantial genetic variation for morphological characters provides a database for genetic improvement of agro-morphological characters given that the argan tree is source of food for a large number of herds especially in times of drought in arid environments.

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1 Introduction

Natural populations of *Argania spinosa*, only representative species of the tropical family Sapotaceae in Morocco, is widely distributed in arid and semi-arid regions of south west Morocco. It had an undisputed ecological and economical value as a species resistant to arid conditions. Argan had a large potential for forestry as well as for domestication as an oil tree of medical, dietetic, cosmetic and industrial uses (Maurin, 1992; Ferradous *et* al., 1996; Charouf & Guillaume, 1999). At the present time, several physical and anthropogenic factors reduce the density and area of Argan ecosystems, so it decreases the biodiversity in the arganeraie ecosystem. Due to the continuous intensify of genetic erosion, it is necessary to save situation of this species (Msanda et al., 2005; Bani-Aameur, 2007). Recent interest for its conservation and development increased following UNESCO declaration of argan area of distribution a MAB (Man & Biosphere) Reserve of Biosphere (Bani-Aameur & Benlahbil, 2004).

The evaluation of plant genetic resources in their area of distribution is critical to their conservation and for their protection against genetic erosion (Behm et al., 1997; Roy & Foote 1997; Mückschel & Otte, 2003; Eckstein et al., 2006). Traditional methods to assess genetic variability in plant species are based on morphological characters (Khurshid et al., 2004; Furat & Uzun, 2010). Morphometric techniques have long been established as valuable tools to discriminate heterogeneous populations, and to explore the development of plants (MacLeod & Forey, 2002). Additionally, they contribute with valuable information for breeding programs as well as for conservation strategies of the species concerned (Sarikamis et al., 2010).

Several researches have been conducted to provide information about the genetic diversity in argan species at different levels by using molecular approach (Bani-Aameur & Hilu, 1996; El Mousadik & Petit, 1996; Majourhat et al., 2008) or morphological studies (Ferradous, 1995; Zahidi, 1997; Bani-Aameur & Ferradous, 2001; Bani-Aameur & Benlahbil, 2004; Ait Aabd et al., 2011). In argan tree, two leaf types coexist on the same tree. Simple leaves observed on twigs of the season or twigs to indefinite growth and Grouped leaves on lignified twigs or branches (Rieuf, 1962; Bani-Aameur & Zahidi, 2005). In both simple and grouped leaves, four forms Viz. Obovate obtuse, spatulate obtuse, lanceolate with acute apex, and lanceolate with mucronate apex were distinguished. The frequencies of each form have varied among the three populations from Ait Melloul Argana and Ait Baha (Zahidi, 1997). So, a good understanding of the variation in this species for other morphological characters of simple and grouped leaves seems to be necessary for good programs design of its domestication, conservation and sustainable management. This work is a contribution in this direction by study of morphological variability and estimate effects of seasonal change, locality and genotype on characters of simple and grouped leaves in three natural populations in south west Morocco.

2 Materials and Methods

2.1 Plant material and measurements

The experiment concerns three populations in south west Morocco; i.e. Ait Melloul, Argana and Ait Baha (Table 1). Rainfalls during the three consecutive seasons of study are often scarce and variable taking place mainly during the cold period while summer is dry (Figure1). Thirty trees were randomly selected from each site and are subject of several previous studies for morphological characters of fruit, branching, flower and yield (Ferradous et al., 1996; Zahidi et al., 1996; Bani-Aameur & Ferradous, 2001). Twenty simple and twenty grouped leaves per tree (genotype) were collected in May for three consecutive seasons according to an optimal sampling method (Zahidi & Bani-Aameur, 1998). Four morphometric characters were observed Viz leaf length (LL), leaf width (LW), petiole length (PL) and midrib length (ML). The leaf ratio (LR = LW / LL) and leaf area (LA = LL x LW) were deduced. Leaf dry matter content (DW) was determined after samples were oven-dried at 60°C for 96 hours (Heitholt, 1989). Number of secondary ribs (SR), stomata density (ST) on underside and upper face were recorded according to the method described by BaniAameur & Zahidi (2005). Clear nail polish was painted at the both opposite central positions of the lamina of each leaf to insure uniformity of samples (Hilu & Randal, 1984). The leaf impression was removed by using a fine forceps and mounted in a drop of water on a microscope slide for viewing. Stomata density was recorded at 400x magnification with the help of Olympus BHV-RFC photomicroscope.

2.2. Statistical analysis

An analysis of variance (ANOVA) with three factors (seasonal variation, tree and locality) in hierarchical model was adopted. Tree was hierarchical to locality because trees are not repeated within the same locality and between sites. Seasonal variations and locality were crossed. The Least Significant Difference Test (LSD α = 5 %) of equality of means was used to compare differences between means (Steel & Torrie, 1960; Dagneli, 1984; Sokal & Rohlf, 1995). The phenotypic variation ($\sigma^2 T$) for each trait was partitioned into components due to genetic (hereditary) and non-genetic (environmental) factors and estimated using the following formula (Montgomery, 1984; Lentner & Bishop, 1993) (Table 2a, b).

$$\sigma^2_T = \sigma^2_A + \sigma^2_l + \sigma^2_{A\,x\,l} + \sigma^2_{a/l} + \sigma^2_{A\,x\,a/l} + \sigma^2_e$$

Whereas σ^2_A: variance due to seasonal variation, σ^2_l: variance due to locality, $\sigma^2_{A\,x\,l}$: variance due to season x locality interaction, $\sigma^2_{a/l}$: is the part of the phenotypic variance that can be attributed to genotypic differences among trees (tree / locality), $\sigma^2_{A\,x\,a/l}$: variance due to genotype x environment interaction (seasonal variations x tree / locality) et σ^2_e: variance due to the error.

Table 1 Ecological characterization of the three sites.

Site	Geographical location	Altitude	Latitude	Longitude	Flying distance to	Soil type	Mean annual temperatures		
		(m)	(N)	(E)	Atlantic Ocean (km)		°C (min, mean, max)		
Ait Melloul	Souss plain	32	30°36'	9°39'	12.5	Brown calcic	13.0	18.4	23.0
Argana	Southern slope of High Atlas mountains	620	30°45'	9°08'	60	Red fersialitic	9.1	18.9	28.7
Ait Baha	Northern slope of Anti Atlas mountains	550	30°30'	9°10'	50	Brown	10.7	20.1	29.4

Table 2 Expectations of mean squares and estimated variance components of simple and grouped leaves observed in the three localities (a) and by site (b)

(a)

Source of variation	DF	Mean square	Expectation of mean squares
Seasonal variations	2	CM A	$\sigma^2 e + 20\sigma^2 Aal + 600\sigma^2 Al + 1800\sigma^2 A$
Locality	2	CM l	$\sigma^2 e + 20\sigma^2 Aal + 600\sigma^2 Al + 60\sigma^2 al + 1800\sigma^2 l$
Tree /locality	87	CM al	$\sigma^2 e + 20\sigma^2 Aal + 60\sigma^2 al$
Seasonal variations x locality	4	CM Al	$\sigma^2 e + 20\sigma^2 Aal + 600\sigma^2 Al$
Seasonal variations x tree / locality	174	CM Aal	$\sigma^2 e + 20\sigma^2 Aal$
Error	5130	CM e	$\sigma^2 e$

(b)

Source of variation	DF	Mean square	Expectation of mean squares
Seasonal variations	2	CM A	$\sigma^2 e + 20\sigma^2 Aa + 600\sigma^2 A$
Tree	29	CM a	$\sigma^2 e + 20\sigma^2 Aa + 60\sigma^2 a$
Seasonal variations x tree	58	CM Aa	$\sigma^2 e + 20\sigma^2 Aa$
Error	1710	CM e	$\sigma^2 e$

The relative percentage of each factor in the total variance per station (σ^2_{Ts}) was expressed as:

$$\sigma^2_{Ts} = \sigma^2_A + \sigma^2_a + \sigma^2_{Axa} + \sigma^2_e$$

Whereas σ^2_A: variance due to seasonal variations, σ^2_a: variance due to tree, σ^2_{Axa}: variance due to seasonal variations x tree interaction and σ^2_e: variance per station due to the error.

Broad sense heritability was estimated as the ratio of variance tree / locality to the total phenotypic variance): global repeatability (r^2 g) calculated for the three stations and repeatability (r^2 s) in each of three localities taken individually using the formula below assimilating the season at replication in time (Kempthorne, 1973; Pfahler et al., 1996; Zahidi, 1997; Bani Aameur & Ferradous, 2001).

$$r^2_g = 100 \text{ x } (\sigma^2_{a/l} / \sigma^2_{a/l} + \sigma^2_{Axa/l} + \sigma^2_e) .$$

The sum ($\sigma^2_{a/l} + \sigma^2_{Axa/l} + \sigma^2_e$) represents the total phenotypic variance in the three localities, and ($\sigma^2_{a/l}$) is the genetic variance in broad sense.

Repeatability per station is calculated using the following model:

$$r^2_s = 100 \text{ x } (\sigma^2_a / \sigma^2_a + \sigma^2_{Axa} + \sigma^2_e),$$

the sum ($\sigma^2_a + \sigma^2_{Axa} + \sigma^2_e$) is considered as total phenotypic variance per station (σ^2_a) variance of tree.

