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# Inter-population variability of leaf morpho-anatomical and terpenoid patterns of *Pistacia atlantica* Desf. ssp. *atlantica* growing along an aridity gradient in Algeria

Samir Ait Said<sup>a,b</sup>, Catherine Fernandez<sup>b</sup>, Stéphane Greff<sup>b</sup>, Arezki Derridj<sup>a</sup>, Thierry Gauquelin<sup>b</sup>, Jean-Philippe Mevy<sup>b,\*</sup>

<sup>a</sup> Faculté des Sciences Biologiques et des Sciences Agronomiques, Université Mouloud, MAMMERI BP 17, Tizi-Ouzou, Algeria

<sup>b</sup> Aix-Marseille Université, Institut Méditerranéen d'Ecologie et Paléoécologie IMEP, UMR CNRS 6116, Equipe Diversité Fonctionnelle des Communautés Végétales, Centre Saint Charles, 3, place Victor Hugo, 13331 Marseille cedex 3, France

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Monoterpenes  
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## A B S T R A C T

Three Algerian populations of female *Pistacia atlantica* shrubs were investigated in order to check whether their terpenoid contents and morpho-anatomical parameters may characterize the infraspecific variability. The populations were sampled along a gradient of increasing aridity from the Atlas mountains into the northwestern Central Sahara.

As evidenced by Scanning Electron Microscopy, tufted hairs could be found only on seedling leaves from the low aridity site as a population-specific trait preserved also in culture. Under common garden cultivation seedlings of the high aridity site showed a three times higher density of glandular trichomes compared to the low aridity site. Increased aridity resulted also in reduction of leaf sizes while their thickness increased. Palisade parenchyma thickness also increases with aridity, being the best variable that discriminates the three populations of *P. atlantica*.

Analysis of terpenoids from the leaves carried out by GC-MS reveals the presence of 65 compounds. The major compounds identified were spathulenol ( $23 \mu\text{g g}^{-1}$  dw),  $\alpha$ -pinene ( $10 \mu\text{g g}^{-1}$  dw), verbenone ( $7 \mu\text{g g}^{-1}$  dw) and  $\beta$ -pinene ( $6 \mu\text{g g}^{-1}$  dw) in leaves from the low aridity site; spathulenol ( $73 \mu\text{g g}^{-1}$  dw),  $\alpha$ -pinene ( $25 \mu\text{g g}^{-1}$  dw),  $\beta$ -pinene ( $18 \mu\text{g g}^{-1}$  dw) and  $\gamma$ -amorphene ( $16 \mu\text{g g}^{-1}$  dw) in those from medium aridity and spathulenol ( $114 \mu\text{g g}^{-1}$  dw),  $\alpha$ -pinene ( $49 \mu\text{g g}^{-1}$  dw), germacrene D ( $29 \mu\text{g g}^{-1}$  dw) and camphene ( $23 \mu\text{g g}^{-1}$  dw) in leaves from the high aridity site. Terpene concentrations increased with the degree of aridity: the highest mean concentration of monoterpenes ( $136 \mu\text{g g}^{-1}$  dw), sesquiterpenes ( $290 \mu\text{g g}^{-1}$  dw) and total terpenes ( $427 \mu\text{g g}^{-1}$  dw) were observed in the highest arid site and are, respectively, 3-, 5- and 4-fold higher compared to the lower arid site. Spathulenol and  $\alpha$ -pinene can be taken as chemical markers of aridity. Drought discriminating compounds in low, but detectable concentrations are  $\delta$ -cadinene and  $\beta$ -copaene. The functional roles of the terpenoids found in *P. atlantica* leaves and principles of their biosynthesis are discussed with emphasis on the mechanisms of plant resistance to drought conditions.

## Introduction

Plants respond to environmental variations, particularly to water availability through morphological, anatomical and biochemical adjustments that help them cope with such variations (Lukovic et al., 2009). Plants are adapted to drought stress by developing xeromorphic characters based mainly on reduction of leaf size (Trubat et al., 2006) and increase in thickness of cell walls, a more dense vascular system, greater density of stomata and an increased development of palisade tissue at the expense of the

spongy tissue (Bussotti et al., 2002; Bacelar et al., 2006; Syros et al., 2006).

Terpenes are one of the most diverse family of chemical compounds found in plant kingdom and they exhibit several roles in plant defense and communication (Kirby and Keasling, 2009). In response to drought conditions, significant changes of terpene emissions were shown in many Mediterranean species (Ormeño et al., 2007a; Lavoit et al., 2009). Similar results were reported regarding the occurrence of terpenic components from *Erica multiflora* and *Globularia alypum* (Llusià et al., 2009). It has been shown also that monoterpenes and sesquiterpenes have a role in protecting plants from thermal damage (Peñuelas and Llusià, 2002; Loreto et al., 2004; Llusià et al., 2005; Peñuelas et al., 2005). Terpenes are recognized as being relatively stable and also as precursors

\* Corresponding author. Tel.: +33 04 13 55 07 66; fax: +33 04 13 55 11 51.  
E-mail address: jean-philippe.mevy@univ-provence.fr (J.-P. Mevy).

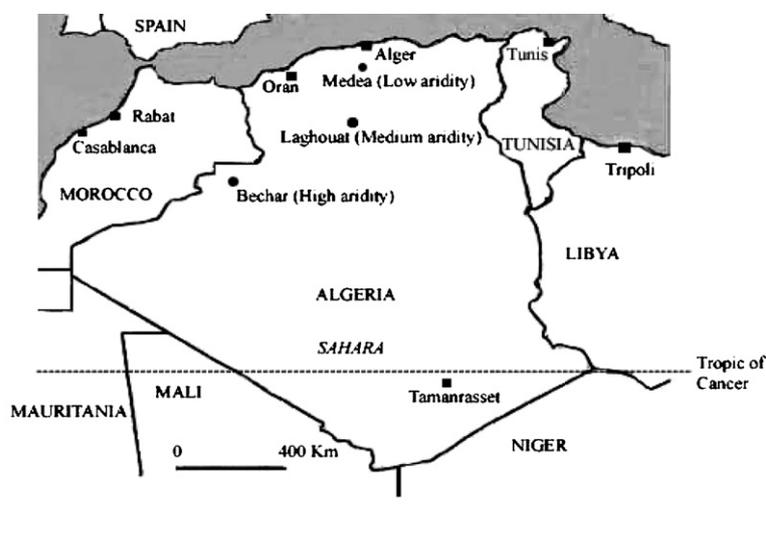


Fig. 1. Geographical location of the investigated *P. atlantica* populations. Sites: ●.