Multivariate analysis (Principal component analysis) and Cluster analysis were performed on annual averages of nine characters per tree (Frontier, 1981; Bernstein et al., 1988). We opted for annual averages since trees response to seasonal variations of temperatures and rainfalls is not homogeneous. The analysis is performed using Statistix, Statitcf, NTCYS-pc package of computer programs (Version1.5, Rohlf, 1988) localities and genotypes were grouped using UPGMA (Unweighted Pair-Group Method Arithmetic Average).

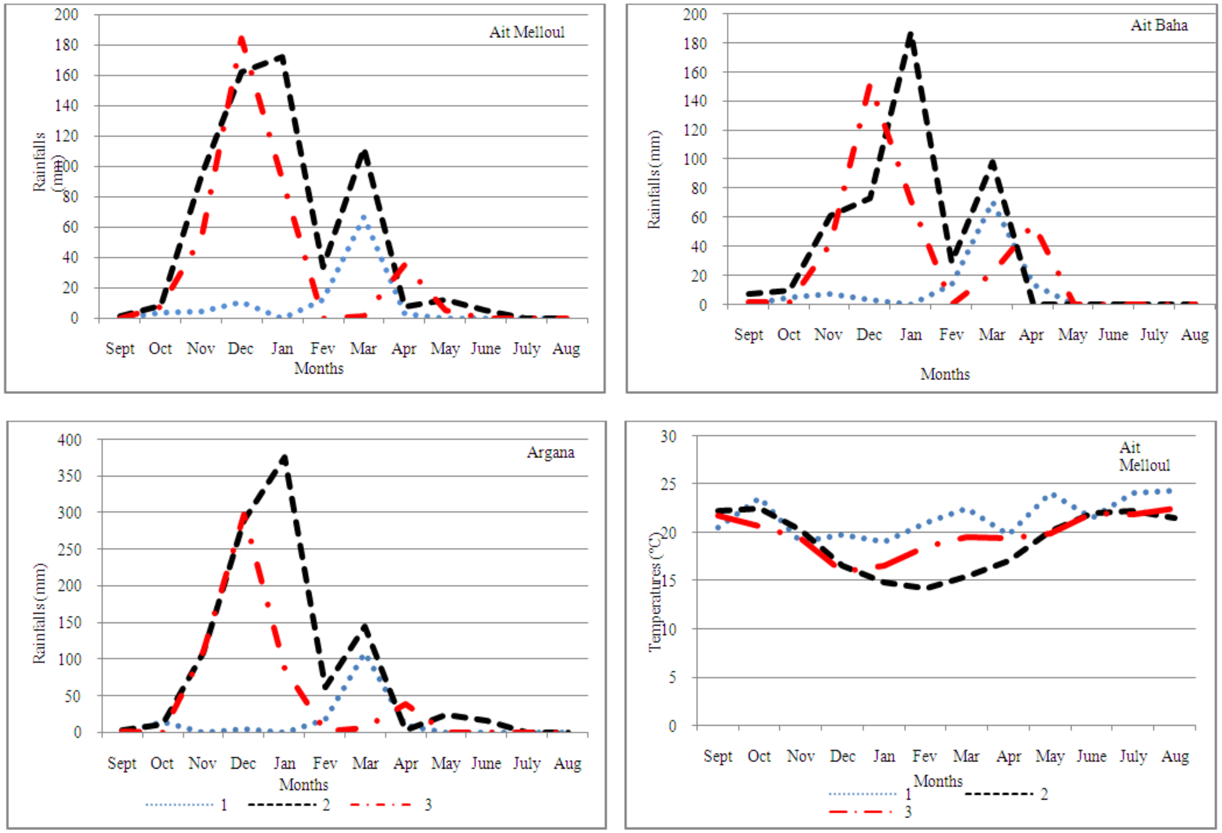
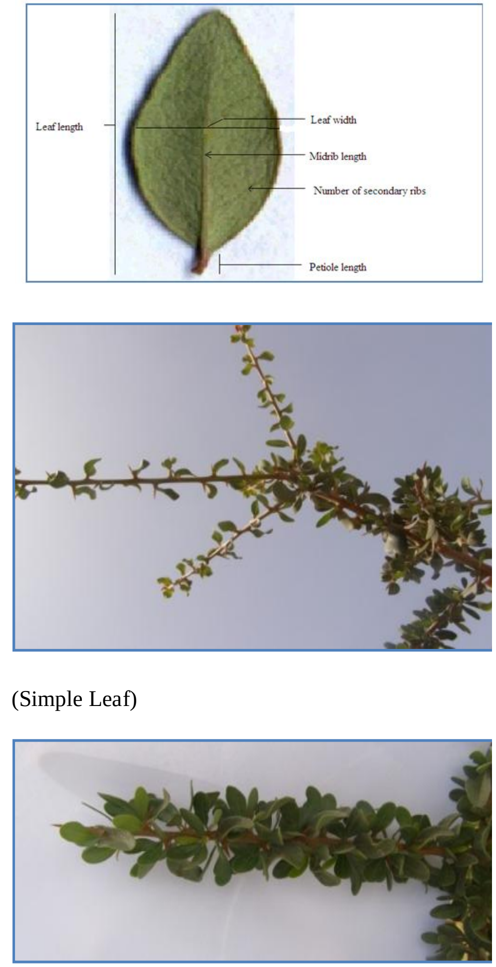


Figure 1 Climatic data from meteorological stations: mean monthly rainfalls (mm) and temperatures recorded at Ait Melloul (AM), Argana (AR) and Ait Baha.
(1: first season, 2: second season, 3: third season).



(Simple Leaf)

(Grouped Leaf)

Figure 2 Morphological characters of simple and grouped leaves from Ait Melloul, Argana and Ait Baha.

Table 3a & 3b Variance analysis of morphological characters of simple (a) and grouped leaves (b) from Ait Melloul, Argana and Ait Baha.

a										
Source of variation	DF				Means square					
		LL	LW	LR	LA	PL	SR	ML	DW	ST
Seasonal variations	2	131.4 ns	7.59 *	0.02 ns	191.2 *	0.24 ns	263.33 ns	131.4 ns	0.009 *	479.04 **
Locality	2	593.5 **	7.33 **	0.96 **	396.3 **	0.31 **	892.71 **	593.5 **	0.01 **	290.05 ns
Tree / locality	87	10.6 **	0.36 **	0.06 *	8.69 **	0.03 **	112.43 **	10.6 **	0.0006 **	214.11 **
Seasonal variations x locality	4	21.42 **	0.48 **	0.14 **	15.67 **	0.1 **	136.36 **	21.42 **	0.0006 **	90.95 ns
Seasonal variations x tree / locality	174	2.0 **	0.11 **	0.02 **	2.58 **	0.009 **	35.74 **	2 **	0.0001 **	124.02 **
Error	5130	0.01	0.01	0.000232	0.01	0.000113	1.04	0.01	0.000166	7.76

b										
Source of variation	DF				Means square					
		LL	LW	LR	LA	PL	SR	ML	DW	ST
Season variations	2	39.43 ns	2.89 **	0.06 ns	59.03 **	0.15 ns	412.31 ns	39.43 ns	0.004 ns	214.27 ns
Locality	2	313.44 **	5.75 **	0.27 **	206.02 **	0.46 **	1027.5 **	313.44 **	0.009 **	183.12 ns
Tree / locality	87	7.26 **	0.27 **	0.05 **	4.77 **	0.03 **	141.65 **	7.26 **	0.0004 **	162.19 **
Season x locality	4	6.52 **	0.07 *	0.06 **	0.92 *	0.04 **	243.92 **	6.52 **	0.0006 **	57.76 ns
Season x tree / locality	174	1.26 **	0.07 **	0.01 **	1.27 **	0.008 **	47.55 **	1.26 **	0.00008 **	76.10 **
Error	5130	0.01	0.000907	0.000144	0.01	0.000103	0.99	0.01	0.00000142	8.04

DF: degree of freedom, ns: Not significant, *: Significant at 5%, **: Significant at 1%.

Table 4a Tree mean (Avg), minimum (Min), maximum (Max) and coefficient of variation (CV) of the observed characters of simple leaves in the three localities,