of numerous potential physiological components including growth regulators (Byrd et al., 1999). Another property of these compounds is their great variability in time and depending on the geographic distribution of species as shown by many studies in literature (Lang, 1994; Staudt et al., 2000; Hillig, 2004; Smelcerovic et al., 2007). As a result, many studies relate terpenic constituents with plant systematic and population issues (Adams, 1999; Naydenov et al., 2006).

The genus *Pistacia* (Anacardiaceae) consists of at least eleven dioecious species (Zohary, 1952; Kokwaro and Gillett, 1980) that all intensely produce terpenes. There are three wild *Pistacia* species in Algeria: *P. atlantica* Desf. ssp. *atlantica* which exhibits high morphological variability (Belhadj et al., 2008), *P. lentiscus* L. and less frequently *P. terebinthus* L. *Pistacia atlantica* is considered to be an Irano-Turanian species which is distributed from south-west Asia to north-west Africa (Zohary, 1952). In Algeria, it occurs in the wild from sub-humid environments to extreme Sahara sites (Monjauze, 1967; Quézel and Médail, 2003; Benhassaini et al., 2007). As a thermophilous xerophyte *P. atlantica* grows in dry stony or rocky hill sides, edges of field, roadsides, near the base of dry stone walls and other similar habitats (Tzakou et al., 2007). The species grows well on clay or silty soils, although it can thrive also on calcareous rocks where roots develop inside cracks. Hence, *P. atlantica* has a wide ecological plasticity as also shown by Belhadj et al. (2007) through leaf epidermis analysis. For all these reasons, *P. atlantica* is used in re-planting projects in Algeria but only few studies are carried out on the infraspecific variability of this plant.

Regarding the phytochemistry of *P. atlantica*, essential oils from samples harvested in Greece (Tzakou et al., 2007) and Morocco (Barrero et al., 2005) were described. Recently also a study was published describing essential oils and their biological properties from *P. atlantica* harvested in Algeria (Gourine et al., 2009). However, to the best of our knowledge, there is no detailed study on

the relationship between the phytochemistry of *P. atlantica* and its ecological conditions of growth.

The aim of this work is to investigate the intraspecific diversity of three populations of *P. atlantica* growing wild in arid zones of Algeria through terpenoid analysis and leaf morpho-anatomical traits. We also examined the possible links that may exist between plant chemical composition and aridity conditions of these three locations.

## Material and methods

### Sampling sites

*Pistacia atlantica* Desf. ssp. *atlantica* was harvested in June 2008 from three Algerian sites chosen along a Northeast-Southwest transect of increasing aridity: Oued-Besbes (Medea)-Low aridity, Tilghemt (Laghouat)-Medium aridity and Beni-Ouniff (Bechar)-High aridity (Fig. 1). Specimens were deposited at the herbarium of the University of Provence Marseille and referred as Mar-PA1-2008; Mar-PA2-2008; Mar-PA3-2008 for the locations of Medea, Laghouat and Bechar, respectively. Ecological factors of samplings sites are described in Table 1.

For all the sites, sampling was carried out during fructification stage in order to take into account the phenological shift due to local climatic conditions. Ten healthy female individuals with the same age were chosen per site. Plants density and soil conditions were similar for the different sites.

### Leaf morphology and anatomy

From each of the three locations, ten female trees were selected and thirty leaves fully sun exposed were harvested per tree. Once harvested, these leaves were carefully dried and kept in herbarium

**Table 1**  
Ecological factors of the *Pistacia atlantica* collection sites, selected to define the aridity gradient.

Site	Mean annual precipitation (mm)	Maximal temperature M (°C) of the driest month	Drought duration in months (Bagnouls and Gausson, 1953)	Emberger, $Q_2^a$	Latitude	Elevation (m)
Medea low aridity	393.10	31.00	4	15.40	36°11'–36°22' north 3°00'–3°10' east	720
Laghouat medium aridity	116.60	39.40	10	04.34	28°00' north 3°00' east	780
Bechar high aridity	57.70	40.70	12	02.36	31°38'–32°03' north 1°13'–2°13' west	790

<sup>a</sup> Emberger's pluviothermic quotient.

**Table 2**

Morphological data (cm) of female *P. atlantica* ssp. *atlantica* leaves from low, medium and high aridity sites in Algeria. Mean of 30 measurements per tree with standard errors.

Leaf biometry (cm)	Low aridity site (Medea)	Medium aridity site (Laghouat)	High aridity site (Bechar)	<i>p</i>
Leaf length	9.63 ± 0.19 a	9.17 ± 0.17 b	8.92 ± 0.18 c	<0.001
Leaf width	7.61 ± 0.16 a	7.16 ± 0.14 b	6.65 ± 0.17 c	<0.001
Rachis length	4.09 ± 0.10 a	3.78 ± 0.07 b	3.72 ± 0.08 b	<0.001
Petiole length	2.13 ± 0.04	2.11 ± 0.05	2.05 ± 0.06	>0.05
Terminal leaflet length	3.41 ± 0.03 a	3.29 ± 0.03 b	3.14 ± 0.02 c	<0.001
Terminal leaflet width	1.58 ± 0.03 a	1.49 ± 0.01 b	1.45 ± 0.02 c	<0.001
Number of leaflet pairs	3.09 ± 0.07 b	3.12 ± 0.08 b	3.26 ± 0.10 a	<0.05

prior to biometric measurements: leaf length and width, petiole length, rachis length and the terminal leaflet length and width.

For anatomical parameters, cross sections were prepared across the middle part of three fresh leaflets per leaf, stained with carmino-green, and thickness measured using light microscopy of abaxial and adaxial epidermis, cuticle, palisade and spongy parenchyma and total leaflet.

#### Scanning Electron Microscopy (SEM) of seedling leaves

Seeds were collected in August 2008 at Medea and Bechar sites. After germination the seedlings were transplanted in pots filled with peat and sand, then kept in a growth chamber at constant temperature of 25 °C. The photoperiod was set at 11/13 h and the light irradiance was 500 μmol photons m<sup>-2</sup> s<sup>-1</sup>. After 11 months of culture eight plants from each location were randomly selected, then three leaves per plant were harvested and carefully dried prior to SEM observations. Micromorphological observations were carried out on three leaflet samples (adaxial and abaxial surfaces) per leaf. These were gold coated before scanning through an electronic microscope: FEI XL30 ESEM (USA).

#### Terpenoids extraction

Mature and sun exposed leaves were harvested in the field, dried in dark at ambient air temperature conditions until constant weight, then, 100 g per tree were grounded and stored until use. Shade-drying method has no significant effect on the qualitative composition of volatile oils compared to fresh, sun-drying and oven-drying at 40 or 45 °C (Omidbaigi et al., 2004; Sefidkon et al., 2006; Ashafa et al., 2008). The extraction method used consisted of suspending leaf dry matter in dichloromethane according to a ratio of 1:2 (w/v), for 30 min, under constant shaking at room temperature. 50 μl of dodecane (5 mg ml<sup>-1</sup>) were added as internal standard for quantification.

#### Quantitative and qualitative analysis of terpenoids

Extracts were filtered on RC syringe filter (regenerated Cellulose, 0.45 μm, 25 mm; Phenomenex, Le Pecq, France) then analyzed with a gas chromatograph Hewlett Packard® GC 6890 coupled to a mass selective detector 5973 Network. The system was fitted with an HP-

5MS capillary column 30 m, 0.25 mm, 0.25 μm. 2 μl of extracts was injected through an automatic injector ALS 7683 in splitless mode. Purge was set at 50 min ml<sup>-1</sup> after 1 min. Injection temperature was maintained at 250 °C. Helium was used as carrier gas. A constant flow rate of 1 ml min<sup>-1</sup> was set throughout the run. The oven temperature initially set at 40 °C was increased to 270 °C at a rate of 4 °C min<sup>-1</sup> and remained constant for 5 min. The MSD transfer line heater was maintained at 280 °C.

Terpenes were identified by comparison of their arithmetic index (AI) and mass spectra with those obtained from authentic samples and literature (Adams, 2007).

#### Statistical analysis

The data were analyzed by a one-way ANOVA model. Newman-Keuls test was used to test for significant differences in monoterpene, sesquiterpene, total terpene concentrations and morpho-anatomical measurement data between the three populations. In order to evaluate the information contained in the collected chemical data, Principal Component Analysis was carried out. The statistical analyses were performed using R statistical software and packages "ade4".

## Results

#### Morpho-anatomical measurements

Among the biometric parameters studied it appears that leaf length and width as well as terminal leaflet length and width highly discriminate statistically the three populations of *P. atlantica* (Table 2). The population from the most arid site shows the lowest leaf and terminal leaflet sizes. However the number of leaflet pairs increases with aridity. Regarding the anatomical data, the thickness of palisade parenchyma is the major discriminating variable and it increases with aridity (Table 3).

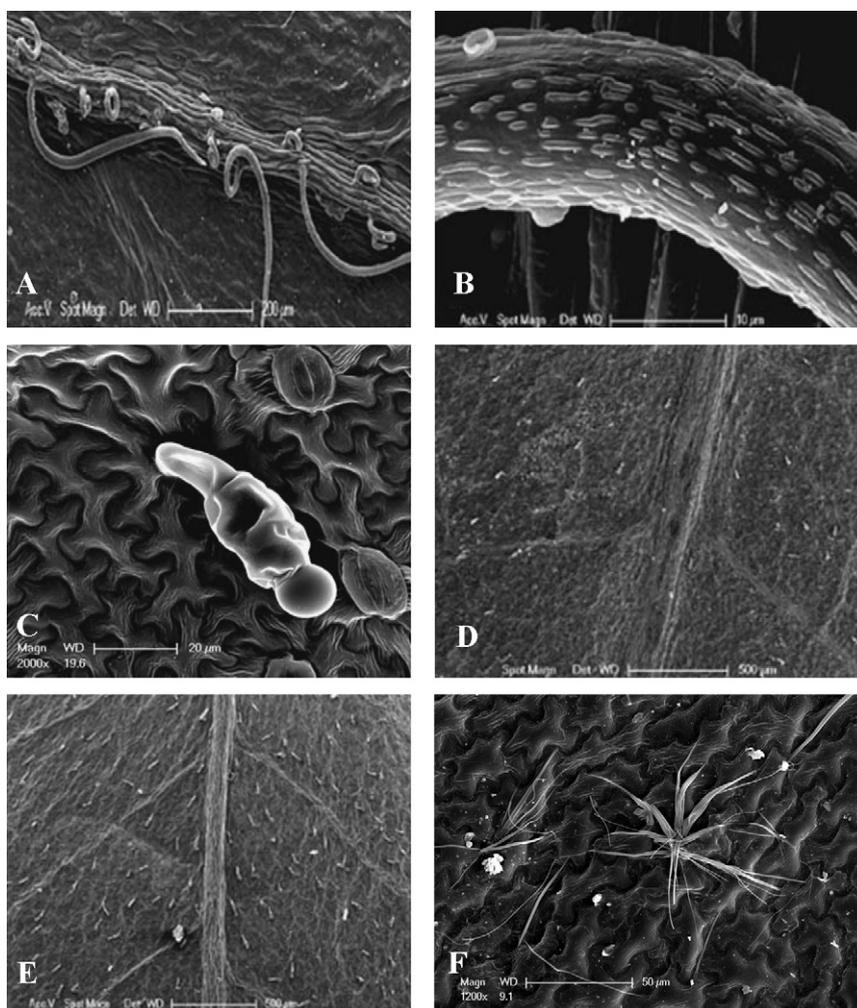
#### SEM observations

The epidermis of seedling leaves has markedly sinuous walls in both Medea and Bechar populations. Abaxial and adaxial leaf surfaces of each population are covered with two types of trichomes, elongated hairs and glandular trichomes. The former are essen-

**Table 3**

Anatomical data (μm) of female *P. atlantica* ssp. *atlantica* leaves from low, medium and high aridity sites in Algeria. Mean of 30 measurements per plant (3 replicates per leaf) with standard errors.