Character	Season	Ait Melloul				Argana				Ait Baha				Average			
		Max	Min	Avg	CV	Max	Min	Avg	CV	Max	Min	Avg	CV	Max	Min	Avg	CV
LL (cm)	1	4.92	1.72	2.97 c	19.27	3.16	1.10	1.69 c	24.02	3.85	1.72	2.74 c	12.28	3.98	1.51	2.47	18.53
	2	5.15	2.34	3.45 a	18.36	3.53	1.44	2.21 b	20.58	4.98	1.92	3.33 a	17.84	4.55	1.90	3.00	18.93
	3	5.25	1.98	3.10 b	17.84	3.83	1.28	2.42 a	18.25	3.80	2.17	2.95 b	12.27	4.29	1.81	2.82	16.12
	Average	5.11	2.01	3.17 a	18.49	3.51	1.27	2.11 c	20.95	4.21	1.94	3.01 b	14.13	4.27	1.74	2.76	17.86
LW (cm)	1	0.89	0.28	0.52 c	20.83	0.66	0.17	0.40 b	26.11	0.82	0.26	0.52 b	16.12	0.79	0.24	0.48 c	21.02
	2	1.14	0.37	0.68 a	23.34	0.74	0.30	0.52 a	17.99	0.98	0.29	0.61 a	19.54	0.95	0.32	0.61 a	20.29
	3	0.84	0.43	0.59 b	12.05	0.71	0.36	0.52 a	13.65	0.86	0.45	0.62 a	11.57	0.80	0.41	0.58 b	12.42
	Average	0.96	0.36	0.60 a	18.74	0.70	0.28	0.48 c	19.25	0.89	0.33	0.58 b	15.74	0.85	0.32	0.55	17.91
SR	1	24.00	7.00	13.41 b	18.57	18.00	7.00	11.79	16.04	20.00	7.00	11.97	16.64	20.67	7.00	12.39	17.08
	2	20.00	9.00	13.75 a	11.78	17.00	8.00	12.45	12.95	19.00	8.00	12.23	15.87	18.67	8.33	12.81	13.53
	3	22.00	8.00	12.43 c	16.59	20.00	7.00	11.24	18.69	18.00	8.00	12.46	16.52	20.00	7.67	12.04	17.27
	Average	22.00	8.00	13.19 a	15.65	18.33	7.33	11.83 c	15.89	19.00	7.67	12.22 b	16.34	19.78	7.67	12.41	15.96
DW (g)	1	0.02	0.01	0.013 c	27.10	0.02	0.00	0.007 b	40.64	0.02	0.00	0.012 c	28.32	0.02	0.00	0.011 c	32.02
	2	0.04	0.01	0.017 a	34.00	0.02	0.01	0.012 a	24.85	0.05	0.01	0.018 a	34.45	0.04	0.01	0.015 a	31.10
	3	0.03	0.01	0.014 b	28.04	0.02	0.01	0.012 a	23.14	0.03	0.01	0.014 b	24.69	0.02	0.01	0.013 b	25.29
	Average	0.03	0.01	0.014 a	29.71	0.02	0.00	0.009 c	29.54	0.03	0.01	0.014 b	29.15	0.03	0.01	0.013	29.47
LR=(LW/LL)	1	0.30	0.08	0.18 b	22.30	0.45	0.10	0.24 a	22.78	0.67	0.11	0.19 b	20.15	0.47	0.10	0.20	21.74
	2	0.33	0.13	0.20 a	21.82	0.38	0.14	0.24 a	21.32	0.30	0.11	0.19 b	21.38	0.34	0.12	0.21	21.51
	3	0.33	0.10	0.12 a	18.61	0.36	0.14	0.22 b	15.64	0.30	0.14	0.21 a	14.79	0.33	0.13	0.21	16.35
	Average	0.32	0.10	0.19 c	20.91	0.40	0.13	0.23 a	19.91	0.42	0.12	0.20 b	18.77	0.38	0.12	0.21	19.87
PL (cm)	1	0.22	0.01	0.11	31.62	0.16	0.04	0.07 b	27.95	0.18	0.03	0.08 c	32.02	0.19	0.03	0.08	30.53
	2	0.17	0.05	0.11	25.29	0.15	0.05	0.08 b	21.60	0.26	0.06	0.12 a	37.35	0.19	0.05	0.10	28.08
	3	0.23	0.06	0.11	28.87	0.18	0.05	0.01 a	32.01	0.16	0.05	0.10 b	26.14	0.19	0.05	0.11	29.01
	Average	0.21	0.04	0.11 a	28.60	0.16	0.05	0.08 c	27.19	0.20	0.05	0.10 b	31.83	0.19	0.04	0.10	29.21
ML (cm)	1	4.92	1.72	2.97 c	19.27	3.16	1.10	1.69 c	24.02	3.85	1.72	2.74 c	12.28	3.98	1.51	2.47	18.53
	2	5.15	2.34	3.45 a	18.36	3.53	1.44	2.21 b	20.58	4.98	1.92	3.33 a	17.84	4.55	1.90	3.00	18.93
	3	5.25	1.98	3.10 b	17.84	3.83	1.28	2.42 a	18.25	3.80	2.17	2.95 b	12.27	4.29	1.81	2.82	16.12
	Average	5.11	2.01	3.17 a	18.49	3.51	1.27	2.11 c	20.95	4.21	1.94	3.01 b	14.13	4.27	1.74	2.76	17.86
LA (cm^2)	1	2.92	0.60	1.55 c	30.20	1.86	0.28	0.70 c	45.61	2.25	0.48	1.44 c	21.47	2.34	0.45	1.23 c	32.43
	2	5.17	0.94	2.40 a	35.86	2.37	0.57	1.18 b	34.60	4.39	0.56	2.05 a	29.97	3.98	0.69	1.88 a	33.47
	3	3.06	1.13	1.85 b	22.47	2.36	0.52	1.27 a	28.91	3.10	1.11	1.84 b	18.34	2.84	0.92	1.65 b	23.24
	Average	3.72	0.89	1.93 a	29.51	2.20	0.46	1.05 c	36.37	3.25	0.72	1.78 b	23.26	3.05	0.69	1.59	29.71
ST	1	33.00	9.00	23.3 b	26.56	32.00	19.00	24.30	15.06	31.00	17.00	22.68	16.65	32.00	15.00	23.43	19.42
	2	35.00	13.00	23.73 a	19.20	28.00	18.00	23.14	9.15	28.00	18.00	22.82	10.04	30.33	16.33	23.23	12.80
	3	30.00	11.00	23.18 c	18.61	27.00	19.00	23.07	7.53	24.00	20.00	22.41	13.78	27.00	16.67	22.89	13.31
	Average	32.67	11.00	23.40	21.46	29.00	18.67	23.50	10.58	27.67	18.33	22.64	13.49	29.78	16.00	23.18	15.18

Means followed by letters are significantly different at 5%.

Table 4b Tree mean (Avg), minimum (Min), maximum (Max) and coefficient of variation (CV) of the observed characters of grouped leaves in the three localities.

Character	Season	Ait Melloul				Argana				Ait Baha				Average			
		Max	Min	Avg	CV	Max	Min	Avg	CV	Max	Min	Avg	CV	Max	Min	Avg	CV
LL (cm)	1	4.48	1.78	2.94	15.46	3.34	1.46	2.13 c	16.92	3.90	2.16	2.91 b	11.79	3.91	1.80	2.66	14.72
	2	4.78	2.34	3.28 a	14.25	3.78	1.60	2.40 b	16.76	4.16	2.40	3.15 a	12.03	4.24	2.11	2.94	14.34
	3	4.88	2.24	3.17 b	16.40	3.57	1.50	2.50 a	14.80	4.07	2.01	2.92 b	12.04	4.17	1.92	2.86	14.42
	Avg	4.71	2.12	3.13 a	15.37	3.56	1.52	2.35 c	16.16	4.04	2.19	2.99 b	11.96	4.11	1.94	2.82	14.50
LW (cm)	1	0.73	0.22	0.48 b	18.30	0.73	0.13	0.39 c	23.85	0.85	0.34	0.51 b	18.16	0.77	0.23	0.46 b	20.10
	2	0.78	0.35	0.53 a	15.86	0.70	0.32	0.48 a	17.09	0.91	0.31	0.58 a	17.32	0.80	0.33	0.53 a	16.76
	3	0.80	0.40	0.55 a	14.37	0.67	0.29	0.46 b	17.44	0.74	0.40	0.58 a	13.62	0.74	0.36	0.53 a	15.15
	Avg	0.77	0.32	0.52 b	16.18	0.70	0.25	0.45 c	19.46	0.83	0.35	0.56 a	16.37	0.77	0.31	0.51	17.33
SR	1	25.00	8.00	13.18 b	19.75	20.00	9.00	12.30 a	14.14	20.00	7.00	12.14 b	18.67	21.67	8.00	12.54	17.52
	2	23.00	8.00	13.51 a	17.82	15.00	7.00	11.74 b	13.10	18.00	7.00	11.68 c	23.97	18.67	7.33	12.31	18.30
	3	18.00	7.00	12.22 c	15.19	15.00	6.00	10.40 c	19.61	21.00	7.00	12.24 a	17.01	18.00	6.67	11.62	17.27
	Avg	22.00	7.67	12.97 a	17.59	16.67	7.33	11.48 c	15.62	19.67	7.00	12.02 b	19.88	19.44	7.33	12.16	17.70
DW (g)	1	0.03	0.01	0.012 b	25.95	0.02	0.00	0.008 c	40.24	0.02	0.01	0.012 c	26.80	0.02	0.00	0.011	31.00
	2	0.03	0.01	0.015 a	26.86	0.02	0.01	0.011 a	26.37	0.04	0.01	0.015 a	28.19	0.03	0.01	0.014	27.14
	3	0.02	0.01	0.014 a	20.53	0.02	0.01	0.011 a	18.26	0.02	0.01	0.013 b	14.62	0.02	0.01	0.012	17.80
	Avg	0.02	0.01	0.014 a	24.45	0.02	0.01	0.009 b	28.29	0.03	0.01	0.013 a	23.20	0.02	0.01	0.01	25.31
LR=(LW/LL)	1	0.29	0.08	0.17	22.68	0.30	0.07	0.19 b	21.35	0.33	0.11	0.18 c	19.57	0.31	0.09	0.18	21.20
	2	0.27	0.09	0.17	19.85	0.32	0.13	0.20 a	18.98	0.28	0.12	0.19 b	17.54	0.29	0.11	0.19	18.79
	3	0.26	0.09	0.18	21.64	0.33	0.12	0.19 b	16.96	0.30	0.13	0.20 a	15.37	0.30	0.11	0.19	17.99
	Avg	0.27	0.09	0.17	21.39	0.32	0.11	0.19 a	19.10	0.30	0.12	0.19 a	17.49	0.30	0.10	0.18	19.33
PL (cm)	1	0.21	0.03	0.11	33.88	0.19	0.03	0.07 c	33.72	0.16	0.03	0.08 b	28.44	0.19	0.03	0.09	32.01
	2	0.24	0.06	0.11	30.30	0.16	0.04	0.08 b	28.84	0.26	0.06	0.11 a	36.01	0.22	0.05	0.10	31.72
	3	0.18	0.05	0.12	25.29	0.18	0.05	0.09 a	29.18	0.17	0.06	0.10 a	24.69	0.18	0.05	0.10	26.39
	Avg	0.21	0.05	0.11 a	29.82	0.18	0.04	0.08 b	30.58	0.20	0.05	0.10 a	29.71	0.19	0.05	0.10	30.04
ML (cm)	1	4.48	1.78	2.94 c	15.46	3.34	1.46	2.13 c	16.92	3.90	2.16	2.91 b	11.79	3.91	1.80	2.66	14.72
	2	4.78	2.34	3.28 a	14.25	3.78	1.60	2.40 b	16.76	4.16	2.40	3.15 a	12.03	4.24	2.11	2.94	14.34
	3	4.88	2.24	3.17 b	16.40	3.57	1.50	2.50 a	14.80	4.07	2.01	2.92 b	12.04	4.17	1.92	2.86	14.42
	Avg	4.71	2.12	3.13 a	15.37	3.56	1.52	2.35 c	16.16	4.04	2.19	2.99 b	11.96	4.11	1.94	2.82	14.50
LA (cm²)	1	2.51	0.54	1.40 c	23.21	2.39	0.23	0.86 c	37.15	2.48	0.78	1.50 c	23.01	2.46	0.51	1.25 c	27.79
	2	2.76	0.88	1.75 a	20.65	2.60	0.60	1.18 a	30.18	3.37	0.81	1.84 a	24.27	2.91	0.77	1.59 a	25.04
	3	2.96	1.14	1.73 b	20.51	2.29	0.59	1.17 b	29.48	2.95	0.99	1.71 b	21.43	2.73	0.91	1.53 b	23.81
	Avg	2.74	0.85	1.63 b	21.46	2.43	0.47	1.07 c	32.27	2.93	0.86	1.68 a	22.90	2.70	0.73	1.46	25.54
ST	1	29.00	13.00	22.70	20.48	34.00	16.00	23.94	17.66	28.00	15.00	22.38	14.92	30.33	14.67	23.01	17.69
	2	29.00	13.00	22.57	17.60	25.00	18.00	22.82	7.09	27.00	19.00	22.96	9.04	27.00	16.67	22.78	11.24
	3	26.00	14.00	22.63	13.92	26.00	18.00	22.73	6.81	24.00	21.00	22.61	3.80	25.33	17.67	22.66	8.18
	Avg	28.00	13.33	22.63	17.33	28.33	17.33	23.16	10.52	26.33	18.33	22.65	9.25	27.56	16.33	22.82	12.37