Leaf anatomy (μm)	Low aridity site (Medea)	Medium aridity site (Laghouat)	High aridity site (Bechar)	<i>p</i>
Abaxial cuticle	4.98 ± 0.08 b	5.99 ± 0.12 a	6.08 ± 0.13 a	<0.001
Adaxial cuticle	4.32 ± 0.06 b	4.88 ± 0.16 a	4.91 ± 0.12 a	<0.01
Abaxial epidermis	12.70 ± 0.18 b	12.69 ± 0.18 b	14.07 ± 0.20 a	<0.001
Adaxial epidermis	13.26 ± 0.16 b	13.30 ± 0.14 b	13.45 ± 0.18 a	<0.05
Palisade parenchyma	64.66 ± 1.5 c	72.77 ± 1.38 b	95.76 ± 1.42 a	<0.001
Spongy parenchyma	98.67 ± 1.64	102.45 ± 1.81	106.34 ± 1.86	>0.05
Leaf thickness	198.53 ± 2.78 c	212.08 ± 2.97 b	240.61 ± 3.51 a	<0.001



**Fig. 2.** Scanning electron micrographs showing epidermis and trichomes of *P. atlantica* seedling leaves. (A) Midrib of adaxial leaf surface, covered by elongated trichomes. Bar = 200  $\mu\text{m}$ . (B) Elongated trichomes in parallel rows. Bar = 10  $\mu\text{m}$ . (C) Glandular trichome. Bar = 20  $\mu\text{m}$ . (D and E) Low density of glandular trichomes in Medea population (D) compared to Bechar population (E). Bar = 500  $\mu\text{m}$ . (F) Tufted hairs at the adaxial leaf surface in Medea population. Bar = 50  $\mu\text{m}$ .

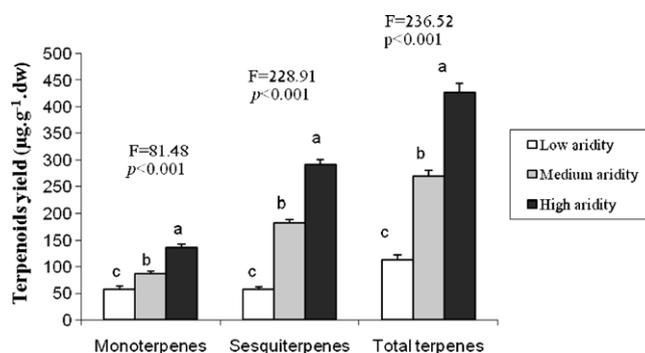
tially located at midrib of the adaxial leaf surface (Fig. 2A) and at the rachis forming parallel rows (Fig. 2B). The latter (Fig. 2C) are distributed over the entire leaf surface (essentially at the abaxial surface) with high density ( $18.31 \pm 0.29 \text{ mm}^{-2}$ ) in plants that seeds were sampled from the population of the most arid site (Bechar). Trichome density of the plants raised from seeds sampled from the population that grows under less arid conditions (Medea) was  $6.15 \pm 0.21 \text{ mm}^{-2}$  when both seedling lots were cultivated in the same environment (Fig. 2D and E). The Medea population could further be discriminated by the presence of tufted hairs which never were observed in the Bechar population on *P. atlantica*, neither in seedlings nor in adult plants (Fig. 2F).

#### Terpenoid analysis

*P. atlantica* leaves contain forty nine compounds identified (Table 4). Among these, twenty two were monoterpenes (8 hydrocarbons and 14 oxygenated) and twenty five were sesquiterpenes (16 hydrocarbons and 9 oxygenated). In the high aridity site, the major compounds identified were spathulenol ( $114 \mu\text{g g}^{-1} \text{ dw}$ ),  $\alpha$ -pinene ( $49 \mu\text{g g}^{-1} \text{ dw}$ ), germacrene D ( $29 \mu\text{g g}^{-1} \text{ dw}$ ) and camphene ( $23 \mu\text{g g}^{-1} \text{ dw}$ ) while from the low aridity site spathulenol ( $23 \mu\text{g g}^{-1} \text{ dw}$ ),  $\alpha$ -pinene ( $10 \mu\text{g g}^{-1} \text{ dw}$ ), verbenone ( $7 \mu\text{g g}^{-1} \text{ dw}$ ) and  $\beta$ -pinene ( $6 \mu\text{g g}^{-1} \text{ dw}$ ) were the dominant constituents. For the medium aridity site situated between these two extreme con-

ditions of aridity, spathulenol ( $73 \mu\text{g g}^{-1} \text{ dw}$ ),  $\alpha$ -pinene ( $25 \mu\text{g g}^{-1} \text{ dw}$ ),  $\beta$ -pinene ( $18 \mu\text{g g}^{-1} \text{ dw}$ ) and  $\gamma$ -amorphene ( $16 \mu\text{g g}^{-1} \text{ dw}$ ) were the main terpenes found.