Means followed by letters are significantly different at 5%.

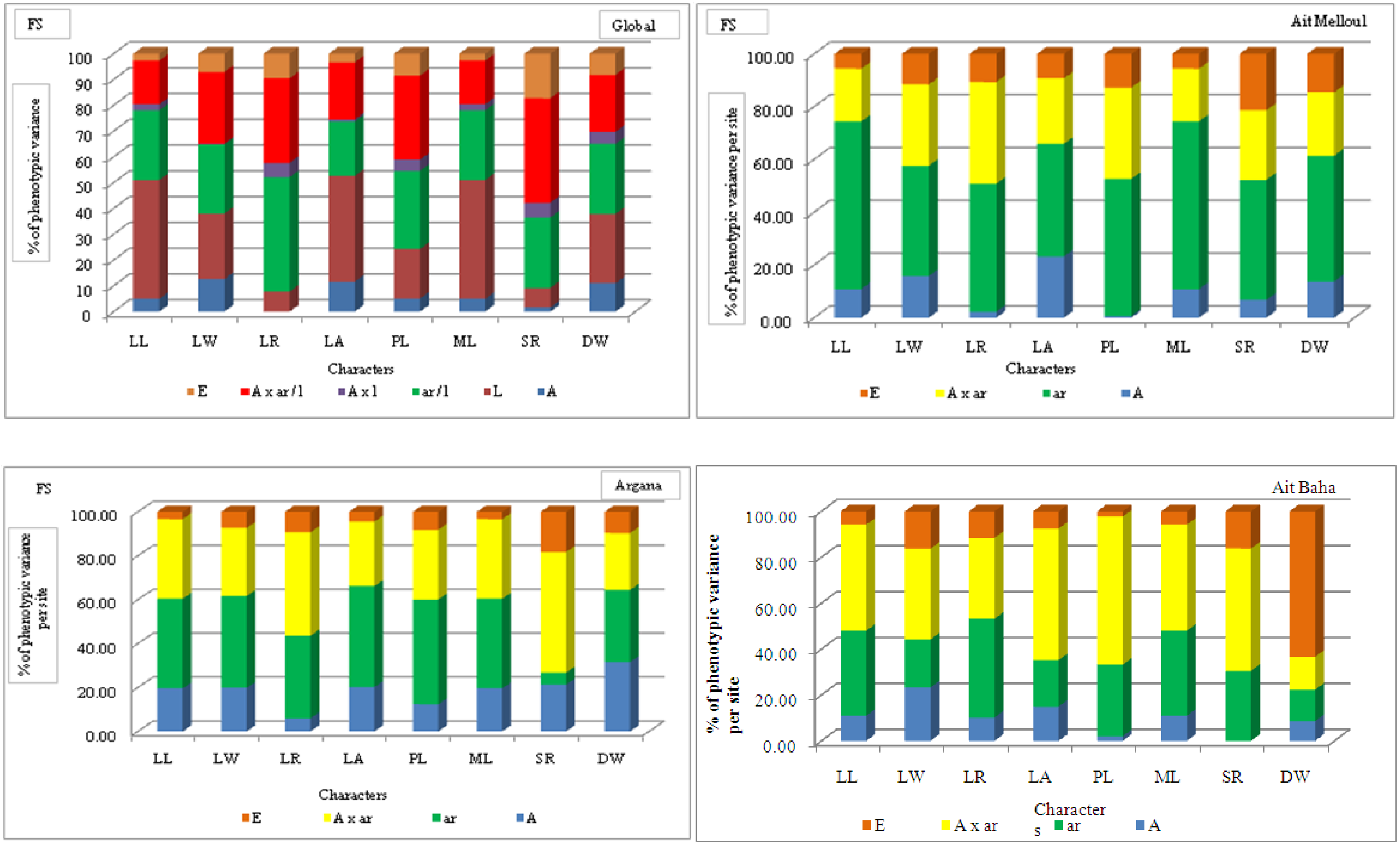


Figure 3a Percentages of total variance (global) and by station for characters of simple leaves observed in Ait Melloul (AM) Argana (AR) and Ait Baha (AB). A: seasonal variations, l: locality, ar/l: Tree/locality, E: Error, A x l: seasonal variations x locality interaction, A x ar / l: seasonal variations x tree / locality interaction.

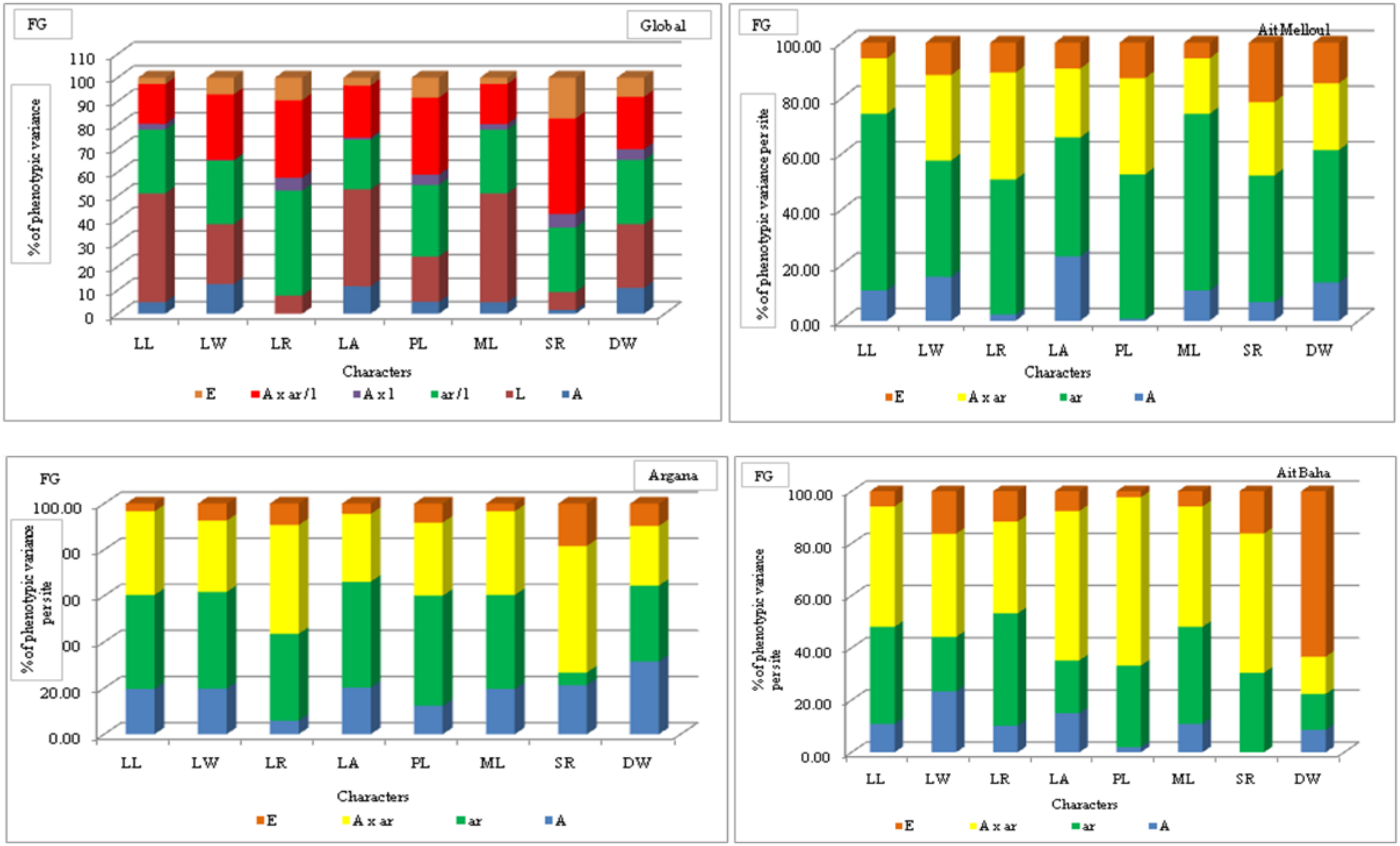


Figure 3b Percentages of total variance (global) and by station for characters of grouped leaves observed in Ait Melloul (AM) Argana (AR) and Ait Baha (AB). A: seasonal variations, l: locality, ar/l: Tree/locality, E: Error, A x l: seasonal variations x locality interaction, A x ar / l: seasonal variations x tree / locality interaction.

3 Results

3.1 The phenotypic variability

3.1.1 Seasonal variations

Seasonal variations was found significant for leaf width (LW), leaf area (LA) of simple and grouped leaves, for dry matter content (DW) and stomata numbers (ST) of simple leaves. It was not significant for the remaining characters (Table 3a, b). Simple leaves were larger, having area and dry matter content greater during the very humid season (2nd); than in humid (3rd) and dry seasons (1st). The four characters are more variable in 1st and 2nd season than in 3rd season (Table 4a). Similar findings are observed in grouped leaves. But, there was less variation than in simple leaves (Table 4b).

Table 5.1 Variances and broad sense heritabilities in percentages of simple (a) and grouped (b) 1eaves characters from Ait Melloul, Argana and Ait Baha.

a

Sources of variation	Variances								
	LL	LW	LR	LA	PL	SR	ML	DW	ST
Seasonal variations σ^2 A	0.06110	0.00395	0.00000	0.09752	0.00008	0.07054	0.06110	4.66E-06	0.215
Locality σ^2 l	0.31782	0.00381	0.00046	0.21146	0.00012	0.42019	0.31782	5.22E-06	0.1106
Tree / locality σ^2 al	0.14333	0.00417	0.00067	0.10183	0.00035	1.27817	0.14333	8.33E-06	1.5015
Seasonal variations x locality σ^2 Al	0.03237	0.00062	0.00020	0.02182	0.00015	0.16770	0.03237	8.33E-07	0.00
Seasonal variations x tree / locality σ^2 Aal	0.09950	0.00500	0.00099	0.12850	0.00044	1.73500	0.09950	0.00000	5.813
Seasonal variations x tree / locality x leaf (error) σ^2 e	0.01000	0.01000	0.00023	0.01000	0.00011	1.04000	0.01000	0.00017	7.76
r^2 g = 100 x ($\sigma^2_{a/l}$ / σ^2_e + $\sigma^2_{a/l}$ + $\sigma^2_{A \times a/l}$)	**56.69**	**21.74**	**35.33**	**43.37**	**38.57**	**31.54**	**56.69**	**4.78**	**9.96**

b

Sources of variation	Variances								
	LL	LW	LR	LA	PL	SR	ML	DW	ST
Seasonal variations σ^2 A	0.01828	0.00157	0.00000	0.03228	0.00006	0.09355	0.01828	1.901E-06	0.08695
Locality σ^2 l	0.17051	0.00316	0.00012	0.11394	0.00023	0.43532	0.17049	4.68E-06	0.0696
Tree / locality σ^2 al	0.10000	0.00333	0.00067	0.05833	0.00037	1.56833	0.10000	4.72E-06	1.4348
Seasonal variations x locality σ^2 Al	0.00877	0.00000	0.00008	0.00000	0.00005	0.32728	0.00877	8.04E-07	0.00
Seasonal variations x tree / locality σ^2 Aal	0.06250	0.00345	0.00049	0.06300	0.00039	2.32800	0.06250	3.85E-06	3.403
Seasonal variations x tree / locality x leaf (error) σ^2 e	0.01000	0.00091	0.00014	0.01000	0.00010	0.99000	0.01000	1.42E-06	8.40
r^2 g = 100 x ($\sigma^2_{a/l}$ / σ^2_e + $\sigma^2_{a/l}$ + $\sigma^2_{A \times a/l}$)	**57.97**	**43.32**	**51.15**	**44.42**	**42.41**	**32.10**	**57.97**	**47.25**	**11.14**

Table 5.2 Repeatabilities in percentages for characters of simple (a) and grouped leaves (b) per locality.

a

Character	LL	LW	LR	LA	PL	SR	ML	DW	ST
Ait Melloul	64.13	37.34	41.42	45.30	42.35	32.88	64.13	41.19	35.10
Argana	67.31	50.61	29.85	**11.05**	47.55	36.44	67.31	**67.32**	38.30
Ait Baha	35.39	26.05	40.40	18.71	35.81	25.70	35.39	45.05	26.74

b

Character	LL	LW	LR	LA	PL	SR	ML	DW	ST
Ait Melloul	**71.31**	49.66	49.67	55.63	52.32	48.82	71.31	55.21	46.13
Argana	50.73	52.18	39.97	57.53	54.45	6.97	50.73	48.00	18.12
Ait Baha	41.65	**0.00**	47.94	23.77	0.00	30.46	41.65	15.03	24.4

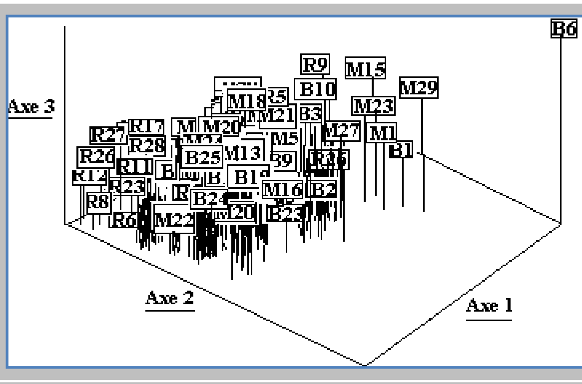


Figure 4a Principal components analysis using morphological traits of simples leaves of the different genotypes from Ait Melloul (M), Argana (R) and Ait Baha (B)

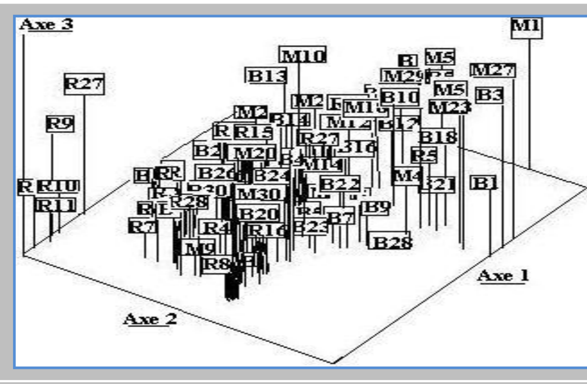


Figure 4b Principal components analysis using morphological characters of grouped leaves of the different genotypes from Ait Melloul (M), Argana (R) and Ait Baha (B)

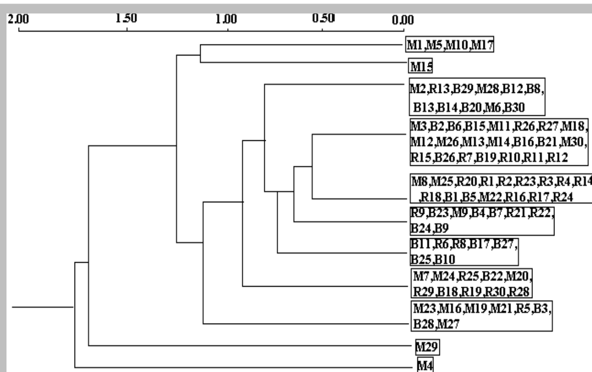


Figure 5a UPGMA dendrogram of 90 genotypes based on Euclidean Distance, using morphological traits of simple leaves of three natural populations from Ait Melloul (M), Argana (R), and Ait Baha (B)