The quantitative analysis showed significant differences in both monoterpene, sesquiterpene and total terpene concentrations of the *P. atlantica* leaves according to the sites investigated (Fig. 3). Three distinct groups were obtained (Newman-Keuls test, 5% level). Terpene concentrations increase with the degree of aridity. The



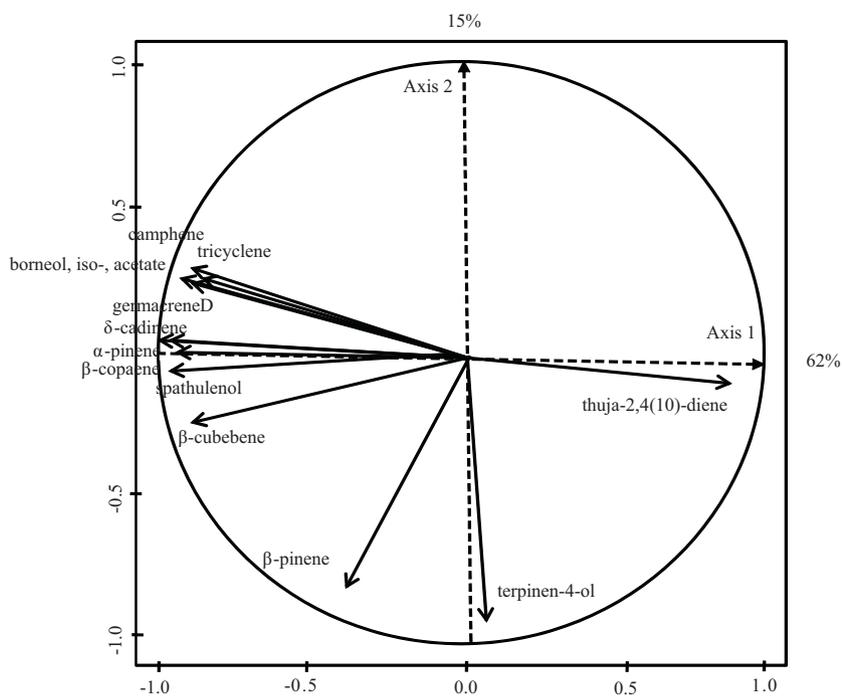
**Fig. 3.** Variance analysis of monoterpene, sesquiterpene and total terpene contents found in female *Pistacia atlantica* ssp. *atlantica* leaves from low, medium and high aridity sites in Algeria. Means of  $n = 10$  with standard errors,  $p < 0.05$ .

**Table 4**

Concentrations of terpenoids ( $\mu\text{g g}^{-1}$  dw) found in female *Pistacia atlantica* ssp. *atlantica* leaves from low, medium and high aridity sites in Algeria. Mean of 10 extractions per site with standard errors.