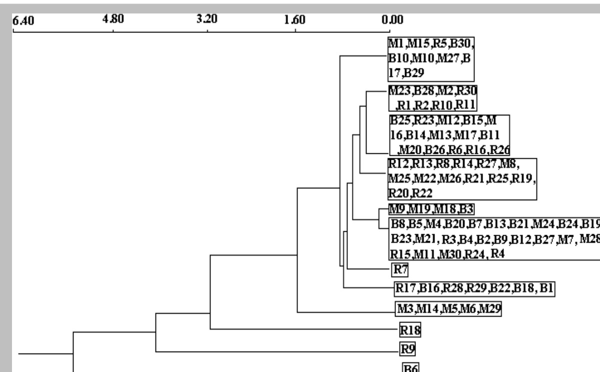


Figure 5b UPGMA dendrogram of 90 genotypes based on Euclidean Distance, using morphological traits of grouped leaves of three natural populations from Ait Melloul (M), Argana (R), and Ait Baha (B)

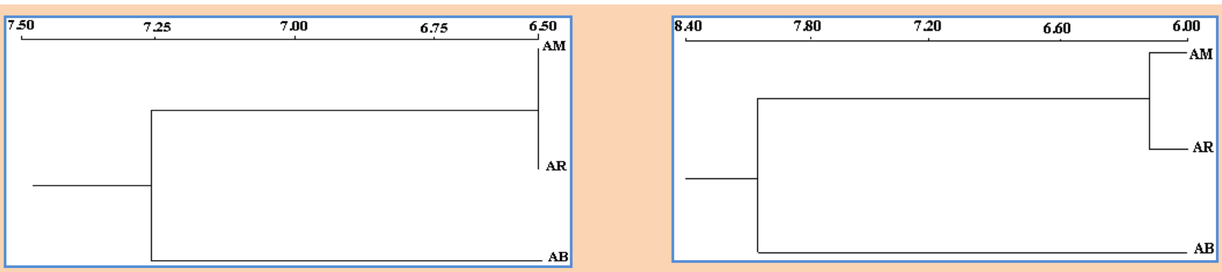


Figure 6 Classification of localities Ait Melloul (AM), Argana (AR), and Ait Baha (AB) for characters of simple (left) and grouped leaves (right).

Table 6a Correlation matrix of morphological characters in simple leaves observed for three consecutive seasons in the three localities.

Character	LL1	LL2	LL3	LW1	LW2	LW3	SR1	SR2	SR3	DW1	DW2	DW3	PL1	PL2	PL3	LR1	LR2	LR3	ML1	ML2	ML3	LA1	LA2	LA3	ST1	ST2	ST3
LL1	-																										
LL2	0.84	-																									
LL3	0.73	0.71	-																								
LW1	**0.60**	**0.51**	0.43	-																							
LW2	0.42	**0.56**	0.41	0.63	-																						
LW3	**0.50**	0.49	0.48	0.64	0.42	-																					
SR1	0.48	0.42	0.35	0.34	0.28	0.15	-																				
SR2	0.33	0.43	0.39	0.16	0.33	0.10	0.54	-																			
SR3	0.43	0.45	**0.61**	0.26	0.21	0.31	0.39	0.44	-																		
DW1	**0.60**	**0.56**	0.41	**0.61**	0.35	0.48	0.37	0.17	0.31	-																	
DW2	**0.54**	**0.62**	0.38	0.49	0.36	0.43	0.28	0.24	0.27	0.51	-																
DW3	0.34	0.34	0.45	0.39	0.35	0.40	0.26	0.23	0.32	0.45	0.44	-															
PL1	**0.57**	0.46	0.41	0.23	0.20	0.02	0.41	0.34	0.23	0.32	0.22	0.23	-														
PL2	**0.52**	**0.56**	0.32	0.35	0.22	0.24	0.14	0.10	0.27	0.32	0.42	0.24	0.47	-													
PL3	0.28	0.26	0.46	0.14	0.18	0.08	0.12	0.19	0.28	0.07	0.09	0.25	0.49	0.37	-												
LR1	**-0.64**	**0.52**	-0.46	0.17	0.04	-0.07	-0.17	-0.18	-0.23	-0.11	-0.20	-0.05	-0.43	-0.30	-0.22	-											
LR2	**-0.56**	**-0.61**	-0.44	-0.07	0.25	-0.23	-0.22	-0.16	-0.29	-0.31	-0.32	-0.09	-0.34	-0.42	-0.17	0.62	-										
LR3	-0.38	-0.39	**-0.71**	0.01	-0.13	0.22	-0.20	-0.31	0.37	-0.03	-0.07	-0.13	-0.43	-0.19	-0.45	0.45	0.32	-									
ML1	**1.00**	**0.84**	**0.73**	**0.60**	0.42	**0.50**	0.47	0.33	0.43	**0.60**	**0.54**	0.34	**0.57**	**0.52**	0.28	**-0.64**	**-0.56**	-0.38	-								
ML2	**0.84**	**1.00**	**0.71**	**0.51**	**0.56**	0.49	0.41	0.43	0.45	**0.56**	**0.62**	0.36	0.46	**0.56**	0.26	**-0.52**	**-0.61**	-0.39	0.84	-							
ML3	**0.73**	**0.71**	**1.00**	0.43	0.41	0.48	0.35	0.39	**0.61**	0.41	0.39	0.45	0.41	0.32	0.46	-0.46	-0.44	**-0.71**	0.73	0.71	-						
LA1	**0.91**	**0.76**	**0.66**	**0.86**	**0.58**	**0.62**	0.47	0.29	0.39	**0.68**	**0.60**	0.46	0.48	0.49	0.24	-0.31	-0.35	-0.23	**0.91**	**0.76**	**0.66**	-					
LA2	**0.69**	**0.87**	**0.63**	**0.59**	**0.87**	0.49	0.39	0.45	0.34	0.49	**0.55**	0.44	0.37	0.42	0.24	-0.23	-0.21	-0.30	**0.69**	**0.87**	**0.63**	0.72	-				
LA3	**0.71**	**0.70**	**0.90**	**0.59**	0.48	**0.81**	0.31	0.31	**0.56**	**0.51**	0.47	**0.54**	0.28	0.32	0.34	-0.32	-0.38	-0.36	**0.71**	**0.70**	**0.90**	0.73	0.66	-			
ST1	-0.14	-0.18	-0.16	-0.27	-0.18	-0.06	0.01	0.09	-0.12	-0.09	-0.19	-0.16	0.13	-0.06	0.16	-0.11	-0.01	0.14	-0.14	-0.18	-0.16	-0.22	-0.17	-0.15	-		
ST2	0.03	-0.03	0.02	-0.19	-0.13	-0.05	0.05	0.21	0.02	-0.11	-0.21	-0.07	0.23	0.02	0.20	-0.22	-0.08	-0.05	0.03	-0.03	0.02	-0.08	-0.08	-0.02	0.66	-	
ST3	0.02	-0.02	-0.01	-0.22	-0.15	0.04	0.06	0.10	-0.02	-0.06	-0.03	-0.02	0.17	0.11	0.17	-0.22	-0.12	0.09	0.02	-0.02	-0.01	-0.01	-0.07	0.02	0.72	0.57	-

1: first season, 2: second season, 3: third season.

Table 6b Correlation matrix of morphological characters in grouped leaves observed for three consecutive seasons in the three localities.

Charac	LL1	LL2	LL3	LW1	LW2	LW3	SR1	SR2	SR3	DW1	DW2	DW3	PL1	PL2	PL3	LR1	LR2	LR3	ML1	ML2	ML3	LA1	LA2	LA3	ST1	ST2	ST3
LL1	-																										
LL2	0.92	-																									
LL3	0.66	0.68	-																								
LW1	0.48	0.42	0.20	-																							
LW2	0.37	0.38	0.17	0.77	-																						
LW3	0.43	0.41	0.41	0.53	0.51	-																					
SR1	0.43	0.31	0.30	0.09	0.01	0.07	-																				
SR2	0.43	0.43	0.42	0.12	0.27	0.09	0.60	-																			
SR3	0.42	0.35	**0.52**	0.16	0.05	0.39	0.40	0.24	-																		
DW1	**0.54**	**0.52**	0.36	0.47	0.39	0.20	0.18	0.26	0.13	-																	
DW2	**0.54**	**0.61**	0.43	0.46	0.44	0.27	0.06	0.24	0.16	0.56	-																
DW3	0.33	0.38	0.40	0.26	0.18	0.17	0.08	0.28	0.14	0.43	0.43	-															
PL1	**0.55**	**0.51**	0.43	0.16	-0.01	0.01	0.34	0.35	0.27	0.27	0.32	0.35	-														
PL2	**0.51**	**0.52**	0.32	0.34	0.25	0.12	0.20	0.19	0.17	0.38	**0.51**	0.33	0.63	-													
PL3	0.36	0.37	0.45	0.19	0.17	0.06	0.13	0.23	0.17	0.25	0.29	0.38	0.53	0.52	-												
LR1	-0.42	-0.42	-0.37	**0.59**	0.41	0.10	-0.30	-0.25	-0.20	0.02	-0.02	-0.03	-0.32	-0.17	-0.14	-											
LR2	**-0.53**	**-0.61**	-0.45	0.22	0.46	-0.01	-0.25	-0.13	-0.26	-0.12	-0.18	-0.18	-0.48	-0.33	-0.20	0.74	-										
LR3	-0.13	-0.17	-0.49	0.33	0.29	**0.56**	-0.14	-0.25	-0.05	-0.10	-0.11	-0.16	-0.32	-0.14	-0.33	0.42	0.34	-									
ML1	**1.00**	**0.92**	**0.66**	0.48	0.38	0.42	0.43	0.43	0.42	**0.54**	**0.54**	0.33	**0.55**	**0.51**	0.36	-0.42	**-0.53**	-0.13	-								
ML2	**0.92**	**1.00**	**0.68**	0.42	0.38	0.41	0.31	0.43	0.35	**0.52**	**0.61**	0.38	**0.51**	**0.52**	0.37	-0.42	**-0.61**	-0.17	0.92	-							
ML3	**0.66**	**0.68**	**1.00**	0.20	0.17	0.41	0.30	0.42	**0.52**	0.36	0.43	0.40	0.44	0.32	0.45	-0.37	-0.45	-0.49	0.66	0.68	-						
LA1	**0.84**	**0.75**	0.48	**0.87**	**0.68**	**0.55**	0.28	0.32	0.31	**0.59**	**0.60**	0.35	0.40	**0.51**	0.32	0.10	-0.16	0.12	**0.84**	**0.75**	0.48	-					
LA2	**0.76**	**0.83**	0.49	**0.7**	**0.83**	**0.53**	0.17	0.42	0.22	**0.55**	**0.65**	0.35	0.29	0.48	0.32	-0.02	-0.10	0.07	**0.76**	**0.83**	0.49	0.85	-				
LA3	**0.62**	**0.63**	**0.83**	0.43	0.41	**0.84**	0.20	0.30	**0.53**	0.34	0.42	0.34	0.24	0.24	0.29	-0.14	-0.24	0.04	**0.62**	**0.63**	**0.83**	0.59	0.60	-			
ST1	-0.07	-0.14	-0.02	-0.14	-0.01	-0.13	0.05	-0.01	-0.07	-0.08	-0.16	-0.06	-0.01	-0.03	0.13	-0.09	0.10	-0.13	-0.07	-0.13	-0.02	-0.13	-0.08	-0.08	-		
ST2	-0.05	-0.11	-0.13	-0.07	-0.07	-0.11	0.05	0.06	-0.03	-0.22	-0.23	-0.15	-0.02	-0.02	-0.05	-0.02	0.02	0.04	-0.05	-0.11	-0.13	-0.07	-0.11	-0.17	0.34	-	
ST3	0.03	0.02	-0.16	-0.15	-0.10	-0.09	0.11	0.11	-0.09	-0.16	-0.13	-0.18	0.03	0.03	-0.06	-0.20	-0.13	0.07	0.03	0.01	-0.16	-0.08	-0.06	-0.18	0.12	0.55	-