Group	Compounds	AI	Compound content in leaves ( $\mu\text{g g}^{-1}$ dw) and location				
			Low aridity	Medium aridity	High aridity	p	
Hydrocarbon monoterpenes	1	Tricyclene	914	1.2 ± 0.2 b	2.4 ± 0.4 b	8.7 ± 0.5 a	<0.001
	2	$\alpha$ -Pinene	926	10.0 ± 0.4 c	24.5 ± 0.8 b	49.4 ± 1.0 a	<0.001
	3	Camphene	941	3.1 ± 0.5 b	5.5 ± 0.9 b	23.2 ± 1.1 a	<0.001
	4	Thuja-2,4(10)-diene	948	1.0 ± 0.1 a	0.6 ± 0.0 b	–	<0.001
	5	$\beta$ -Pinene	971	6.5 ± 2.3 b	18.1 ± 0.9 a	12.6 ± 0.7 ab	<0.001
	6	Mentha-1,3,5-triene, p-	1007	0.7 ± 0.1 a	0.1 ± 0.0 b	–	<0.001
	7	Cymene, p-	1023	0.7 ± 0.1 b	1.9 ± 0.2 a	0.2 ± 0.0 b	<0.001
	8	$\gamma$ -Terpinene	1058	0.6 ± 0.3 ab	1.6 ± 0.3 a	0.3 ± 0.0 b	<0.001
Oxygenated monoterpenes	9	Sabinene hydrate, cis- (IPP vs OH)	1067	0.4 ± 0.2 b	1.6 ± 0.3 a	0.2 ± 0.0 b	<0.001
	10	NI	1088	0.6 ± 0.1 a	0.5 ± 0.0 a	0.2 ± 0.0 b	<0.001
	11	Sabinene hydrate, trans- (IPP vs OH)	1098	0.6 ± 0.2 b	1.5 ± 0.2 a	0.1 ± 0.0 b	<0.001
	12	NI	1101	5.8 ± 1.5	3.9 ± 0.5	4.9 ± 0.4	>0.05
	13	$\alpha$ -Campholenic aldehyde	1125	1.9 ± 0.4	2.1 ± 0.3	1.9 ± 0.3	>0.05
	14	Nopinone	1133	0.3 ± 0.1	–	–	<0.05
	15	Pinocarveol, trans-	1138	1.8 ± 0.4 ab	1.5 ± 0.1 b	3.2 ± 0.4 a	<0.01
	16	Verbenol, trans-	1146	6.0 ± 1.6	3.9 ± 0.6	6.1 ± 0.8	>0.05
	17	3-Pinocarvone, trans-	1157	1.2 ± 0.4 ab	1.9 ± 0.4 a	–	<0.001
	18	Pinocarpone	1161	0.8 ± 0.1 b	0.7 ± 0.1 ab	1.2 ± 0.2 a	<0.05
	19	Terpinen-4-ol	1177	1.3 ± 0.3 b	3.8 ± 0.4 a	1.3 ± 0.2 b	<0.001
	20	Myrtenal	1194	0.4 ± 0.2 b	0.6 ± 0.2 b	1.4 ± 0.2 a	<0.001
	21	Myrtenol	1197	1.4 ± 0.3	1.9 ± 0.4	1.5 ± 0.2	>0.05
	22	Verbenone	1208	7.0 ± 1.7	3.9 ± 0.7	5.1 ± 0.9	>0.05
	23	Carveol, trans	1221	0.8 ± 0.2 b	0.4 ± 0.1 ab	0.9 ± 0.1 a	<0.05
	24	Borneol, iso-, acetate	1285	2.6 ± 0.5 b	3.9 ± 0.4 b	13.9 ± 0.7 a	<0.001
Hydrocarbon sesquiterpenes	25	$\delta$ -Elemene	1337	1.4 ± 0.6 b	14.0 ± 3.7 a	22.0 ± 1.8 a	<0.001
	26	$\alpha$ -Cubebene	1349	0.3 ± 0.0 b	0.7 ± 0.2 b	1.5 ± 0.2 a	<0.001
	27	$\alpha$ -Copaene	1375	0.2 ± 0.0 b	0.5 ± 0.1 ab	1.0 ± 0.1 a	<0.001
	28	$\beta$ -Bourbonene	1383	0.9 ± 0.2	1.2 ± 0.2	0.8 ± 0.2	>0.05
	29	$\beta$ -Cubebene	1389	0.2 ± 0.0 b	0.5 ± 0.1 ab	0.8 ± 0.1 a	<0.001
	30	$\beta$ -Elemene	1392	0.1 ± 0.0 b	0.5 ± 0.1 ab	0.8 ± 0.2 a	<0.001
	31	$\beta$ -Ylangene	1418	1.6 ± 0.2 b	9.0 ± 1.3 a	7.2 ± 0.9 a	<0.001
	32	$\beta$ -Copaene	1429	0.5 ± 0.1 b	1.2 ± 0.1 b	3.8 ± 0.6 a	<0.001
	33	$\gamma$ -Elemene	1433	0.5 ± 0.0 b	2.6 ± 0.8 b	7.4 ± 0.9 a	<0.001
	34	Guaia-6,9-diene	1438	0.3 ± 0.1 b	2.0 ± 0.2 a	1.9 ± 0.4 a	<0.001
	35	NI	1444	0.1 ± 0.0 b	0.6 ± 0.1 b	1.3 ± 0.2 a	<0.001
	36	NI	1453	0.2 ± 0.1 b	1.4 ± 0.3 a	2.2 ± 0.3 a	<0.001
	37	Caryophyllene, 9-epi-	1461	0.8 ± 0.2 b	4.0 ± 0.6 a	3.8 ± 0.3 a	<0.001
	38	NI	1470	0.8 ± 0.4	0.4 ± 0.0	1.1 ± 0.1	>0.05
	39	Germacrene D	1482	3.0 ± 0.4 b	5.2 ± 1.0 b	29.0 ± 2.9 a	<0.001
	40	$\gamma$ -Amorphene	1496	1.8 ± 0.6 b	15.5 ± 2.8 a	20.5 ± 2.2 a	<0.001
	41	$\alpha$ -Muurolene	1501	0.3 ± 0.0 b	3.7 ± 0.4 a	0.9 ± 0.1 b	<0.001
	42	$\gamma$ -Cadinene	1515	0.3 ± 0.0 b	0.7 ± 0.1 b	2.0 ± 0.3 a	<0.001
	43	$\delta$ -Cadinene	1524	1.0 ± 0.1 b	2.0 ± 0.2 b	4.9 ± 0.6 a	<0.001
Oxygenated sesquiterpenes	44	Cubebol	1518	1.1 ± 0.1 b	1.2 ± 0.1 ab	1.7 ± 0.1 a	<0.01
	45	NI	1527	0.7 ± 0.4	1.4 ± 0.2	1.6 ± 0.3	>0.05
	46	Elemol	1552	0.7 ± 0.1 b	2.1 ± 0.6 b	5.8 ± 0.9 a	<0.001
	47	NI	1557	1.0 ± 0.2 b	2.0 ± 0.6 ab	3.1 ± 0.7 a	<0.05
	48	NI	1568	0.4 ± 0.0	0.5 ± 0.2	0.8 ± 0.2	>0.05
	49	Spathulenol	1581	23.2 ± 1.1c	72.9 ± 1.9 b	114.4 ± 2.2 a	<0.001
	50	NI	1586	3.5 ± 0.8 b	10.1 ± 0.6 a	3.4 ± 0.3 b	<0.001
	51	NI	1590	0.4 ± 0.1 b	1.2 ± 0.3 ab	2.4 ± 0.5 a	<0.01
	52	Salvial-4(14)-en-1-one	1595	0.8 ± 0.1 b	1.5 ± 0.2 ab	2.4 ± 0.3 a	<0.001
	53	NI	1609	0.6 ± 0.2 b	1.1 ± 0.2 ab	1.9 ± 0.5 a	<0.05
	54	NI	1615	1.4 ± 0.2 b	2.8 ± 0.3 b	5.1 ± 0.8 a	<0.001
	55	NI	1620	0.7 ± 0.2	1.0 ± 0.5	0.8 ± 0.1	>0.05
	56	Germacrene D-4-ol	1623	0.3 ± 0.0 b	0.8 ± 0.2 b	2.1 ± 0.3 a	<0.001
	57	$\gamma$ -Eudesmol	1634	0.2 ± 0.0 b	0.7 ± 0.1 b	1.3 ± 0.2 a	<0.001
	58	NI	1641	1.4 ± 0.3 b	9.5 ± 1.2 a	12.8 ± 1.3 a	<0.001
	59	$\alpha$ -Muurolol	1645	0.3 ± 0.1 b	1.1 ± 0.1 ab	1.6 ± 0.3 a	<0.001
	60	Cedr-8(15)-en-10-ol	1650	0.5 ± 0.1 b	1.3 ± 0.3 ab	2.7 ± 0.4 a	<0.001
	61	$\beta$ -Eudesmol	1653	0.4 ± 0.0 b	2.0 ± 0.3 b	4.7 ± 0.5 a	<0.001
	62	NI	1657	2.0 ± 0.2 b	3.7 ± 0.8 b	10.2 ± 1.3 a	<0.001
	63	NI	1677	0.7 ± 0.3 b	2.3 ± 0.3 a	0.7 ± 0.1 a	<0.001
Others	64	Hex-3-en-1-ol benzoate, (Z)-	1572	tr	0.5 ± 0.1	1.0 ± 0.1	
	65	Actinolid, dihydro-	1530	2.0 ± 0.3	2.8 ± 0.3	2.3 ± 0.1	

NI: non-identified; AI: arithmetic index of Adams (2007) calculated with the formula of Van den Dool and Kratz (1963); tr: trace.