1: first season, 2: second season, 3: third season.

Table 7a Correlations between principal components and morphological characters of simple leaves observed for three consecutive seasons in the three localities.

Variables	PC1	PC2	PC3
LL1	**-0.92**	-0.09	0.01
LL2	**-0.92**	-0.02	-0.04
LL3	**-0.85**	-0.15	-0.25
LW1	**-0.66**	0.52	0.27
LW2	**-0.58**	0.41	0.21
LW3	**-0.60**	0.33	0.39
SR1	-0.51	-0.08	0.12
SR2	-0.45	-0.22	0.07
SR3	-0.57	-0.10	-0.16
DW1	**-0.64**	0.25	0.25
DW2	**-0.63**	0.25	0.13
DW3	**-0.52**	0.21	0.14
PL1	-0.55	-0.42	0.02
PL2	-0.55	-0.12	0.06
PL3	-0.39	-0.38	-0.05
LR1	0.49	0.58	0.23
LR2	0.52	0.40	0.20
LR3	0.46	0.40	**0.62**
ML1	**-0.92**	-0.09	0.00
ML2	**-0.92**	-0.01	-0.03
ML3	**-0.85**	-0.15	-0.25
LA1	**-0.89**	0.22	0.15
LA2	**-0.83**	0.20	0.10
LA3	**-0.85**	0.08	0.03
ST1	0.18	**-0.63**	**0.63**
ST2	0.02	**-0.68**	0.47
ST3	0.01	**-0.62**	**0.60**
Eigen values	11.56	3.20	2.03
Percentages explained (%)	**42.80**	**11.80**	**7.50**
Cumulative percentages (%)	**42.80**	**54.60**	**62.10**

PC1: First principal component, PC2: Second principal component, PC3: Third principal component.

3.1.2 Locality

Locality and seasonal variations x locality interaction are significant for all traits of simple and grouped leaves except ST (Table 3a, b). During the three seasons, leaves were longer, wider, with area and dry matter content higher in Ait Melloul and Ait Baha (Table 4a, b). But in Argana site, leaves were shorter, narrower, with area and dry matter content lower in both types of leaves. Variability for the four characters is more remarkable in Argana, than Ait Melloul and Ait Baha (Table 4a). During the 2nd season, leaves were longer, wider, with much secondary veins; the midrib is longer, with area and dry matter content higher in Ait Melloul and Ait Baha than in Argana site. But, during the first season, leaves were shorter, narrower, with a relatively small number of secondary veins, with lower surface and dry matter content. The same observations were found in Grouped leaves even though values are quite different depending on characters (Table 4b).

3.1.3 Genotype

Genotype (tree / locality) is significant for all traits of simple and grouped leaves, showing a great potential genetic diversity in field for the studied characters (Table 3a, b). Some genotypes such as (11 and 25) from Ait Melloul, (20 and 21) from Argana and (20 and 23) from Ait Baha had simple leaves longer, wider, with surface and dry mass content higher. Genotype x environment interaction (seasonal variations x tree / locality) is highly significant for all characters (Table 3a, b).

Table 7b Correlations between principal components and morphological traits of grouped leaves observed for three consecutive seasons in the three localities.

Variables	PC1	PC2	PC3
LL1	**0.93**	-0.09	-0.18
LL2	**0.93**	-0.10	-0.09
LL3	**0.79**	-0.28	0.33
LW1	0.56	**0.72**	-0.12
LW2	0.50	**0.74**	-0.17
LW3	0.52	**0.50**	0.10
SR1	0.39	-0.29	-0.28
SR2	0.50	-0.18	-0.23
SR3	0.47	-0.14	0.15
DW1	**0.61**	0.20	0.06
DW2	**0.67**	0.20	0.09
DW3	**0.50**	0.01	0.22
PL1	**0.58**	-0.37	-0.12
PL2	**0.60**	-0.07	-0.19
PL3	**0.50**	-0.18	0.06
LR1	-0.28	**0.80**	0.07
LR2	-0.45	**0.70**	-0.01
LR3	-0.17	**0.68**	-0.23
ML1	**0.93**	-0.09	-0.18
ML2	**0.93**	-0.10	-0.09
ML3	**0.79**	-0.28	0.33
LA1	**0.85**	0.39	-0.17
LA2	**0.84**	0.38	-0.15
LA3	**0.77**	0.14	0.29
ST1	-0.10	-0.16	-0.30
ST2	-0.15	-0.13	**-0.71**
ST3	-0.09	-0.22	**-0.75**
Eigenvalues	10.50	3.89	1.96
Percentages explained (%)	**38.90**	**14.40**	**7.30**
Cumulative percentages (%)	**38.90**	**53.30**	**60.60**

PC1: First principal component, PC2: Second principal component, PC3: Third principal component.

Thus, changes in length, width, area, leaf ratio and dry matter content of simple leaves showed that sensitivity to seasonal variation in temperatures and rainfalls depends on tree genotype. Even in better conditions such in 2nd season, tree response was very heterogeneous depending on characters (Table 4a, b). Trees (8, 20 and 30) of Ait Melloul, (8, 23 and 27) Argana and individuals (5, 18, 19 and 26) of Ait Baha had produced shorter and narrower leaves with very low leaf area, ratio and dry matter. But, even in very dry season, characterized as dry and hot, trees (10, 13, 15 and 27) of Ait Melloul, (1, 5, 15 and 24) of Argana and trees (2, 9, 21 and 23) of Ait Baha have produced the longest leaves, largest leaves and whose area and dry matter content were remarkable. The similar type of results have been reported in grouped leaves, since even under best conditions (2nd season), trees (7 and 16) of Ait Melloul, (9, 10 and 13) from Argana and (6, 7, 14 and 24) of Ait Baha have produced shorter, narrower leaves with lower leaf area, leaf ratio and dry matter content. However, in dry season, trees (1, 5, 10, 15 and 17) of Ait Melloul, (5 and 25) from Argana and trees (3, 9, 12, 21 and 29) of Ait Baha had produced longest leaves, largest units with higher leaf area and dry matter.

3.2 Variance components

Relative contribution of variance related to seasonal variations and seasonal variations x locality interaction on the phenotypic variance was low (0% to 17.1%) for all characters of simple and grouped leaves (Figure 3a, b). Relative contribution of variance related to locality was low for LR, ST, PL and DW (2.8% to 19.3%), but higher for the others characters (25.4% to 47.6%). Relative contribution of variance related to genotype x environment interaction (season x tree / locality) on the phenotypic variance was higher (17% to 40.5%) then the seasonal variation and seasonal variation x locality interaction for all traits except LL, ML and DW in simple leaves (0% to 14.9%). Relative contribution of variance related to genotype (tree / locality) on the total variance was greater (15.1% to 44.3%) except dry matter content of simple leaves (4.5%). In all three sites taken individually, percentages related to tree factor in the total variance were ranged from 25.9% to 54.9% in Ait Melloul, between 9.9% and 39.4% in Argana and from 12.9% to 36.2% in Ait Baha. Similar types of results were observed for grouped leaves.