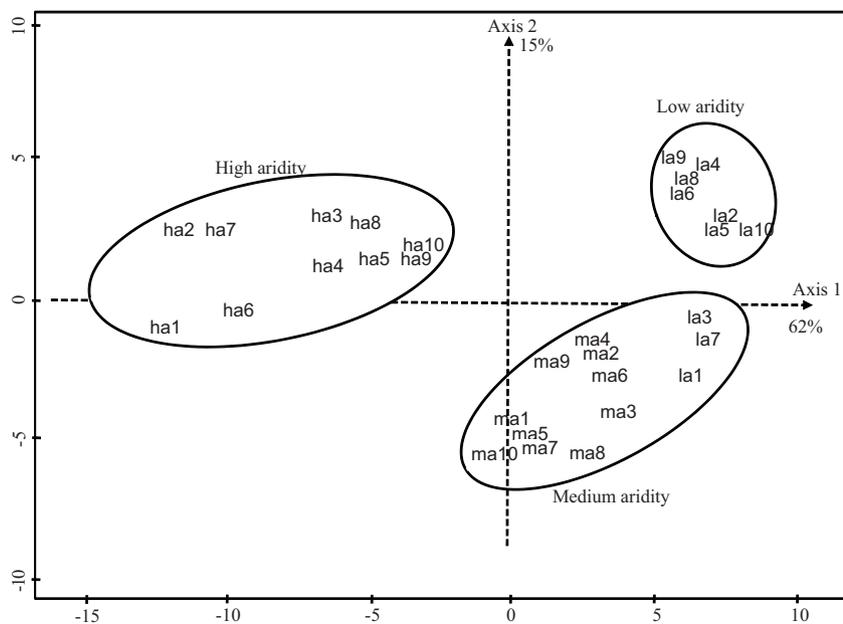
**Fig. 4.** Correlation of occurrences of terpenoid compounds ( $\mu\text{g g}^{-1}$  dw) from female *Pistacia atlantica* ssp. *atlantica* leaves from low, medium and high aridity sites in Algeria; shown are only those terpenoids among which high correlation could be found.

highest mean concentrations of monoterpenes ( $136 \mu\text{g g}^{-1}$  dw), sesquiterpenes ( $290 \mu\text{g g}^{-1}$  dw) and total terpenes ( $427 \mu\text{g g}^{-1}$  dw) were observed in the high aridity site, whereas these figures were:  $57 \mu\text{g g}^{-1}$  dw,  $57 \mu\text{g g}^{-1}$  dw and  $113 \mu\text{g g}^{-1}$  dw, respectively, at the low aridity site.

Multivariate analysis was applied to the terpenoid contents of 30 solvent extracts. Fig. 4 shows the two-dimensional mapping of the Principal Component Analysis which comprises 77% of the total inertia. Axis 1 represents 62% of the information and is characterized on the positive side by thuja-2,4(10)-diene and on the negative side by a couple of compounds, essentially tricyclene, camphene, isoborneol acetate,  $\beta$ -cubebene,  $\beta$ -copaene,

germacrene D,  $\delta$ -cadinene and spathulenol. Axis 2 representing 15% of the information is characterized on the negative side by  $\beta$ -pinene and terpinen-4-ol.

Positions of the individual samples from leaf extractions in the two-axes space show an overall homogeneity between leaf extracts belonging to the same study site (Fig. 5). Three main groups which are characterized by the geographical provenances can be distinguished. The first group is situated on the positive side of Axis 1 and includes samples from individuals of the low aridity site. The second group is located on the negative side of Axis 1 and includes all individuals of the high aridity site. The third group situated on the negative side of Axis 2, between the points related



**Fig. 5.** Two dimensional PCA of *Pistacia atlantica* ssp. *atlantica* individual samples originating from low (la), medium (ma) and high (ha) aridity sites in Algeria.

to samples from the two extreme sites, includes in the majority samples from individuals of the medium aridity site. These three groups are clearly separated along Axis 1 which can be interpreted as indicating the aridity gradient. The most discriminating variables encompass  $\alpha$ -pinene, spathulenol,  $\delta$ -cadinene and  $\beta$ -copaene.

## Discussion

Increase of epidermis, cuticle, palisade parenchyma and total leaf thickness with the degree of aridity may enhance survival and growth of *P. atlantica* by improving water relations and providing higher protection for the inner tissues in the high aridity site. Such patterns were observed in many species submitted to water stress (e.g., Bussotti et al., 2002; Bacelar et al., 2006; Guerfel et al., 2009). Also, a pronounced decrease of leaf size reduces transpiration in sites where water is scarce, as also reported for other plants (Huang et al., 2009; Macek et al., 2009). The high morpho-anatomical plasticity of *Pistacia atlantica* in response to aridity may explain its wide ecological distribution in northern Africa. Trichomes are considered as important taxonomic characters (Krak and Mraz, 2008; Salmaki et al., 2009; Shaheen et al., 2009). The absence of tufted hairs in Bechar population suggests the existence of genetic differences between the populations studied.