Repeatabilities (broad-sense heritability) were remarkable for all characters (31.5% and 57.9%) except dry matter content (4.8%) in simple leaves (Table 5b.1). Repeatabilities at each site varied in large proportions (25.7% and 67.3%). These values were higher in Ait Melloul and Argana than in Ait Baha in simple leaves. In grouped leaves, repeatability at Ait Melloul and Argana populations were higher than in Ait Baha population (Table 5b.2).

3.3 Diversity analysis

Correlations between characters of simple and grouped leaves were varied in large proportions over the three seasons. In simple and grouped leaves, annual values of leaf length, midrib length, leaf area and stomata number are correlated in positive direction whatever the seasonal variation (Table 6a, b). For these characters, trees response in 1st season was similar to reactions observed in 2nd and 3rd seasons although levels reached were different. These variations can be explained by differential responses of trees with respect to seasonal changes illustrated by coefficients of variation that have varied over the three seasons between 15.9% and 29.5% for simple leaves and between 14.5% and 30.1% in Grouped leaves.

Principal component analysis (PCA) showed that 62.1% of the total variance could be explained using three first principal components (Table 7a). First PC, explaining about 42.8% of variation, was linked to variables related to leaf length, midrib length, leaf area, leaf width and dry matter content. Second PC that is responsible for 11.8% of variations was linked to stomata number. Third PC, explained 7.5% of total variability, was attributed to leaf ratio in the third season. The ordering of individuals revealed that genotypes are not grouped according to their original populations. But a high variability for morphological characters both within and between the three populations is observed (Figure 4a). For Grouped leaves, even if values and direction of correlation between different characters and axis of PC, results were similar to simple leaves (Table 6b). Also, no clear separation between individuals for morphological characters observed in three sites in south west Morocco (Figure 4b).

The dendrogram generated on the basis on all the morphological data, showed a similar pattern. Two major clusters of genotypes were distinguished. The first sub-cluster is formed by only one genotype from Ait Baha (tree 6) and characterized by shorter and narrower leaves with a low number of stomata (Figure 5a, b). The second sub-group contained 89 individuals. The sub-clusters were formed by individuals from all the three populations and contained 90% of genotypes from Ait Melloul, 93.3% from Argana and 96.6% of trees from Ait Baha. So, in simple and grouped leaves, genotypes from the three populations Ait Melloul, Argana and Ait Baha were not grouped according to belonging to original locality, but remarkable mixture of genotypes was observed. Thus, differentiation for the morphological characters is not established in argan tree. Classification of the three populations distinguishes two groups. A first group formed by loality Ait Baha and a second group containing Ait Melloul and Argana (figure 6).

4 Discussions

The present work is an attempt for study the genetic diversity of argan tree based on morphological leaf traits. The results indicate that variability in field was the fruit of influence of seasonal variations, geographical site, genotype and genotype x environment interaction (season x tree / locality). Changes in weather conditions (temperatures and rainfalls) especially in dry season are manifested by a clear reduction in leaf width and leaf area than in very humid season. The relative contribution of variance related to season and season x locality interaction in the total variance is low (0% to 17.1%). Similar results are also observed in argan tree for characters of fruit and kernel (Bani-Aameur & Ferradous, 2001), and for the branching characters. Rainfall in autumn and winter were associated with sweet ambient temperatures and were decisive for the annual production of branches, twigs and leaves (Zahidi et al., 2013). The influence of seasonal variations was also observed among varieties of pine (*Pinus clausa*), in which rainfalls are more strongly related to radial growth than temperatures (Parker et al., 2001). In *Pinus pinea* (L.), a significant correlation was established between the rainfall of June and needles growth and thickness branches growth of current season or shoots length and scope flowering next season (Mutke et al., 2003). In several species, the significant limiting factor on tree growth, stomata density, leaf dry matter content, leaf area, fruit and leaf biometric characteristics is spring precipitations and soil water deficits (Abbad et al., 2004; Gou et al., 2005; Al Afas et al., 2005;

Camposeo et al., 2011; Dinis et al., 2011; Michelot et al., 2012). In addition, leaf characteristics showed a high variability which is more significant, as populations are geographically distant and are subject to important seasonal variations. Thus, the morphological and phenological differences among ecotypes are not related to the small genetic differences, but were simply phenotypic adaptations to different climatic conditions.

The relative contribution of locality in the total variance was important. This variance can be decomposed into a variance related to differences between trees in the three localities (σ^2 between populations) and a component related to geographic site (geographical σ^2). Significant differences between four populations for photosynthesis, transpiration and stomatal conductance were observed during two years of study. Characters associated with leaf morphology also differed between populations, including leaf size, chlorophyll content and specific leaf weight of *Populus deltoides* var. wi-slizenii (S. Wats.) (Rowland, 2001). In argan tree, these two components for leaf length, length of secondary vein, leaf width and leaf area were remarkable. In Argana site, with autumn and winter cold, even in better conditions such as in the second and third season, leaves were shorter (1.44 to 3.83 cm), narrower (0.30 to 0.74 cm), with leaf area (0.52 to 2.36) and dry matter content (0.01 to 0.02 g) lower. In Ait Melloul, to milder temperatures, under such conditions, leaves were longer (1.92 to 5.25 cm), wider (0.29 to 1.14 cm), with leaf area (0.56 to 5.17 cm²) and dry matter contents (0.01 to 0.04 g) greater. The small size of leaves observed in Argana, can be explained by high frequency (86%) of obovate obtuse shape (OT) characterized by small size (Zahidi, 1997). Ait Melloul and Ait Baha were characterized by the existence of LA and LM shapes relatively longer, wider and with higher leaf area than OT and ST forms. In simple and grouped leaves, variability between populations (difference between localities) was very important. It was twice higher than variance within populations (difference between trees in the same locality) for LL, ML and LA. For LW, LR, LP and SR; variance within populations was more important than variability between populations. Repeatabilities are higher for all characters except DW in simple leaves. Similar results have found in argan for charactes of the fruit and kernel (Bani-Aameur & Ferradous, 2001), but lower than value for oil content in seeds (0.93) observed in fruit collected in five areas in south west Morocco (Ait Aabd et al., 2011).

Responses of trees to seasonal variations were highly heterogeneous, since the proportions of variance related to genotype x environment interaction (tree x season x locality) were remarkable. This contribution has proved as important as that observed for characters of fruit and kernel (4.4% and 14.7%) (Bani-Aameur & Ferradous, 2001). A significant correlation was reported between the genotype x environment interaction and season x tree interaction on each site. Thus, season x tree interaction explained a percentage between (19.9% and 44.2%) at Ait Melloul and (0.4% and 55.1%) at Argana. At Ait Baha, this variance ranged from 14.3% and 64.5%. Ait Baha which creates the contrast, would considered as a environment for selection of resistant genotypes to seasonal variations in temperatures and rainfalls as it has been reported by Ferradous (1995) for characters of fruit and kernel. These findings are in conformity with the findings of Sultan (2000) and Mückschel & Otte (2003) that argan tree shows a high adaptive plasticity with respect to his living environment as has been noticed in other plant species.

Hamrick et al. (1992) reported that woody perennial species maintain generally most of their variation within populations, which holds true for tropical trees in particular. These variations were associated with the life history and ecological characteristics of the woody species. Results of present study were congruent with such as description since multivariate analysis of all genotypes on the basis of leaf characters did not show a grouping of individuals congruent with source population. In fact, the two main groups identified containing a mixture of trees from the three geographical origins. These patterns do not appear to be the result of geographic isolation of populations which will constitute an adaptation to local environmental conditions. Both populations of Ait Melloul and Argana were slightly distant from population of Ait Baha. Much of the diversity observed was related to differences between genotypes illustrated by percentage of variance related to tree factor in the total variance in each site. High levels of similarities between populations are observed for characters of fruit and kernel (Bani-Aameur & Ferradous, 2001; Ait Aabd et al., 2011). Similar findings were also observed in nine mixed stands of oak (*Quercus petraea* and Q. robur) for leaf morphology (Kremer et al., 2001), and for four natural *Arbutus unedo* L. populations in Portugal, a high morphological variation between and within-populations is observed. The dry-weight, length, width and peduncle length of leaves are the most discriminating characters, being responsible for 52.6% of the total variation (Lopes et al., 2012).

5 Conclusions

Responses of trees to seasonal variations are very heterogeneous, since the proportions of variance related to genotype x environment interaction are remarkable. Drought effect induced a net reduction in leaf width, leaf area and dry mass in the three localities. Ait Baha, which creates the contrast, would consider as an environment for selection of resistant genotypes to seasonal variations in temperatures and rain falls. *Argania spinosa* shows a high adaptive plasticity with respect to his living environment, as has been noticed in other plant species. Although human activities have resulted in reductions in density and acreage in recent decades which will induce the genetic erosion. We found that this species currently maintains a relative high degree of genetic diversity especially within populations than between populations. Leaf length, midrib length, leaf width, leaf area and stomata number were the most discriminating characters, being responsible for 54,6% of the total variation. Argan tree is also contributing to the local economy, making it a potential candidate for domestication and a useful source of drought resistance.

However, there is a real danger related to the combined effects of increased drought and intensity of human activities that may result in a reduction in the size of populations and thus reduce the level of diversity currently present if conservation strategies are not implemented. In fact, the high level of genetic variation attributed to within populations was higher than among populations. For a better understanding of the genetic diversity and genetic structure of natural populations of argan, further studies by using molecular markers and perhaps DNA sequencing data are needed to improve our conclusions.

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