Regarding the phytochemistry of *P. atlantica*, no data were reported before on extractable terpenoids composition of the pistacia leaves. However, qualitative and quantitative analyses of essential oils from leaves of *P. atlantica* were reported by several authors. Oils from female plants originating from Greece contained myrcene (17.8–24.8%), sabinene (7.8–5.2%) and terpinene (6–11.6%) as major components (Tzakou et al., 2007). Some compounds found in our samples like  $\gamma$ -amorphene, p-mentha-1,3,5-triene, cis- and trans-sabinene hydrate,  $\alpha$ -campholenic aldehyde, trans-verbenol, myrtenal, myrtenol, verbenone,  $\alpha$ -muurolene and spathulenol were not found in leaves of *P. atlantica* from Greece. A provenance from Morocco whose sex was not specified was rich in terpinen-4-ol (21.7%) and elemol (20.0%) (Barrero et al., 2005). These compounds were found in small amounts (less than 1.1%) also in our samples. Recently, Gourine et al. (2009) have identified 31 compounds from samples harvested at Laghouat with  $\beta$ -pinene (19.1%),  $\alpha$ -terpineol (12.8%), bicyclogermacrene (8.2%) and spathulenol (9.5%) as the principal molecules. Qualitative and quantitative differences between literature data and our results may be explained by such factors as sex of the plants (Tzakou et al., 2007), period of plant collection (Barra et al., 2007; Gardeli et al., 2008; Hussain et al., 2008), plant competition (Ormeño et al., 2007b), position of leaves in the trees (Gambliel and Cates, 1995; Barnola et al., 1997), soil nutrient availability (Yang et al., 2005; Ormeño et al., 2008; Blanch et al., 2009) and water availability (Turtola et al., 2003; Blanch et al., 2009). Moreover, according to the method of extraction used, recovering the true components of the plant *in vivo* still remains a matter of debate. Indeed through hydrodistillation, thermal hydrolysis in acid medium may be a source of artifacts in terms of the essential oil composition (Adams, 1991).

However, the chemical analysis indicated that there are significant differences between the three populations which were analyzed by the same method. These differences comprise both the quantitative and the qualitative composition of the terpenoids. Spathulenol and  $\alpha$ -pinene are the dominant compounds that clearly discriminate quantitatively the three stations. Although being identified in minor contents from samples of low and medium aridity stations, thuja-2,4(10)-diene, p-mentha-1,3,5-triene, nopinone and trans-3-pinocarvone were not registered from high arid station samples. This raises the question of the role of indi-

vidual terpenoid components in plant responses to aridity and the central issue of phenotypic/genotypic diversity of the investigated populations.

Allelopathic properties of  $\alpha$ -pinene are reported in literature. This hydrocarbon monoterpene inhibits radicle growth of several species, enhances root solute leakage and increases level of malondialdehyde, proline and hydrogen peroxide indicating lipid peroxidation and induction of oxidative stress (Singh et al., 2006). It is likely that, the high content of  $\alpha$ -pinene found in the leaves from the driest site may influence interspecific competition for water resources. For all sites investigated, the understory diversity was low, composed mainly of *Ziziphus lotus*. Hence,  $\alpha$ -pinene might play direct and indirect roles in *P. atlantica* responses to drought situations.

Spathulenol is an azulenic sesquiterpene alcohol that occurs in several plant essential oils (Mévy et al., 2004; Cavar et al., 2008). Azulenes are also known as allelochemicals (Inderjit et al., 1999). Especially their bactericidal activity has been proven as well as their function as plant growth regulator precursors (Muir and Hansch, 1961; Konovalov, 1995). Azulene is a polycyclic hydrocarbon, consisting of an unsaturated five member ring linked to an unsaturated seven member ring. This molecule absorbs red light 600 nm for the first excited state transition and UVA 330 nm light for the second excited state transition producing a dark blue color in aqueous medium (Tetreault et al., 1999). The high content of spathulenol found from leaves collected in the high arid station may be interpreted as a defense mechanism against deleterious effects of biotic interactions and UV-light during summer.

Our results are in accordance with several authors who reported increased terpene concentrations in plants under high temperature and water stress conditions (Llusià and Peñuelas, 1998; Loreto et al., 2004; Peñuelas et al., 2005; Llusià et al., 2009). For instance, 54 and 119% increases of total terpene contents under drought treatment were recorded from *Pinus halepensis* and *Quercus ilex*, respectively (Blanch et al., 2009). Because monoterpene biosynthesis is strictly dependent on photosynthesis (López et al., 2008) the increase of their content along with aridity suggests an involvement of specific metabolic pathways that sustain photosynthesis in harsh environmental conditions. In our study, the high thickness of palisade parenchyma can be mentioned in favor of this assumption. On the other hand, monoterpenes act as plant chloroplast membrane stabilizers and protectors against free radicals due to their lipophilicity and the presence of double bonds in their molecules (Peñuelas and Llusià, 2002; Chen et al., 2009). Hence, the increase of monoterpenes may be considered as a regulatory feedback loop that protects photosynthesis machinery from oxidative and thermal damages.

Glandular trichomes are one of the most common secretory structures that produce and store essential oil in plants (Covello et al., 2007; Giuliani and Bini, 2008; Biswas et al., 2009). The high terpenoid contents in Bechar population could be related to the high density of glandular trichomes in this population, which would be also in accordance with other results found by several authors (Mahmoud et al., 2004; Fridman et al., 2005; Ringer et al., 2005).

$\delta$ -cadinene and  $\beta$ -copaene are two compounds found in low contents (0.5–3.8 and 1–4.9  $\mu\text{g g}^{-1}$  dw, respectively) and are similarly as spathulenol and  $\alpha$ -pinene correlated with the increased aridity the populations are experiencing. Except for antibacterial effects (Townsend et al., 2005; Bakkali et al., 2008), no information is available about specific ecological roles of  $\beta$ -copaene and  $\delta$ -cadinene. It should be noted that they are germacrene D derivatives (Bülow and König, 2000) which is found in high concentration in *Cupressus sempervirens* after long-term water stress (Yani et al., 1993). Also, the content of germacrene D from *Pistacia lentiscus* was shown to increase four times during the summer season compared to spring (Gardeli et al., 2008).

The different terpenoids can be appreciated as aridity markers characterizing the three *P. atlantica* populations. It is not clear whether they are constitutively synthesized or induced by the environmental conditions. Morphological data of leaves indicate that the three populations significantly differ. Scanning electronic microscopy of leaves of seedlings from the high aridity and low aridity provenances grown under controlled conditions reveals that the two populations keep their morphological differences with respect to trichome typology and density. Hence it is likely that the three populations investigated indeed are genetically different. Therefore the chemical variability observed might be as well genetically based. This should be tested in the future by submitting clones selected from the three populations to the same conditions of drought.

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